IMPACT OF OILFIELD WASTEWATERS FROM SOKU SWAMP RIG ON THE POPULATION AND DIVERSITY MICROBES OF SOKU RIVER IN NIGER DELTA

Abstract

Oilfield wastewater contains toxic and hazardous substances that are detrimental to water quality and microorganisms. Accelerated water quality change due to oilfield wastewater from operations of oil industries in Nigeria discharging into water has long become an environmental issue in Niger Delta. The microbiological evaluation of the impact of oilfield wastewater from Soku swamp oil rig on Soku River in Rivers State in the Niger Delta was studied bi-weekly for a period two months. Water samples collected from up-stream, down-stream, drilling point, Deck drainage, and a control point 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 0.2 to 2.4 (log10cfu/ml), total fungal (TF) count ranged from 0.1 to 0.95 (log10cfu /ml), while the total hydrocarbon utilizing bacterial (THUB) count ranged from 0.01 to 0.25 (log10cfu/ml), and the total hydrocarbon utilizing fungal (THUF) count ranged from 0.01 to 0.85 (log10cfu/ml). Statistical analysis showed that there was no significant difference in the THB between the control and the sampling stations. The types of bacteria isolated in the study included *Streptococcus* spp, *Bacillus* spp, *Pediococcus* spp, *Kurthia, Staphylococcus* and *Micrococcus* spp. The fungi isolated included, *Aspergillus flavus*, *Penicillium adamatzii*, *Rhizopus oligosporus* and *Penicillium* 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 body studied contained active indigenous hydrocarbon utilizers which can be harnessed for bioremediation process.

Keywords: Oilfield wastewater, drilling point, deck drainage, hydrocarbon utilizing bacteria and 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.

Offshore drilling for oil and gas produces large amount of oil field waste water which is usually discharged into the aquatic environment though it may undergoes some form of treatment before being discharged. Oilfield wastewater 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).

Nigeria oilfield formation waters contain 3,000 to 9000mgL-1 chloride ions (Ibiebele, 1985; Oteri, 1985) and the continuous discharge of such wastewaters into a freshwater environment could cause major damage to aquatic and agricultural resources. In recent years, oilfield formation water has been regarded as a major pollutant of the aquatic environment in Nigeria (Ibiebele, 1985; Garbado *et al.*, 2011). To reduce the impact of oilfield formation water, it is subjected to some form of treatment before it is discharged into the aquatic or terrestrial environment (Garbado *et al.*, 2011).

Produced water is a complex mixture with many variables influencing its characteristics, including the age and location of the oilfield, 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). Environmental regulations in most jurisdictions dictate the allowable water quality parameters for discharged waters and often include maximum oil-in-water concentration limits, ranging between 14 mg/L and 39 mg/L (OGP, 2005). Current treatment methods are not entirely effective, and small suspended oil particles, micro-emulsions, dissolved elements, and organic chemicals are often still present in treated produced water (Fakhru'l-Razi *et al*., 2009).

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). Oilfield wastewater contains toxic and hazardous substances that are detrimental to water quality and microorganisms. Therefore, this study investigated the impact of oilfield wastewater from Soku swamp oil rig on the population and diversity of microorganisms of Soku River in Rivers State.

MATERIALS AND METHODS

Description of study Area

Soku is a community in Akuku- Toru Local Government area, Rivers State. It is located in a coastal swamp zone around 40km South West and known to be part of the Niger Delta oil producing community. Its geographical coordinates are Latitude 4.683,4^0 41. 0" North and Longitude 4.683, 6^0 41. 0" East. The oilfield site is known as OML 23, where all the oil production processes are carried out and oilfield wastewater is discharged.

Collection of Samples

Oilfield wastewater samples were collected from Soku flow Station (OML, 23); an onshore oil production platform located in Soku in Akuku-Toru local government Area (AKULGA) of Rivers State, Nigeria. The Oilfield wastewater samples were collected using sterilized glass bottles and were appropriately labeled immediately after collection and stored in an ice packed cooler. On the other hand, the upstream and downstream were taken from a point 500m to the left and to the right from the drilling point. The drilling point sample was collected from the drilling point of the oil rig, while the deck drainage sample was collected from the deck on the point of drilling. The Control sample on the other hand was collected from 2km upstream from the point of drilling. The collected and appropriately labeled oilfield wastewater samples 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 – [$MgSO_4.7H_2O$ (0.42g), KCl (0.29g), KH_2PO_4 (0.83g), Na_2HP0_4 (1.25g) $NaNO_3$ (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 45ºC 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^{-4} for oilfield wastewater and of 10^{-5} for the other river water samples were saparately 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 or river water (Obire and Wemedo, 1996).

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).

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^{-3}) on mineral salt agar. The Vapor Phase Transfer method was adopted by the use of sterile filter paper discs that were impregnated with 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 as colony forming units per ml (cfu/ml) of sample.

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 ($10^{-1)}$ 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 water sample 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

The results of Total Heterotrophic Bacteria Count (THB) ranged from 0.02 Log_{10}cfu/ml to 2.4 Log_{10}cfu/ml. The highest count was recorded in the Downstream (2.4 Log_{10}cfu/ml), while the lowest was observed in the Deck Drainage (0.2 Log_{10}cfu/ml). The total fungal counts (TFC) ranged from 0.05 Log_{10}cfu/ml to 0.95 Log_{10}cfu/ml. The highest count was recorded in the Control (0.95cfu/ml), while the Lowest was observed in the Downstream. The Total Hydrocarbon Utilizing Bacteria (HUB) count ranged from 0.01 Log_{10}cfu/ml to 0.25 Log_{10}cfu/ml. The highest count was observed in the Deck Drainage (0.25 Log_{10}cfu/ml), while the lowest was recorded in Upstream, Downstream and Control (0.01 Log_{10}cfu/ml). The total Hydrocarbon Utilizing Fungi (HUF) counts ranged from 0.015 Log_{10}cfu/ml to 0.85 Log_{10}cfu/ml. The highest was recorded in the Deck Drainage, while the lowest was recorded in the Upstream. The microbiological counts obtained in the various sampling points in Log10cfu/ml are as shown in Fig 1.

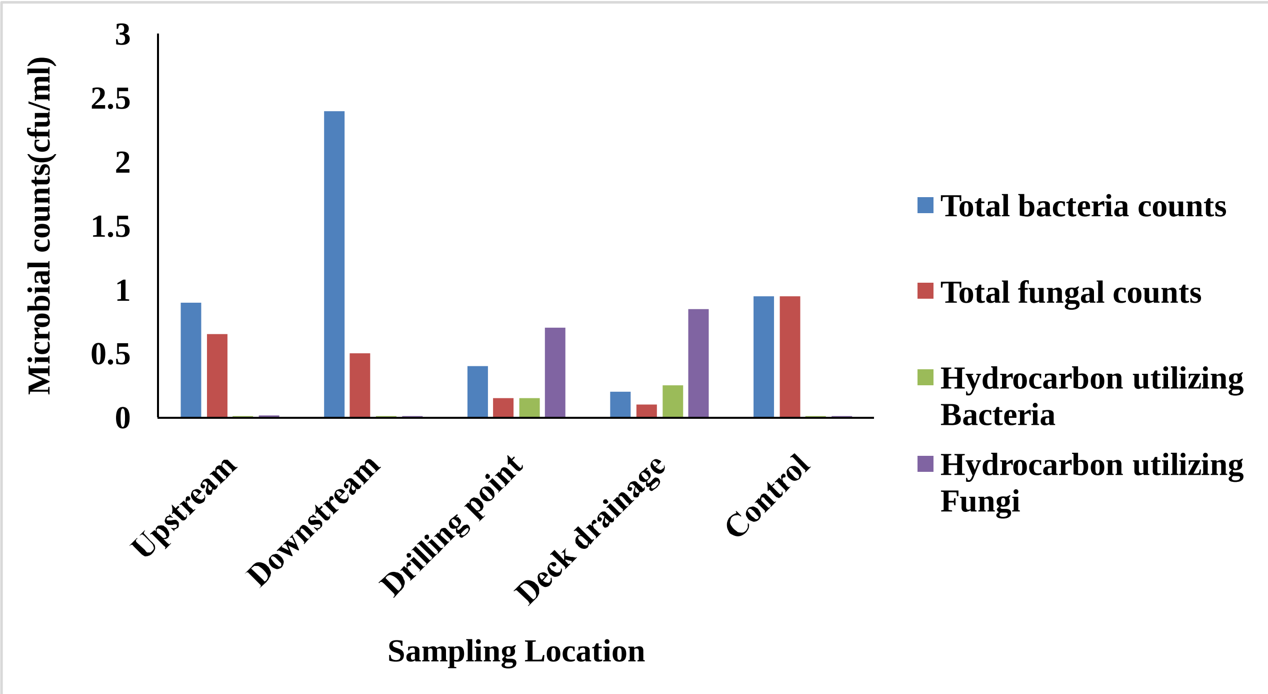

Fig 1: Microbial Counts of Soku

The predominant bacteria are of the genera; *Bacillus*, *Streptococcus*, *Kurthia*, *Staphylococcus*, *Micrococcus* and *Pediococcus* and fungi genera; *Rhizopus*, *Penicillium*, *Aspergillus* that were isolated from the oilfield wastewater from Soku are as shown in Table 1 and Table 2 respectively.

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

Isolates code	**Organism**
DD S11	*Streptococcus* spp
S12	*Bacillus* spp
S13	*Pediococcus* spp
S14	*Micrococcus* spp

S15	*Bacillus* spp
S16	*Bacillus* spp
Hydrocarbon Utilizing Bacteria	
HSU1	*Kurthia* spp
HSU2	*Bacillus* spp
HSDD	*Staphylococcus* spp
HSDP	*Pediococcus* spp
HSC1	*Staphylococcus* spp
HSC2	*Staphylococcus* spp

Keys; DDS- deck drainage Soku, HSU1,2- Hydrocarbon utilizing bacteria upstream 1&2, HSDD; Hydrocarbon bacteria deck drainage, HSDP-Hydrocarbon bacteria drilling point, HSC1 &2- Hydrocarbon bacteria control 1&2

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

Isolates code	Organism
SK7	*Rhizopus oligosporus*
SK10	*Penicillium adamatzii*
SK4	*Aspergillus flavus*
SK15	*Penicillium* spp

Keys; SK- Soku

DISCUSSION

The present study revealed the microbial population and diversity of bacteria and fungi in oilfield wastewater from Soku. Microbial populations play a role in degradation of hydrocarbon contaminations, Atlas (1981) and Leahy and Colwell (1990) reported that the rate of petroleum hydrocarbon biodegradation in nature is determined by the populations of indigenous hydrocarbon degrading microorganisms. 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. Heterotrophic bacteria counts were higher in the oilfield wastewater in the various months of study compared to other samples.

The result showed that more heterotrophic bacteria count was obtained from the downstream, followed by the control and then the least count was obtained from the deck drainage, these can be attributed to the less oilfield wastewater been deposited there than in the deck drainage. The high fungi count was obtained in the control point, followed by upstream and the least found in the downstream, this can attribute to lesser activities being carried out there in the downstream. The high hydrocarbon utilizing bacteria and fungi count was obtained in the Deck drainage, followed by the Drilling point, the least count was obtained in the Upstream, Downstream and Control, this high hydrocarbon utilizing bacteria and fungi count found in the Deck Drainage can be attributed to more hydrocarbon content being deposited there since it is close to the Drilling point and drains the wastewater. The high hydrocarbon utilizing bacteria and fungi found in this study concurs with a research carried out by Aleruchi and Obire (2019) also reported hydrocarbon utilizing bacteria and fungi count in oilfield wastewater which can be attributed to inorganic and organic constituent found in the oilfield wastewater that serves as nutrient for bacteria and fungi growth. The continuous discharge of treated oilfield wastewater will have a deleterious effect on the proper functioning of the aquatic ecosystem thereby affecting aquatic and agricultural resources that are of economic importance (Obire, and Amusan, 2003).

The isolated Bacteria (*Streptococcus, Bacillus, Kurthia, Staphylococcus, Micrococcus* and *Pediococcus*) and Fungi (*Rhizopus oligosporus, Penicillium adamatzii, Aspergillus flavus*) 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 Soku.

CONCLUSION AND RECOMMENDATION

The microbial population of hydrocarbon utilizing microorganisms play a role in the degradation of hydrocarbon contaminated environment. The occurrence of these microorganisms in the waste water may be to the constant exposure of these microorganisms to hydrocarbon (oily) components of the waste water 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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