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Microbiological Evaluation of Various Oilfield Wastewaters from Oben Land Rig Location in Edo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Oilfield wastewater contains poisonous and dangerous compounds that harm microorganisms and the quality of the water. According to estimates, Nigerian oil industry operations generate significant amounts of wastewater that are improperly treated before being released into the environment. For a period of two months, biweekly analyses of the microbiological effects of various oilfield wastes on the microbial population and variety of aquatic environments were conducted. Water samples collected 10 cm 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. 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

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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 study's bacterial and fungal counts showed how oilfield effluent affected aquatic microorganisms. According to the significant frequency of hydrocarbon-using bacteria, the water under investigation contained active native hydrocarbon utilizers that might be used in the bioremediation process.

Keywords: Oilfield wastewater; hydrocarbon utilizing bacteria / hydrocarbon utilizing fungi.

1. INTRODUCTION

Oilfield wastewater, also known as produced water, is the formation and injection water created during the extraction of crude oil and natural gas from onshore and offshore wells [1,2]. The production water is a complex mixture of organic and inorganic substances that are both dissolved and particulate, and it can range in composition from concentrated saltwater brine to practically freshwater. Water soluble low molecular weight organic acids and monocyclic aromatic hydrocarbons are the two types of organic compounds that are most prevalent in produced waters. The age and location of the oil field, the geological features 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 are just a few of the many factors that can affect the characteristics of produced water [2]. Although the major constituents of generated water are comparable throughout oil production facilities [3] the composition of this fluid is thought to be highly variable [4] and constituent concentrations can vary between sources by orders of magnitude. According to [3], the constituents of produced water include solids like formation solids, corrosion and scale materials, bacteria, waxes, and asphaltenes, as well as dissolved gases and heavy metals and radioactive materials. It also contains production chemicals, which are typically synthetic additives. Depending on their solubility and structural characteristics, hydrocarbons, which make up the majority of oil molecules, may be present in generated water as distributed droplets or dissolved in the water phase [5]. Carboxylic acids are often found in the dissolved phase, whereas aliphatic hydrocarbons are typically found in the dispersion phase. Depending on their molecular weight and structural complexity, aromatic compounds can exist in either or both phases. Lower molecular weight compounds tend to be substantially more water soluble and are therefore more frequently found in the water (dissolved) phase [5]. Large amounts of produced water are produced during the production stage of conventional oil wells. According to Neff et al. [1], there are approximately 1.1 m3 of waste generated for every m3 of oil produced globally, making this the largest waste stream linked with the production process (Arctic Monitoring and Assessment Programme (AMAP), 2010). Before being released into the ocean as trash or reinjected into a sub-sea formation for disposal, produced water is often treated to eliminate the dispersed crude oil content (i.e., droplets of crude oil, typically ranging in size from 1 to 10 um) [6,7].

Large amounts of oil field waste water are created during offshore drilling for oil and gas, albeit they are typically treated before being released into the aquatic environment [8,9,10]. After being separated from the oil extracted from the reservoir, oil field waste water is released into the sea [11]. Oilfield wastewater, which is water generated alongside oil and gas but separated for disposal, can retain up to 50mgL-1 of distinct phase oil as tiny droplets and can also contain up to 35mgL-1 of dissolved hydrocarbons [12]. More dangerous than crude oil itself may be the various inorganic components that are dissolved in formation water [13,14]. The release of hazardous substances and the buildup of these contaminants in these aquatic ecosystems endanger the ecological health of many river systems [15].

The most prevalent organic compounds in most treated generated waters are water-soluble low molecular weight organic acids (mainly mono- and di-carboxylic acids) and monocyclic aromatic hydrocarbons (MAHs) including benzene, ethyl benzene, toluene, and xylenes [1]. 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 [5,16]. The microbiological analysis of several oilfield wastewaters from the Oben Land Rig location in Edo State was hence the focus of this investigation.

2. MATERIALS AND METHODS

2.1 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.

2.2 Collection of Samples

Oben Flow Station, an onshore oil production facility in Oben, Edo State, Nigeria, was used to collect oilfield wastewater. The water samples were taken using a specifically made pail at each Land rig location. Before being collected from each Pit point, the samples were washed in the pail. The samples were placed in sterilized McCartney bottles with correct labels for microbiological analysis, and they were then kept in an ice-filled cooler. Oilfield wastewater was immediately delivered to the lab for processing and testing within 24 hours of being collected and properly labeled.

2.3 Media Preparation

For the total heterotrophic bacterial count, nutrient agar was used; for the total fungal count, potato dextrose agar; and for the isolation of the total hydrocarbon-using bacteria and fungi, mineral salt agar medium prepared in accordance with Mills 's et al [17] modified minimal salts medium (MSM) composition was used. The composition of minimal salts medium (MSM) 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 [18] 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.

2.4 Microbiological Analysis of the Oilfield Wastewater

2.4.1 Determination of Total Heterotrophic Bacterial (THB) count of oilfield wastewater

The nutritional agar and spread plate method, as reported by Prescott et al., was used to count the total heterotrophic bacteria (THB) (2005) [19]. Using dilution ratios of 10-5 for raw wastewater and 10-4 for oilfield wastewater, an aliquot (0.1 ml) of each sample was serially diluted and inoculated in triplicate onto various sterile nutrient agar plates. The plates were incubated for 24 hours at 37°C in an upside-down posture. Only counts of 30 to 300 were recorded when colonies on the plates were counted after incubation. For oilfield wastewater, the average values of replicate plates were calculated and expressed as colony forming units (cfu/ml).

2.4.2 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 [20].

2.4.3 Hydrocarbon Utilizing Bacterial Count (HUB) of samples

Oilfield wastewater samples were used to calculate the total number of hydrocarbon-using bacteria by inoculating 0.1ml of the serially diluted samples (10-4) onto mineral salt agar. The Vapor Phase Transfer technique was used, with the only carbon source in the mineral salt agar being sterile filter paper discs bathed in filter sterilized crude oil [20]. The sterile filter papers were aseptically placed within the inoculated Petri dishes' inner cover and allowed to incubate for five days at room temperature. The number of colonies that form was counted, and the average of duplicate colonies was used to compute the colony forming units per milliliter (cfu/ml) of oilfield wastewater.

2.4.4 Hydrocarbon Utilizing Fungal Count (HUF) of samples

Oilfield wastewater was tested for the total number of hydrocarbon-using fungi by serially diluting samples (10-2) and inoculating 0.1ml of each onto mineral salt agar. To prevent bacterial development, streptomycin (0.1 ml) will be added to the mineral salt medium [20]. Utilizing sterile filter paper discs soaked in filter sterilized crude oil, the only carbon source in the mineral salt agar, the Vapor Phase Transfer technique was used. The sterile filter papers were aseptically placed within the inoculated Petri dishes' inner cover and allowed to incubate for five days at room temperature. The number of colonies that form was counted, and the average of duplicate colonies was used to compute the colony forming units per milliliter (cfu/ml) of oilfield wastewater.

2.4.5 Characterization and identification of bacterial and fungal isolates from samples

The isolates from the investigation were inspected, and their morphological, cultural, and microscopic properties were noted. Gram staining, motility, catalase, oxidase, citrate utilization, sugar fermentation, hydrogen supplied generation, indole production, methyl red, and the Voges Proskauer test were among the morphological and biochemical tests performed using the isolates. Using Bergey's manual of determinative bacteriology, the isolates' morphological and biochemical properties were compared to those of recognized Taxa (1994) [21]. Pure fungal cultures were examined under a compound microscope while still on plates and after being wet mounted in lactophenol on slides for the presumed identification of fungi isolates. Vegetative hyphae and reproductive structures were observed, and they were documented and matched to the established identification key of [22] and [23].

2.5 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.

3. RESULTS

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.

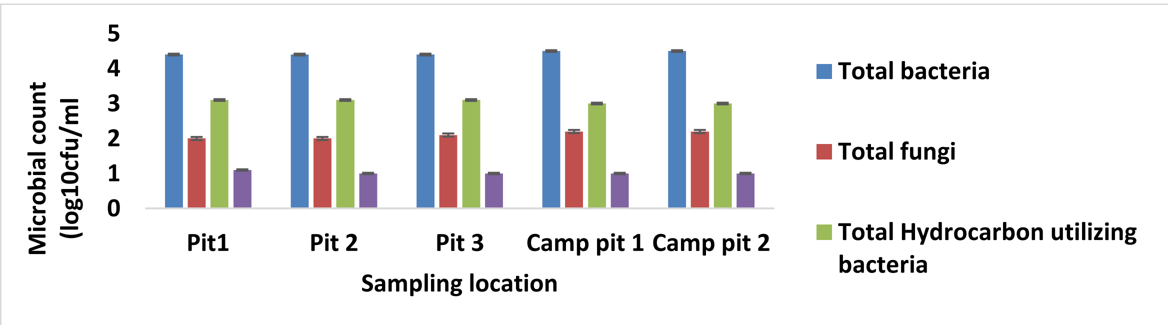

Fig. 1. Microbial counts of Oben

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

4. DISCUSSION

In the oilfield wastewater from Oben, the current study identified the microbial population and variety of bacteria and fungi. Leahy and Colwell [24] found that hydrocarbon biodegradation depends on the makeup of the microbial community and its adaptive response to the presence of hydrocarbons. Microbial populations have a role in the degradation of hydrocarbon contaminations. The number of bacteria and fungus that use hydrocarbons increased over the course of the study's different months in the oilfield wastewater.

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

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. Continuous release of treated oilfield wastewater will negatively impact the health of the soil and aquatic habitats, impacting agricultural and aquatic resources with significant economic value [25,26,27]. 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 [28,29] indicating high hydrocarbon content contained in the oilfield waste water that is been discharged into Oben oilfield.

5. CONCLUSION AND RECOMMENDATION

The prevalence of these bacteria in wastewater may be due to their ongoing exposure to hydrocarbon (oily) wastewater constituents, which may have given rise to their capacity to use and develop in the presence of the hydrocarbon. The high population of hydrocarbon consumers at the sampling stations suggests that the hydrocarbon consumers have become more numerous in the polluted area as a result of adapting to the amount of hydrocarbons in the environment. The investigation also showed that the utilizers comprised the majority of the species recovered as total heterotrophic bacteria and total fungus. The reaction of these microorganisms in the oil-contaminated environment shows that the isolated bacteria and fungus could use the oil as energy and carbon source, which serves as food for their growth and may thus be useful in the bioremediation of the polluted locations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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