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Bacteriological and Physicochemical Evaluation of Petroleum Reservoirs (Flow Stations) in Delta State, Nigeria

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ABSTRACT

The chemical and physical characteristics of crude oil are greatly influenced by microbial activity in petroleum reservoirs, which also affects the oil's economic value and circumstances of exploitation. The physicochemical and bacteriological properties of crude oil samples taken from four flow stations in Delta State, Nigeria, were examined in this study. Total heterotrophic bacteria (THB), hydrocarbon-utilizing bacteria (HUB), and sulfate-reducing bacteria (SRB) were analysed using standard microbiological procedures. Also, using recognized analytical techniques, physicochemical parameters including pH, total nitrogen, organic carbon, dissolved oxygen, and sulfate concentration were ascertained. The findings showed that the crude oils had a pH ranged from 6.20 to 6.70, that of total nitrogen ranged from 0.05 to 0.08 percent, and that the organic carbon ranged from 9.42% to 11.23 percent. Potential hazards for microbial corrosion and biofouling were indicated by the considerable variation in total heterotrophic bacterial counts, which ranged from 1.10×107 to 3.1×107 cfu/ml. Hydrocarbon- utilizing bacterial counts ranged from 0.4×104 to 1.2×104 cfu/ml, indicating active microbial engagement in the biodegradation of crude oil. The samples were classified as sweet crude because of the notable absence of sulfate-reducing bacteria, which is consistent with the reported non-detection of sulfur compounds. The majority of the bacterial isolates found were Pseudomonas aeruginosa and Bacillus subtilis, which are known to contribute to biofilms formation, breakdown of hydrocarbons, and biocorrosion. In order to reduce adverse effects on oil production, environmental integrity, and public health, the study emphasizes the significance of continuous microbiological surveillance and reservoir management.

1. INTRODUCTION

Petroleum reservoirs, the important reservoir of the world energy, are the home to complex microbial communities which have important functions in changing the subsurface environment. Different metabolic activities of the dominating microorganisms (i.e. bacteria) in these reservoirs determine the biogeochemical processes related to hydrocarbon deposits (Sierra-Garcia and de Oliveira, 2013). To develop an understanding of how microbial communities relate to the petroleum reservoirs is essential for the purposes of streamlining extraction of oil, ensuring sustainability of the reservoirs, as well as minimizing negative environmental consequences of the oil recovery (Hu *et al*.,

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2016). Microorganisms existing in petroleum reservoirs are actively included in hydrocarbon interactions, demonstrating a plethora of mechanisms – anaerobic hydrocarbon degradation and biosurfactant production, etc. These microbial activities aid in the change in the physical and chemical properties of reservoir fluids and define the general reservoir dynamics. Such research of these microbial processes is critical enough to unravel the relationship between hydrocarbons and microorganisms in subsurface environments (Dong *et al*., 2020., Balwart *et al*., 2022). There are various challenges experienced in the process of oil recovery, including reduced production rates and increased viscosity of crude oil. Microbial activities in reservoir further aggravates the problems when these activities alter the properties of the reservoir fluids, promote corrosion and cause plugging due to the formation of biofilms which block movement of the fluid. Thus, it is essential to have a profound understanding of the microbial communities that live in petroleum reservoirs to design ways for mitigating these challenges and making the oil recovery process more optimized (Nikolova and Gutierrez, 2020; Saravanan *et al*., 2020; Zhang *et al*., 2020.

The biotechnological development has offered new opportunities for gaining the benefits of microbial communities in the petroleum reservoirs. Methods like bioremediation, bio stimulation, and microbial enhanced oil recovery (MEOR) are based on the biochemical abilities of bacteria to better the oil recovery processes (Hillman *et al*., 2022; Freitas-Silva *et al*., 2023). The study of microbial communities that inhabit the petroleum reservoirs is plagued with challenges that continue to baffle us as to our knowledge today. The unbelievable diversity of microorganisms inhabiting these subsurface settings is among the most pressing problems. The interplay among different bacterial species with distinct metabolic pathways as their tool of trade makes it virtually impossible to understand the collective effect of these microorganisms on the hydrocarbon degradation and the Reservoir dynamics. Also, the limited broad understanding of the exact functions of individual microbial species in such ecosystems prevent us from designing focused strategies to increase oil recovery without adverse effect on environment. The study of the dynamics of the microbial community is essential in solving the issues associated with the change in properties of fluids, corrosion and formation of biofilm, which affects the efficiency of recovery processes of oil (Salgar-Chaparro *et al*., 2020; Rellegadla *et al*., 2020).

The second important challenge in the petroleum reservoir microbiology is that there is little knowledge of how microbial activities directly bear upon oil recovery processes. The challenges that are experienced in the extraction of oil, the declining production rates and the increase in the viscosity of crude oil, are compounded when microbial interactions occur in reservoirs. However, the actual mechanism through which the microorganisms contribute to these challenges is not well known. The lack of a good background understanding to microbial influence hinders development of practical biotechnological products, such as bioremediation and microbial enhanced oil recovery (MEOR). The fact that there is a knowledge gap here serves as a significant barrier to our efforts to develop sustainable and effective methods of oil recovery, and there needs to be concerted research to untangle the mysteries of the microbial contribution to the petroleum reservoirs ecology (Alkan *et al*., 2020).In spite of the growing awareness about the value of microbial communities in petroleum reservoirs, there is a major research gap related to specific roles, interactions and microbial adaptation in the community. The need to bridge this gap is crucial if only to create specific strategies for best optimizing oil recovery in petroleum reservoirs and then maximizing their sustainability (Dopffel *et al*., 2021; Singh *et al*., 2023). It is critical to comprehend the complex interactions of the microorganisms with the reservoir environment to predict their influence on the quality of oil and the souring of the reservoir and the functioning of the ecosystem on the whole. This is the knowledge which is the basis for the development of strategies for controlling the microbial activities and improving the rate of oil recovery in a sustainable way.

The studied microbiology of oil reservoirs can also be used for productivity and enhancement of recovery efficiency. This research work was aimed at filling this knowledge gap, making a contribution to a greater body of the field of petroleum microbiology and beneficial insights for the petroleum industry and environment management. The exploration of the microbial ecology of reservoirs is a vital step, since the dynamics of microbial activity may greatly differ at various geographical locations or under the different natural conditions. This study was to evaluate the bacteriological and physicochemical features of oil reservoirs in selected oil flow stations in Delta State of Nigeria.

2. MATERIALS AND METHODS

2.1. Sample Collection

A sample of crude oil was taken from four chosen Flow Stations in Delta State of Nigeria. A systematic and strict sampling collection protocol was applied. 1 liter of crude oil was carefully sampled from ligament valve located in the Flow Stations using sterile glass bottles. In order to obtain pure sample, an initial 500ml was rejected from the collection point before filling a 1liter sterile glass amber bottle with sample. These bottles were particularly selected because they could block light exposure hence limiting any possible degradation of the crude oil. The samples were well sealed for excluding any external influence that had been stored upright ready for transfer to the laboratory for analyses in chilled cooler.

2.1.1 Microbiological Analyses

Samples of crude oil collected from different Flow Stations were quantified according to the standardized microbial methodology while paying attention to three broad areas: total heterotrophic bacteria (THB), hydrocarbon-utilizing bacteria and the corrosive bacteria, especially sulfate-reducing bacteria. The total heterotrophic bacterial (THB) count of the reservoir oil was done by using ten-fold serial dilution with normal saline. 1ml of the oil was measured into test tubes containing 9 ml normal saline. Then 1ml was transferred from the stock to another test tube which contained 9 ml normal saline and serially diluted. Aliquots of dilution (1ml) were taken and spread on nutrient agar plates in triplicates. (APHA 2018). The plates were left at 37 ^{o}C under incubation for 24 hours.

2.1.2. Hydrocarbon-Utilizing Bacterial (HUB) Enumeration

The enumeration was carried out of the Hydrocarbon-utilzing bacteria (HUB) on the standard procedure. A medium that was selective and which only contained hydrocarbon as the only source of carbon was used (Atlas, 2010). The isolation process can be sued using this approach to isolate bacteria with a particular activity towards the utilization of hydrocarbons. HUB count was carried out in three (3) repetitions on mineral salt agar (bushnell – haas agar). One millilitre of one of the serially diluted aliquots was inoculated in triplicates on the already-made mineral salt agar plates by spread plate method with antifungi added to suppress fungi growth (AP The filter paper soaked with crude oil was the only carbon source and source of energy. Plates were counted after incubation period. Colonies of plates were noted down and colony forming unit was determined. The single colonies were streaked on a new medium for further purification (Maier *et al*., 2014).

2.1.2. *Isolation and Enumeration of Corrosive Bacteria*

As derived from the procedures of microbial methodology, the inventory of corrosive bacteria, with particular emphasis on sulfate-reducing bacteria, was followed. Selective medium such as the sulfate reduction agar or Post gate medium was used. Postgate's B medium was used to explore the number of sulphate reducing bacteria (Videla, 1996; Postgate, 1984; Hamiilton, 1985). The pH of medium was adjusted to 7.6 using 1 M NaOH solution and antifungal (Artcin) was added in order to prevent fungal growth, the medium was sterilized in autoclaving at 121°C for 15 minutes before using it. The medium was then poured in the nitrogen gas for almost an hour to remove the oxygen from it before autoclave.1 ml aliquot of serial diluted sample was poured on the agar prepared already by spread plate method. The incubation was anaerobically done in the anaerobic jars for 7 days at 30 ^{o}C. Colonies formed on plates were counted thereby, determining the colony forming unit. Discrete colonies were streaked onto new medium for more purification.

2.1.3. Identification and Characterization of Isolates

Standard characterization tests were employed. Pure isolates from the corresponding agar slants were characterized and identified using cultural, morphological and biochemical characteristics. (Cheesbrough, 2006).

2.2. Physico-Chemical Analyses

Samples of crude oil were taken from the various places and directly sent to the laboratory for physico-chemical analysis. Parameters analyzed were: pH, total nitrogen, sulphate, dissolved oxygen and total organic carbon. The crude oil pH was ascertained from electrometric method. For the reading Hanna Temperature Meter was used. The temperature and redox potential were obtained by shifting the meter's mode to that of the temperature and redox potential. The method of Walkley and Black (1934) for the chronic acid wet digestion was used to determine the organic carbon. KH2PO4 extraction method as suggested by Gautam (2012) was adopted to determine the Sulphate. Total nitrogen was estimated using the Kjeldahl's method, Kjeldahl (1883) described by Borah *et al*., (2009) and also adopted by Ryan *et al*., (2001).

3. RESULTS AND DISCUSSIONS

3.1. *Physico-Chemical Analyses*

Table 1 gives physical and chemical properties of crude oil samples collected in four locations. The highest pH value observed was at this location B and the lowest pH at this location D with the values 6.70 and 6.20 respectively. The highest total Nitrogen value (0.08%) was recorded at C location while the lowest one (0.05%) was recorded at location D. The highest and the lowest values of total organic carbon were recorded both at location D with the value 11.23% and A with the value 9.42%. Dissolved Oxygen and Sulphate were not observed in all the places sampled.

Table 1.: The Physico-Chemical Characteristics of crude oil Samples in the Oil Exploration and Exploitation areas of Delta State

Parameter	**Source of Oil Sample**			
	Location A	**Location B**	**Location C**	**Location D**
pH	6.30	6.70	6.40	6.20
Total Nitrogen %	0.06	0.07	0.08	0.05
Total Organic Carbon %	9.42	9.81	9.56	11.23
Dissolved Oxygen mg/l	ND	ND	ND	ND
Sulphate content mg/l	ND	ND	ND	ND

ND= Not Detected

3.2. Bacteriological Analysis

Bacteriological properties of crude oil samples collected from various locations have been given as Table 2 below. Highest total heterotrophic count was made in location C of 3.1×10^7, the lowest total heterotrophic count was recorded in location A of 1.10×10^7 Hydrocarbon utilizing bacterial was found to be highest in location D of 1.2×10^4 the lowest hydrocarbon utilizing bacteria. Sulphate Reducing Bacteria were not found in all locations.

Table 2: Bacterial Counts of various Oil Samples collected from different Locations on various Media

Bacterial counts (cfu/ml)	Location A	Location B	Location C	Location D
Total Heterotrophic Bacterial Count (THB) onNutrient agar cfu/ml	1.10×10^7	2.6×10^7	3.1×10^7	1.7×10^7
Hydrocarbon Utilizing Bacterial count on (HUB)Mineral Salt Agar cfu/ml	0.4×10^4	0.8×10^4	0.6×10^4	1.2×10^4
Sulphate Reducing Bacteria (SRB) on Postgate medium cfu/ml	ND	ND	ND	ND

ND= Not Detected

3.3. Identification of Isolates

The identity of the chosen isolates on the basis of their morphological, microscopic (Gram- reaction) and biochemical tests are given in Table 3. Separated and distinguished isolates were Pseudomonas aeruginosa and Bacillus subtilis. Pseudomonas aeruginosa was the leading isolates.

Table 3: Chart for the Identification of Isolates by Microscopic and Gram Test and Biochemical tests.

Bacterial Isolates	Gram Stain	Shape	Motility	Oxidase	Catalase	Coagulase	Citrate	Urease	Indole	Glucose	Lactose	Sucrose	Maltose	Mannitol	VP	MR	Nitrate Reduction	Probable isolates
1	-	**Rod**	+	+	+	-	+	+	-	+	+	+	+	-	-	-	**+**	*Pseudomonas aureginosa*
2	+	**Rod**	+	-	+	-	+	-	-	+	-	+	+	+	+	-	**+**	*Bacillus sp .*
3	-	**Rod**	+	+	+	-	+	+	-	+	+	+	+	-	-	-	**+**	*Pseudomonas aureginosa*

KEYS: Sp. Specie, + Positive – Negative, MR Methyl red, VP Voges proskauer

3.4 Discussion

Physicochemical properties of the crude oil in the different locations or oil reservoirs showed that the oil was slightly acidic. This has been brought about by acidic pollution of contaminants from formation waters, drilling fluids and metabolites from microbial activities. Acidic crude oil can corrode pipelines, storage tanks and equipment that could result into leaks and environmental hazards. The magnitude of biodegradation may affect the pH of the crude oil. API Technical Report 2574, ASTM Special Technical Publication1589 indicated that the normal pH range of crude oil is neutral to slightly alkaline from 7.0 to 8.5. This range is typical for the vast majority of crude oils which shows balanced chemical composition. Although the acceptable range is 6.5 to 9.0, crude oils with the pH value within this range are usually taken to be stable and ready for refining. Biodegraded crude oils normally show pH from 5.5 to 6.5 and are affected by the microbial activity. Some of the factors affecting the pH level of crude oil include biodegradation, contamination, water content, organic matter composition among others. The effect of biodegradation is pronounced on oil composition especially for shallow reservoir. Knowledge of oil composition and geochemistry is important for Petroleum exploration and production, assessments concerning Oil spills and contaminations. (Moldowan *et al.,* 2015). A pH range of 5.5- 8.5 was reported in crude oil by Fingas" (2013), EI-Gendy *et al*., (2017) reported a pH range of 5.8- 7.9 and; Al- Sabagh *et al*., (2020) reported a pH range of 5.9-8.2.

From the research, the Nitrogen content was low at 0.05-0.08% in weight. The normal content range for nitrogen in the crude reeservior is usually rather small i.e. < 0.1-2.0 % weight, API Technical Report 2574, ASTM Special Technical Publication1589. This is so because nitrogen is found almost in all crude oils in very minute quantities. In fact, non-hydrocarbon content of the crude oil, which comprises of nitrogen, sulphur and oxygen, can be very different depending on the

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source of the oil. Some of the oils have been found containing less than 0.05 % nitrogen whereas some crudes were found to have 2 % of nitrogen. The range that is accepted is less than 0.1% – 2.0% hence in all the locations the nitrogen contents were within the acceptable range. The result also indicated carbon content of 9.42-11.23 % and the normal range of 83-87% as per the ISO Technical Report 20962. The range of the organic carbon was below acceptable limit. The contents of sulfur and oxygen were not found. The normal level for sulphur is 0.1-5.0 %, oxygen 0.1-2.0 %. The oils in the reserviors were sweet oils because sulphur was not identified. Sulfur content influences refining complexity. Sulfur content may as well encourage the proliferation of sulphate reducing bacteria and thus microbial corrosion. The lack of sulphur can therefore be related to absence of sulphate reducing bacteria in the oils.

The study showed total heterotrophic bacteria count of 10^7 in the various places. The normal total bacterial count in the range of 10^2 to 10^5 is found in crude oil. 10^5-10^6 may have to be monitored but above 10^6 is corrosion, souring and other related issues according to API RP38, API RP45 (American Petroleum Institute) Recommended Practice for Analysis of Subsurface Microbial Communities and ASTM D6021 'Standard Test Method for Measurement of Total Microbial Growth in Fuels and Fuels Systems'. The bacteria load in crude oils from reservoirs is also dependent on the type of reservoir and geology, water content, temperature and salinity among others. Thus, these factors may affect the bacteria numbers in the various places.

In this study the bacteria that degrade hydrocarbons were isolated and reported. The presence and number of these bacteria calls for constant monitoring of the crude oil. The existence of these bacteria could cause crude oil degradation which thus impairs the quality and value. It might also cause biofouling that contaminates the reservoirs and influences oil production. The presence of bacteria degrading hydrocarbons may also lead to biofilm formation thereby reducing flow of fluid and increasing pressure drop (Dinh *et al*., 2023). The Sulfate Reducing Bacteria were not present in the crude oil and this is within the acceptable range noted to be less than 10^3 cells per millimetre (ml). Above 10^3 to 10^4 needs monitoring and above 10^4 cells /ml is an indication of corrosion and souring. A bacterial load that is less than 10^2 cells/ml is classified as non-problematic. The reported bacteria were *Pseudomonas aeruginosa.* and *Bacillus subtilis.* The presence of these bacteria in reservoir may be attributed to contamination from activities such as drilling, production, injection of fluids and injection of water for secondary oil recovery as earlier reported by Bergstrand et al. (2016). The environment, human health, and the oil and gas sector are all significantly impacted by the presence of these bacteria in crude oil from reservoirs. This could encourage biocorrosion of equipment, pipelines, and storage tanks, requiring expensive repairs and downtime. Additionally, it might encourage the development of biofilms and biofouling. These bacteria have the potential to degrade the crude oil quality leading to deterioration. They can increase the risk of pipeline blockages. These bacteria can contaminate produce water, affecting aquatic ecosystems. Through leaks or spills, these bacteria can potentially pollute the soil. Maintaining clean and dry storage facilities, routinely inspecting and maintaining pipelines and equipment, putting effective water management strategies into practice, using biocides and bacteria-resistant coatings, and educating staff on correct handling and maintenance techniques are all necessary to detect bacteria in crude oil from reservoirs.

4. CONCLUSIONS

Bacteria associated with biocorrosion, biofouling, degradation of oil quality and biofilm formation were found in this study. The chemical composition showed that the oils were sweet oils and within acceptable range of values. The presence of these bacteria may also have significant implications on the environment by causing water pollution and soil pollution. It may also have serious public health implications. Understanding bacterial communities can reduce maintenance and repair costs. Addressing bacterial contamination can minimize costly environmental liabilities.

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