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Bacterial Population of an Oilfield Wastewater in Nigeria

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ABSTRACT

An investigation of bacterial population of an oilfield wastewater was done for 5 months and 2 seasons (dry and wet) in 2009. Freshly-produced oilfield wastewater samples were analyzed for Aerobic Heterotrophic Bacteria (AHB) and Hydrocarbon-utilizing Bacteria (HUB) using standard procedures. Mean monthly counts of aerobic heterotrophic bacteria (x10^4 cfu mL^{-1}) and hydrocarbon-utilizing bacteria (x10^3 cfu mL^{-1}) were: Dec. 13.2±9 and 0.66±0; Jan. 15.2±13 and 13.4±12; Mar. 8.4±6 and 0.18±0; Jul. 14.5±9 and 0.11±0; Aug. 9.9±4 and 0.17±0, respectively. Dry season recorded mean counts (x10^4 cfu mL^{-1}) of 14.3±8 (AHB) and 7.0±9 (HUB) while wet season had mean counts (x10^3 cfu mL^{-1}) of 12.3±5 and 0.14±0 aerobic heterotrophic bacteria and hydrocarbon-utilizing bacteria, respectively. Fourteen species of heterotrophic bacteria isolated included *Pseudomonas* (16%), *Klebsiella* and *Staphylococcus* (13%), *Corynebacterium*, *Enterobacter* and *Proteus* (9%), *Acinetobacter* and *Micrococcus* (6%); *Alcaligenes, Bacillus, Chromobacterium, Citrobacter*, *Clostridium* and *Flavobacterium* (3%). Eleven species of hydrocarbon-utilizing bacteria isolated included *Acinetobacter* and *Bacillus* (15%), *Klebsiella, Micrococcus, Proteus, Pseudomonas* and *Staphylococcus* (10%), *Alcaligenes*, *Corynebacterium*, *Flavobacterium* and *Serratia* (5%). Monthly fluctuations of bacterial population occurred in the wastwater and number of bacteria were higher in dry season than wet season. Bacteria survive in wastewater and hydrocarbon-utilizing bacteria can be isolated from the wastewater and used for clean up of petroleum contaminated soil.

Key words: Investigation, samples analyzed, aerobic heterotrophic, hydrocarbon-utilizing

INTRODUCTION

Oilfield wastewater is effluent co-produced with oil and gas during drilling. At the surface, oilfield wastewater is separated from the hydrocarbons, treated to remove as much oil as possible and then either discharged into the environment or injected back into the wells (Odeigah *et al.*, 1997; Wills, 2000). Oilfield wastewater is composed of formation water and injected water. Formation water is the natural water that occurs in association with oil and gas deposits in reservoirs and being denser, lies under the hydrocarbons (Wills, 2000). Injected water is additional water usually pumped into the reservoirs, during the secondary stage of oil recovery, to maintain reservoir pressure and to help force the oil to the surface, in order to achieve maximum oil recovery (Somerville *et al.*, 1987; Wills, 2000). Offshore oil production platforms as well as onshore oil fields discharge the oily water or oilfield wastewater into the environments as part of their normal operations (Somerville *et al.*, 1987; Wills, 2000).

Offshore drilling for oil and gas produces large amounts of the wastewater which is usually discharged into the aquatic environment. Oilfield wastewater contains inorganic and organic constituents (Wardley-Smith, 1979) as well as hydrocarbon components (Koons *et al.*, 1977). Components of the wastewater are broadly classified into three categories: dissolved mineral salts, dissolved gas and microorganisms (Mony, 1975). The most important microorganisms to biological waste management are bacteria, fungi, algae, protozoa, rotifers and crustaceans (Raras, 1995). Microbes seem to accelerate the corrosion process by locating susceptible areas and producing or accumulating chemicals that promote corrosion (Walsh *et al.*, 1993). This is because of the microscopic heterogeneity of many materials. The rock, a porous medium, is a natural habitat for microbes to cling and to colonise (Microbes, 1977). Even during treatment, chemicals such as water clarifier and biocides are added to reduce microbial populations (Obire and Wemedo, 2002).

In Nigeria, information on the bacterial populations of the oilfield wastewater has been scare. However, Wemedo *et al.* (2009) reported the presence of viable fungi in oilfield wastewater although in low numbers. The objective of this study therefore, was to enumerate and isolate total heterotrophic bacteria associated with oilfield wastewater, determine the level of occurrence of bacteria in the wastewater and highlight the environmental significance of bacteria in the wastewater.

MATERIALS AND METHODS

Sampling: Sampling was done at monthly intervals for a period of five months (December, January, March, July and August, 2009). Samples were collected from Total Fina-Elf Nig. Plc. oilfield flow station located at Obagi in Ogba/Egbema/Ndoni Local Government Area of Rivers State, Southern Nigeria. The oilfield situates in a freshwater swamp forest vegetation of the Niger Delta and the choice of the field was because oil operations have lasted for over 60 years in the area.

Freshly-produced oilfield wastewater samples were collected at the oilfield flow station effluent discharge point before it made contact with the environment. Samples were collected in 250 mL sterilized sample bottles according to the method described in standard methods for water and wastewater analysis (APHA, 1995). Prior to collection of the wastewater, the interior of the nozzle of the outlet valve was flushed by allowing the water to flow to waste for 2 to 3 min, in order to avoid contamination from external sources. After which the sample bottles were filled from a gentle stream of the wastewater. Five replicate samples were collected at each sampling period. During the sampling period, seasonal variation, based on wet and dry months, was considered which also influenced the choice of the sampling months.

Microbiology of oilfield wastewater samples: Microbiology of the wastewater samples involved enumeration and isolation of bacteria from oilfield wastewater samples. Enumeration of bacteria from the wastewater samples was performed using the ten-fold serial dilution method of Harrigan and McCance (1990) and Ofunne (1999). In this method, decimal dilutions of the samples were made by adding 1.0 mL wastewater samples to 9.0 mL of sterile normal saline (diluent) to give an initial dilution of 1:10 (10^{-1}). Subsequent serial ten-fold dilutions were made by transferring 1.0 mL of the last dilution to 9.0 mL of fresh diluent up to 1:1000 (10^{-3}). Finally, 0.1 mL (aliquots) of the appropriate dilutions were plated out in duplicate onto suitable fresh dry sterile agar medium in petri dishes nutrient agar for heterotrophic bacteria and oil agar for hydrocarbon utilizing bacteria. The inoculum was evenly spread out on the surface of the agar with a sterile bent glass rod. Inoculated plates were incubated for 24-48 h (heterotrophic bacteria) and 7-14 days (for hydrocarbon-utilizing bacteria) at temperature of 30±2°C; after which, plates that grew between 30 and 300 colonies (Anonymous, 1995) were counted and recorded. Discrete colonies that developed were randomly picked and purified by sub-culturing onto nutrient agar and incubated at 30±2°C for 24 h to obtain pure cultures.

Identification of bacterial isolates: Identification of bacterial isolates was based on methods of Buchanan and Gibbons (1994), Cowan and Steel (1974), Cruickshank *et al.* (1984) and Winn *et al.* (2006). Standard tests (Gram reaction, motility, catalase, oxidation/fermentation, hydrogen sulphide production, coagulase, oxidase, indole production, Methyl Red-Voges Proskauer (MR-VP) reactions, starch hydrolysis, nitrate reduction and fermentation of the following sugars: glucose, lactose, mannitol, arabinose) on the isolates to identify bacteria associated with the wastewater.

Data analysis: Statistical analysis was carried out on the data obtained during this study. Analysis of variance and Mann-Whitney U-test were used to compare some of the data for the different months and seasons. This was done using a Computer-based programme in Excel.

RESULTS

Monthly counts and means of total aerobic heterotrophic bacteria and hydrocarbon-utilizing bacteria of oilfield wastewater are presented in Table 1 and 2, respectively. Seasonal counts and means of total heterotrophic aerobic bacteria and hydrocarbon-utilizing bacteria of oilfield wastewater are presented in Table 3. The monthly counts of aerobic heterotrophic bacteria ($x10^4$ cfu mL^{-1}) in all the samples analyzed ranged from 0.80-38; mean monthly counts ranged between 8.4 and 15.2, being the lowest counts in March and the highest counts recorded in January, respectively. Dry season recorded mean counts of 14.3 higher than the counts of 12.3 recorded in wet season. The monthly densities of hydrocarbon-utilizing bacteria ($x10^3$ cfu mL^{-1}) in all the samples analyzed ranged from 0-43; mean monthly counts ranged

Table 1: Monthly counts and Mean±SD of total aerobic heterotrophic bacteria of oilfield wastewater (5 replicates)

No. of samples	Dec.	Jan.	Mar.	Jul.	Aug.
1	19.1	10.9	1.00	19.6	5.3
2	23.3	9.0	10.2	23.8	14.6
3	8.4	5.0	3.1	13.1	10.4
4	14.6	38	11.5	15.2	7.5
5	0.80	13.3	16.0	0.85	11.9
Mean	13.2±9	15.2±13	8.4±6	14.5±9	9.9±4

Table 2: Monthly counts and Mean±SD of hydrocarbon-utilizing bacteria of oilfield wastewater (5 replicates)

No. of samples	Dec.	Jan.	Mar.	Jul.	Aug.
1	0.4	0.0	0.2	0.06	0.01
2	0.5	12	0.1	0.11	0.77
3	1.2	3	0.4	0.13	0.02
4	0.2	43	0.1	0.07	0.02
5	1.0	9	0.1	0.17	0.02
Mean	0.66±0	13.4±12	0.18±0	0.11±0	0.17±0

Table 3: Seasonal counts and Mean±SD of total aerobic heterotrophic bacteria and hydrocarbon-utilizing bacteria of oilfield wastewater (5 replicates)

	Dry season		Wet season	
Replicate samples	Aerobic heterotrophic bacteria	Hydrocarbon utilizing bacteria	Aerobic heterotrophic bacteria	Hydrocarbon utilizing bacteria
1	15.0	0.2	12.5	0.04
2	16.2	6.3	19.2	0.44
3	6.7	2.1	11.8	0.07
4	26.3	21.6	11.4	0.04
5	7.1	5.0	6.4	0.09
Mean	14.3±8	7.0±9	12.3±5	0.14±0

between the lowest of 0.11 and the highest of 13.4 in March and January, respectively. The mean seasonal counts showed that dry season recorded counts of 7.0 higher than the counts of 0.14 recorded in wet season.

DISCUSSION

Densities of aerobic heterotrophic bacteria obtained in this study were generally high. Mean counts of aerobic heterotrophic bacteria were lowest in the month of March and highest in January. Activities of aerobic heterotrophic bacteria contributes to corrosion of pipes (Wemedo, 1995). Seasonal counts of aerobic heterotrophic bacteria showed that dry season recorded higher counts than the wet season. This agreed with the findings of Marshall and Devinny (1988) and Obire and Wemedo (2002), who reported that bacterial populations increased during drier months than during the cooler, wetter months. However, statistical analysis showed that there is no significant difference ($p<0.05$) between the monthly counts of aerobic heterotrophic bacteria and between dry season and wet season counts. Counts of hydrocarbon-utilizing bacteria were high in some samples and low or even zero in other samples, showing a wide range between the lowest value and the highest value. Month of January recorded both the lowest and highest counts observed among the samples and also recorded the highest mean monthly counts whereas, the lowest mean monthly counts were recorded in July. Statistical analysis showed that there is no significant difference between the monthly counts of hydrocarbon-utilizing bacteria. This suggested that the variations observed between the monthly counts were insignificant. Counts of hydrocarbon-utilizing bacteria varied between the two seasons with the dry season having higher counts than the wet season. This showed that the drier months supported the growth of hydrocarbon-utilizing bacteria than the cooler, wetter months (Marshall and Devinny, 1988; Obire and Wemedo, 2002). Statistical analysis revealed that there is significant difference ($p<0.05$) between the dry season and wet season counts for hydrocarbon-utilizing bacteria. A strong seasonal influence was observed in this study on the number of hydrocarbon-utilizing bacteria. Oilfield wastewater is composed of hydrocarbon and non-hydrocarbon residues (Somerville *et al*., 1987). It may be explained that the hydrocarbon components could have supported the proliferation of hydrocarbon-utilizing bacteria in the wastewater. The presence of bacterial hydrocarbon-utilizers may be a useful tool for the removal of oil in the wastewater.

Densities of bacteria observed in this study revealed that bacteria can survive in the oilfield wastewater; their survival may be related to the concentrations of the inorganic constituents which may have served as nutrients for the growth of the bacteria. Also, the various levels of concentrations of the inorganic constituents and other parameters may not have been high enough as to inhibit the growth and survival of the microorganisms. Furthermore, the biocide batch treatment (Wemedo, 1995; Wills, 2000), applied to the oilfield wastewater before discharge is aimed at only reducing to safe numbers the populations of the bacteria and not for total elimination. The results of bacterial densities observed during this study showed fluctuations on monthly and hourly basis. Generally speaking, the densities of bacteria in the wastewater were highly unstable. Seasonal variations were also observed for the bacterial population of the wastewater.

The occurrence of bacterial species in the oilfield wastewater was studied by characterization and identification of the isolates obtained during the study period. The results showed that various bacterial species occurred in the wastewater. A total of fourteen (14) heterotrophic bacteria and eleven (11) hydrocarbon-utilizing bacteria were isolated and with varying frequencies. Of the 14 heterotrophic bacterial species observed, *Pseudomonas* species had the highest frequency of 15.6%, followed by *Klebsiella* and *Staphylococcus* species with 12.5% occurrence. *Corynebacterium*, *Enterobacter* and *Proteus species* had 9.4% occurrence each; *Acinetobacter* and *Micrococcus* species recorded 6.3% frequency each. While *Alcaligenes*, *Bacillus*, *Chromobacterium*, *Citrobacter*, *Clostridium* and *Flavobacterium* species had the lowest occurrence of 3.1% each. Of the 11 hydrocarbon-utilizing bacterial species isolated, *Acinetobacter* and *Bacillus* species had the highest occurrence of 15%, followed by *Klebsiella*, *Micrococcus, Proteus, Pseudomonas* and *Staphylococcus* species with occurrence of 10% each while *Alcaligenes*, *Corynebacterium*, *Flavobacterium* and *Serratia* species had the least occurrence of 5% each. The water and sewage bacteria that occurred in the oilfield wastewater include, amongst others, *Chromobacterium, Citrobacter, Klebsiella, Proteus*, *Pseudomonas* and *Staphylococcus species* (Buchanan and Gibbons, 1994; Alexander, 1977). The occurrence of bacteria in the wastewater may be that the constant exposure of these bacteria to hydrocarbon (oily) component of the wastewater could have conferred on the organisms the ability to utilize and grow in the presence of hydrocarbon. In this case, the bacteria could be isolated and introduced into a petroleum-contaminated soil as bioremediation agents.

It has been noted that the oilfield wastewater is subjected to a form of treatment called Biocide Batch Treatment (BBT) during which bactericidal chemicals are injected into the wastewater flowline (Wemedo, 1995). This treatment is aimed at reducing, if not eliminating, certain bacteria such as aerobic heterotrophic bacteria present in the oilfield wastewater, that could cause corrosion, to a safe minimum. The occurrence of bacteria, both in numbers and types, highlighted the fact that bacteria can survive in the wastewater; their survival may be related to the concentrations of the organic and inorganic constituents which may have served as nutrients for bacterial growth. Also, the concentrations of the physicochemical parameters and the hydrocarbon components could not exert inhibitory effects on the bacterial growth. Furthermore, the biocide batch treatment applied to the wastewater only reduced bacterial populations but not complete elimination.

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