



Effect of Season on the Microbial Quality of Oilfield Wastewater and Receiving Pond

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DOI: 10.31080/ASMI.2022.05.1135

Received: July 07, 2022

Published: August 09, 2022

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Abstract

The effect of season on the microbial quality of oilfield wastewater and the effluent receiving pond was investigated. Oilfield wastewater and the recipient pond samples were collected from the Ogbogu flow station, Rivers State, Nigeria for one year. The total heterotrophic bacteria, total heterotrophic fungi, hydrocarbon utilizing bacteria, and fungi of the samples were evaluated using standard microbiological methods. The mean range of the total heterotrophic bacterial, hydrocarbon utilizing bacterial, total heterotrophic fungal, and hydrocarbon utilizing fungal counts of the oilfield wastewater effluents was 1.29×10^8 - 1.98×10^8 , 1.22×10^4 - 1.44×10^4 , 1.5×10^4 - 1.8×10^4 , and 1.3×10^3 - 2.8×10^3 CFU/ml, respectively for the dry season and 3.1×10^7 - 3.6×10^7 , 2.3×10^3 - 2.8×10^3 , 1.5×10^4 - 1.6×10^4 , and 1.2×10^3 - 1.5×10^3 CFU/ml, for the rainy season. The mean range of the total heterotrophic bacterial, hydrocarbon utilizing bacterial, total heterotrophic fungal, and hydrocarbon utilizing fungal counts of the pond effluents was 2.42×10^5 - 2.73×10^5 , 2.01×10^5 - 2.11×10^5 , 2.6×10^4 - 3.8×10^4 , and 1.5×10^3 - 5.7×10^3 CFU/ml, respectively for dry season 4.6×10^4 - 5.0×10^4 , 3.0×10^3 - 3.3×10^3 , 1.5×10^4 - 2.8×10^4 , and 1.4×10^3 - 1.6×10^3 CFU/ml, respectively for the rainy season. *Bacillus* sp, *Aeromonas* sp, *Micrococcus* sp, *Staphylococcus* sp, *Chryseomonas* sp, *Proteus* sp, *Pseudomonas* sp, *Klebsiella* sp, *Actinomyces* sp, *Enterobacter* sp, *Rhodococcus* sp, and *E. coli* were isolated in both samples. *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Saccharomyces cerevisiae*, *Geotricum*, *Fusarium*, and *Penicillium* sp were also isolated from both samples. The HUB, THF, and HUF of the Pond effluent were significantly higher ($P \leq 0.05$) than values obtained for the oilfield wastewater effluent. The microbial population in the pond samples was influenced by the seasons and drier months were characterized by higher microbial populations. These organisms would be very useful for bioremediation.

Keywords: Seasonal Variation; Microbial Quality; Oilfield Wastewater; Recipient Pond

Introduction

Petroleum is a naturally occurring material found in a wide range of rock formations (reservoirs) across the world [1]. In Nigeria, crude oil exploration and extraction operations are major polluters of the Niger Delta's environment [2]. The bulk of crude oil, which is the liquid component of petroleum, is made up of hydrocarbons [3]. However, nitrogen, sulfur, oxygen, and metal compounds may be present [4]. During oil and gas exploration and production, oilfield wastewater is the major wastewater that is

brought up from the hydrocarbon-bearing formation layers and it is a combination of formation water, saltwater, or freshwater that has been trapped beneath hydrocarbons in porous reservoir material for millions of years [5]. Global production of oilfield wastewater is anticipated to be about 60 million barrels per day [6]. The amount of oil produced by each petroleum reservoir is determined by elements such as geological structure, petrophysics, reservoir drive mechanism, well completion technology, and production methods [7]. According to Xie, *et al.* [8], benzene, toluene, ethylbenzene,

xylene, naphthalene, phenanthrene, and dibenzothiophene are among the complex organic chemicals found in oilfield generated water. More so, the presence of harmful chemicals in oilfield-produced water, untreated discharges can damage land and water bodies [8].

Due to the discharge of enormous amounts of oilfield wastewater without sufficient treatment processes, increased petroleum operations, notably in the Niger Delta, have resulted in pollution stress on soil and surface water [9,10]. Oilfield wastewater having significant levels of organic and inorganic pollutants is hazardous to the environment. Total petroleum hydrocarbons, total solids (TS), inorganic compounds such as heavy metals and polycyclic aromatic hydrocarbons (PAHs), biochemical and chemical oxygen demand (BOD and COD), and microorganisms are the most important environmental challenges in oilfield wastewater [11]. The Niger Delta environment is continually polluted with oilfield wastewater and the presence of this waste in the soil has been reported to impact the receiving environment [2]. Over 90% of wastewater from oil industry activities in Nigeria is expected to be released into the land, rivers, and streams without appropriate treatment. This is because most oil firms do not have wastewater treatment plants, or if they have, the facilities are insufficient [12]. The impact on the environment could affect the microbial communities either by supporting the growth of selected species thereby inhibiting the growth of other normal flora or by introducing new microorganisms into the receiving environment. facultative aerobic and strictly anaerobic bacteria have been reported to survive in the oil and water phases of oil wells despite the harsh environmental conditions (i.e., low redox potential in the reservoirs, anoxic, high temperature, high salt) in oil-bearing formations [1]. As exploration activities are still paramount in Nigeria especially the Niger Delta region, the microbial communities in the receiving environment could be affected not just by the wastewater but the seasons too. The previous study has reported the effect of seasonal variation on microbial communities.

This report included that certain microorganisms which were found in the rainy season were not isolated in the dry season of the same year [13]. Other studies have also reported that the introduction of untreated or improperly treated oilfield waste on soil surfaces could alter the microbial communities (8). Thus, this study was carried out to investigate the effect of seasons on the microbial quality of oilfield wastewater and the pond receiving the effluents.

Materials and Methods

The study area

The study was carried out in Ogbogu Flow Station; an onshore oil production platform located in Ogba/Egbema/Ndoni Local government Area (ONELGA) of Rivers State, Nigeria. It lies on Latitude 5.34167°N and Longitude 6.65556°E.

Sample collection

The oilfield wastewater samples and recipient pond effluent samples were collected using 250ml sampling bottles. Samples were collected according to the method described in standard methods for water and wastewater analysis. The samples were collected using 250ml of clean sample bottles which were filled from a gentle stream of the wastewater after flushing the interior of the nozzle of the valve with a flow of the wastewater for 2 minutes, to avoid contamination from external sources. The four replicate samples of the oilfield wastewater were thoroughly mixed to form a composite. The recipient pond effluent was randomly collected at four different points in the pond using a two litres plastic hydrobios water sampler and aseptically transferred to clean two litres plastic containers. The four replicate samples of the pond effluent were thoroughly mixed to form a composite. The plastic bottles were appropriately labeled and stored in an ice-packed cooler. The stored samples were immediately transported to the Microbiology laboratory, Rivers State University within 24 hours for processing and analyses.

Enumeration and characterization of total heterotrophic bacteria

This was determined with the nutrient agar using the spread plate technique. An aliquot (0.1 ml) of 10^{-4} dilution of the serially diluted soil sample, was each inoculated onto different sterile nutrient agar plates in triplicates. The plates were inoculated for 24 hours at 37°C. After incubation, colonies that appeared on the plates were counted and the mean was expressed as cfu/g. discrete colonies on the plates after incubation were sub-cultured on pre-dried nutrient agar plates using a sterile wire loop which was sterilized intermittently by heating the loop to red hot for every streak carried out [14].

Enumeration and characterization total fungal

This was determined using the Potato Dextrose Agar (PDA) onto which sterile streptomycin (50 mg/ml) had been added to

suppress bacterial growth [13]. An aliquot (0.1 ml) of 10⁻² dilution of all samples were inoculated in triplicates onto sterile pre-dried PDA plates and then spread evenly with a sterile glass spreader. The plates were incubated at room temperature for about 3-5 days after which the spores were counted and the mean of the count recorded accordingly.

Hydrocarbon utilizing bacteria and fungi

The population of hydrocarbon utilizing bacteria was determined by inoculating 0.1 ml aliquot of 10⁻¹ dilution of all the samples onto mineral salt agar media while the fungi population was determined by inoculating 0.1 ml aliquot of 10⁻¹ dilution of all the samples onto mineral salt agar media supplemented with streptomycin (50 mg/ml) to suppress bacterial growth [13]. The vapour Phase Transfer method of Wemedo., *et al.* [14] was adopted. It employed the use of sterile filter paper discs 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 then aseptically transferred to the inside covers of the incubated Petri dishes and incubated for 5 days at 37°C for bacteria and 5-10 at 25°C for fungi. After the incubation period, means of the colonies were recorded and discrete colonies were sub-cultured onto pre-dried respective media.

Identification of isolates

Representative colonies of bacteria were picked from different plates after the incubation period. They were streaked on sterile agar plates for purification followed by characterization using their colonial morphology, cellular morphology, and biochemical tests. Biochemical tests carried out were; oxidase test, catalase test, indole test, methyl red test, Voges-Proskauer test, starch hydrolysis test, urease test, citrate test, coagulase test, sugar fermentation test, and Triple Sugar Iron agar test. Reference was made to Bergey's Manual of Determinative Bacteriology. While isolates of fungi were identified using their morphological features followed by microscopic examination of their wet mounts prepared with lactophenol cotton blue.

Statistical analysis

The mean and standard deviations of the microbial populations were calculated while the microbial populations of both stations were compared using a T-test. All analysis was carried out using a statistical package for social science (SPSS v26) ($P < 0.05$) was taken as a significant difference.

Results

Results of the mean microbial counts of the oilfield wastewater are presented in figure 1. The mean range of the total heterotrophic bacterial, hydrocarbon utilizing bacterial, total heterotrophic fungal, and hydrocarbon utilizing fungal counts of the oilfield wastewater effluents in the dry season was 1.29×10^8 to 1.98×10^8 , 1.22×10^4 to 1.44×10^4 , 1.5×10^4 to 1.8×10^4 and 1.3×10^3 to 2.8×10^3 CFU/ml, respectively. The mean range of the total heterotrophic bacterial, hydrocarbon utilizing bacterial, total heterotrophic fungal, and hydrocarbon utilizing fungal counts of the oilfield wastewater effluents in the rainy season was 3.1×10^7 to 3.6×10^7 , 2.3×10^3 to 2.8×10^3 , 1.5×10^4 to 1.6×10^4 and 1.2×10^3 to 1.5×10^3 CFU/ml, respectively. Results of the mean microbial counts of the pond effluent for the dry and rainy seasons are presented in figure 2. Results showed that the mean range of the total heterotrophic bacterial, hydrocarbon utilizing bacterial, total heterotrophic fungal, and hydrocarbon utilizing fungal counts of the pond effluents in the dry season was 2.42×10^5 to 2.73×10^5 , 2.01×10^5 to 2.11×10^5 , 2.6×10^4 to 3.8×10^4 and 1.5×10^3 to 5.7×10^3 CFU/ml, respectively. The mean range of the total heterotrophic bacterial, hydrocarbon utilizing bacterial, total heterotrophic fungal, and hydrocarbon utilizing fungal counts of the pond effluents in the rainy season was 4.6×10^4 to 5.0×10^4 , 3.0×10^3 to 3.3×10^3 , 1.5×10^4 to 2.8×10^4 and 1.4×10^3 to 1.6×10^3 CFU/ml, respectively. Results showing the comparison of the microbial counts in the oilfield wastewater and pond effluent for the different seasons are presented in figure 3.

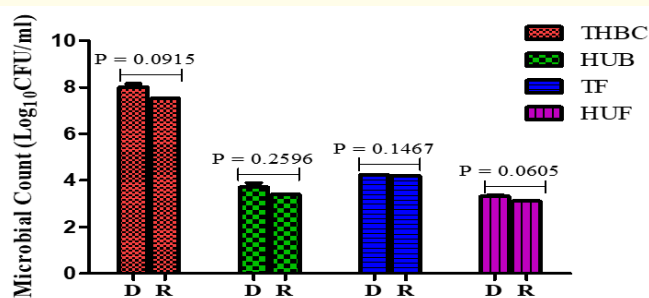


Figure 1: Mean microbial counts of oilfield wastewater during Dry (D) and Rainy (R) season.

KEY: $P \leq 0.05$ are Significantly Different, THBC = Total Heterotrophic Bacteria Count, HUB = Hydrocarbon Utilizing Bacteria, TF = Total Fungi, HUF = Hydrocarbon Utilizing Fungi.

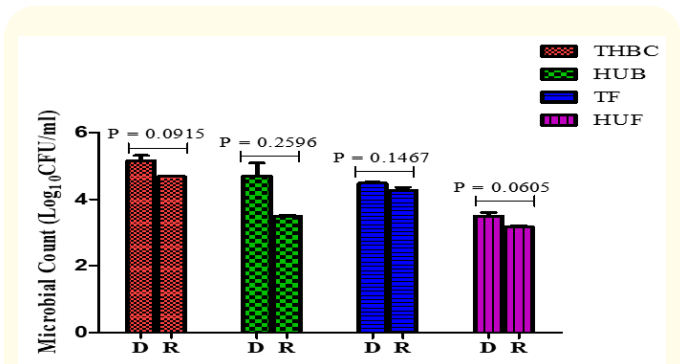


Figure 2: Mean Microbial counts of pond effluent during Dry (D) and Rainy (R) season.

KEY: P ≤ 0.05 are significantly different, THBC = Total Heterotrophic Bacteria Count, HUB = Hydrocarbon Utilizing Bacteria, TF = Total Fungi, HUF = Hydrocarbon Utilizing Fungi.

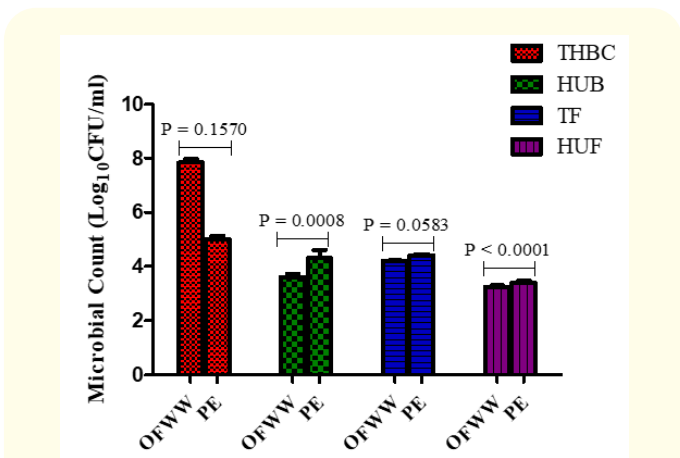


Figure 3: Mean Microbial counts comparing Oilfield Wastewater (OFWW) and Pond Effluent (PE).

KEY: P ≤ 0.05 are significantly different, THBC = Total Heterotrophic Bacteria Count, HUB = Hydrocarbon Utilizing Bacteria, TF = Total Fungi, HUF = Hydrocarbon Utilizing Fungi.

Characterization of bacterial isolates

A total of one hundred and fifty-nine bacterial isolates belonging to twelve genera were isolated from the oilfield wastewater effluent and pond water effluent. The bacterial isolates were *Bacillus* sp, *Aeromonas* sp, *Micrococcus* sp, *Staphylococcus* sp, *Chryseomonas* sp, *Proteus* sp, *Pseudomonas* sp, *Klebsiella* sp, *Actinomyces* sp, *Enterobacter* sp, *Rhodococcus* sp, and *E. coli*. Except for *Aeromonas*

sp, *Klebsiella* sp, *Actinomyces* sp, *Enterobacter* and *Chryseomonas* the rest of the isolates were identified also as hydrocarbon utilizing bacteria. The total number of fungal isolates characterized from the pond and oilfield effluent was one hundred and ninety-eight. These fungal isolates include *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Saccharomyces cerevisiae*, *Geotricum*, *Fusarium*, and *Penicillium* sp. Hydrocarbon utilizing fungi that were isolated includes *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* sp, *Penicillium* sp, and *Saccharomyces cerevisiae*. The distribution of bacterial and fungal isolates across the respective samples is presented in tables 1 and 2, respectively. Results of the percentage occurrence of the bacterial and fungal isolates across the samples are presented in figure 4 and 5, respectively. The percentage occurrence of bacterial isolates in the oilfield wastewater was *Bacillus* sp (10%), *Aeromonas* sp (0%), *Micrococcus* sp (12.5%), *Staphylococcus* sp (10.7%), *Chryseomonas* sp (2.7%), *Proteus* sp (7.1%), *Klebsiella* sp (6.3%), *Pseudomonas* sp (17.9%), *Rhodococcus* sp (11.6%), *E. coli* (5.4%), *Actinomyces* sp (7.1%) and *Enterobacter* sp (8.0%). While the percentage occurrence of bacterial isolates in the pond effluent is *Bacillus* sp (9.1%), *Aeromonas* sp (7.3%), *Micrococcus* sp (10%), *Staphylococcus* sp (10.9%), *Chryseomonas* sp (8.2%), *Proteus* sp (10.9%), *Klebsiella* sp (8.2%), *Pseudomonas* sp (10.9%), *Rhodococcus* sp (7.3%), *E. coli* (5.5%), *Actinomyces* sp (9.1%) and *Enterobacter* sp (2.7%).

The percentage occurrence of fungal isolates in the oilfield wastewater is given as *Aspergillus niger* (20.7%), *Aspergillus fumigatus* (19.6%), *Aspergillus flavus* (18.4%), *Penicillium* sp (17.4%), *Fusarium* sp (10.9%), and *Saccharomyces cerevisiae* (13.0%). While the percentage occurrence of fungal isolates in the pond is given as *Aspergillus niger* (11.5%), *Aspergillus fumigatus* (14.6%), *Aspergillus flavus* (12.5%), *Penicillium* sp (16.7%), *Fusarium* sp (18.8%), and *Saccharomyces cerevisiae* (11.5%) and *Geotricum* sp (14.6%).

Isolates	Oilfield wastewater	Pond Effluent
<i>Bacillus</i> sp	+	+
<i>Aeromonas</i> sp	-	+
<i>Micrococcus</i> sp	+	+
<i>Staphylococcus</i> sp	+	+
<i>Chryseomonas</i> sp	+	+
<i>Proteus</i> sp	+	+
<i>Klebsiella</i> sp	+	+

<i>Pseudomonas</i> sp	+	+
<i>Rhodococcus</i> sp	+	+
<i>E. coli</i>	+	+
<i>Actinomyces</i> sp	+	+
<i>Enterobacter</i> sp	+	+

Table 1: Bacteria Isolated from Samples.

KEY: + = Isolated, - = Not Isolated.

Isolates	Oilfield Wastewater	Pond Effluent
<i>Aspergillus niger</i>	+	+
<i>Aspergillus fumigatus</i>	+	+
<i>Aspergillus flavus</i>	+	+
<i>Penicillium</i> sp	+	+
<i>Fusarium</i> sp	+	+
<i>Saccharomyces cerevisiae</i>	+	+
<i>Geotricum</i> sp	-	+

Table 2: Fungi Isolated from Samples.

KEY: + = Isolated, - = Not Isolated.

Discussion

The microbial populations of oilfield wastewater and the receiving pond in the present study are generally very high. The total heterotrophic bacterial counts of the oilfield wastewater were very high in January and very low in September. This was contrary to the high values of the hydrocarbon utilizing bacteria, hydrocarbon utilizing fungal and heterotrophic fungal counts which were recorded in September. The microbial counts of the receiving pond in the dry and rainy season fluctuated and observed a similar trend to those recorded for the oilfield wastewater with January having higher heterotrophic bacterial counts while September recorded higher counts in the hydrocarbon utilizing bacterial, hydrocarbon utilizing fungal, and total heterotrophic fungal counts, respectively. High bacterial counts in oilfield wastewater have been reported in a previous study [13,14] and this agreed with the findings of the present study. The total heterotrophic bacterial counts which were reportedly high in January in the present study also agreed with Wemedo., *et al.* [14] who in their study reported high mean counts of aerobic heterotrophic bacteria in January. However, the least heterotrophic bacterial counts recorded in March in their study were contrary to the present study which had low bacterial counts in September. Findings also showed that the counts in the total heterotrophic bacteria in the oil field wastewater effluent was higher than the counts recorded for the pond effluents for both seasons while counts in the hydrocarbon utilizing bacteria, hydrocarbon utilizing fungi, and total heterotrophic fungi were higher in the pond effluents than those recorded for the oilfield wastewater effluents. The high total heterotrophic bacterial counts observed in the oilfield wastewater could be attributed to the presence of microbial communities inherent in the oilfield or contamination with other microbial communities from the oil well. According to Tüccar., *et al.* [1], many microorganisms are capable of surviving in the oil and water phases of the oil wells, and these oilfields harbour diverse facultative aerobic and strictly anaerobic microorganisms due to the low redox potential in the reservoirs. Furthermore, oilfield wastewater and pond effluent recorded the highest values in total heterotrophic, hydrocarbon utilizing bacteria, total fungi, and hydrocarbon utilizing fungi count in the dry season than in the rainy season. This agreed with previous studies [13]. Also, Wemedo., *et al.* [14] reported an increase in microbial populations in oilfield wastewater than in wet/rainy seasons and this agreed with the present study. This suggests that the dry season supports the growth of microorganisms better than the wet seasons which is characterized by heavy rainfall. The

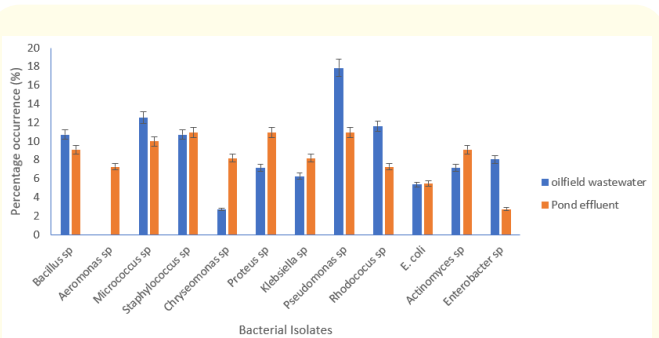


Figure 4: Percentage occurrence of bacterial isolates across the samples.

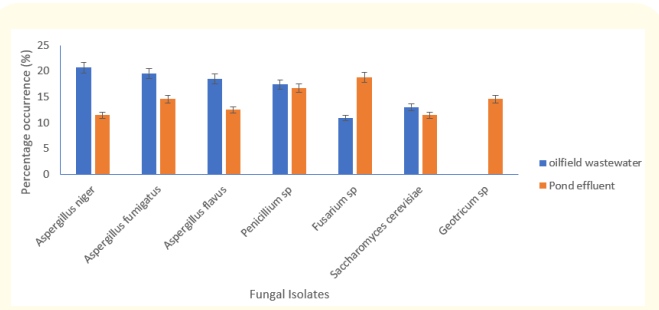


Figure 5: Percentage occurrence of Fungal isolates across the samples.

high rainfall could have altered the microbial populations either by affecting the oxygen content or other parameters required for the growth of the microbial populations. In a previous study, it was reported that climatic conditions selectively favours the growth of different physiological types of microorganisms [15]. Despite the high microbial loads recorded in the dry season, there were no significant differences ($P > 0.05$) in the microbial populations between both seasons. This could suggest that seasonal variation has no significant impact on microbial populations. Also, there were no significant differences in the heterotrophic bacterial counts obtained for oilfield wastewater and pond effluent. However, the hydrocarbon utilizing bacterial, total fungal, and hydrocarbon utilizing fungal counts of Pond effluent was significantly higher ($P \leq 0.05$) than values obtained for the oilfield wastewater effluent. This could be attributed to the continued discharge of hydrocarbon effluent into the receiving pond. Thus, leading to the introduction of hydrocarbon utilizing microorganisms into the pond as well as the adaptation of other microbial types in the pond to hydrocarbon products. Continuous discharges of crude oil into the ecosystem have been reported to cause selective increase or decrease in microbial population [15]. Wemedo, *et al.* [14] suggested that the increased hydrocarbon utilizing bacterial population in the wastewater receiving body could be attributed to the presence of nutrients that selected and supported their growth in this environment. Thus, this could be similar to the present study which showed that the hydrocarbon utilizing microorganisms including heterotrophic fungal populations were higher in the receiving pond.

The distribution of bacterial isolates across the samples showed that both samples of the oilfield wastewater and the receiving pond have similar bacterial types except the presence of *Aeromonas* sp in pond water effluent which was not isolated in oilfield wastewater. The distribution of fungal isolates also showed that *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium* sp, *Fusarium* sp, and *Saccharomyces cerevisiae* were all isolated from both the oilfield wastewater and pond effluent while *Geotrichum* sp was only isolated from the pond effluent. This suggests that there were not many differences in the microbial types between the oilfield wastewater and the receiving pond and this could be due to the presence of these microorganisms in the oilfield wastewater which was discharged into the pond. It has been reported that oilfield wastewater is treated before it is discharged into an

environment and that these treatments are only effective in the reduction of aerobic bacteria which could cause corrosion of pipes [14]. Thus, this could describe the high similarity of microbial types associated with both samples and the absence of a few microbial types could either be due to the effect of the treatment of the oilfield wastewater effluent before it was discharged or the seasonal fluctuation.

The percentage occurrence of bacterial isolates in the oilfield wastewater showed that *Pseudomonas* sp (17.9%) was the most frequent bacterial isolates followed by *Micrococcus* sp (12.5%) while *Staphylococcus* sp (10.9%), *Proteus* sp (10.9%), and *Pseudomonas* sp (10.9%) were the most occurring bacterial isolates in the receiving pond. Results also showed that the frequency of *Bacillus* sp, *Micrococcus* sp, *Pseudomonas* sp, and *Rhodococcus* sp was higher in the oilfield wastewater than in the pond effluent. While the frequency of *Aeromonas* sp, *Chryseomonas* sp, *Proteus* sp, *Klebsiella* sp, and *Actinomyces* sp in the pond effluent was higher than the oilfield wastewater. The percentage occurrence of fungal isolates showed that *Aspergillus niger* (20.7%) was the most frequently isolated fungal isolates in the oilfield wastewater while *Penicillium* sp (16.7%) was the most frequently isolated fungal isolates in the receiving pond. The bacterial and fungal isolates in the present study have been reported not only to be associated with hydrocarbon but to biodegrade hydrocarbon products thereby eliminating pollutions caused by hydrocarbons in the environment [16-19].

Conclusion

The microbial population in oilfield wastewater, as well as the receiving pond, is to a large extent influenced by the seasons, and drier months are usually characterized by higher microbial populations as revealed in the present study. Despite the influence in microbial populations observed by the seasonal variation, the study has revealed that seasons do not have much effect on the microbial diversity but rather the availability of nutrient which supports the microbial types. Also, the presence of varying microbial types in the oilfield wastewater and the receiving pond suggest that these microorganisms possess the capability to utilize or degrade hydrocarbon and its product in the environment. Thus, the microorganisms in the present study are recommended for further biotechnological research in their use as treatment or removal of hydrocarbon products in oilfield waste or water bodies that are receiving terminals of effluents from oil companies.

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