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Treatment of Warri Refinery and Petrochemical Company Pollution Effluent Using Biostimulant

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Abstract

The use of microorganisms to convert harmful organic compounds in wastes has been recognized to be effective in treating industrial wastes. This study investigated the use of inorganic liquefied fertilizer NPK (Nitrogen, potassium, and phosphorus) to biostimulate the native microorganisms in the hazardous Warri refinery and petrochemical company effluent. The refinery sample effluents *A, B,* and C were subjected to treatment with different concentrations of biostimulants (*NPK) of* 10 mg/L, 20 mg/L, and 30 mg/L respectively. pH, temperature, turbidity, electrical conductivity, total dissolved solids, total hydrogen content, dissolved oxygen and Hydrocarbon utilizing, and biochemical and chemical oxygen demand of the effluent were determined in both untreated and treated samples. The treated samples were examined for one week. From the results, pollution indicator parameters chemical oxygen demand (COD) was observed to have dropped from 71.62 mg/L to 22.35 mg/L (samples B) and 15.81 mg/L (sample C), total hydrocarbon content (THC) 86.27 mg/L to 31.63 mg/L (samples B) and 17.4 mg/L (sample C), and biochemical oxygen demand (BOD) from 36.38 mg/L to 12.71 (samples B) and 8.96 mg/L (sample C). Similarly, the microbial count of Hydrocarbon Utilizing Bacteria (HUB) (0.89×10^4) increased as the treatment dosage increased from 4.01×10^4 to 6.27×10^4. The isolated hydrocarbon degraders were *Pseudomonas sp, staphylococcus sp, Micrococcus sp, Enterobacter, Proteus sp, and Bacillus sp.* It was observed that treated sample C (containing 30mg/L biostimulants) was most effective in the study. The result of treated sample C complied with the DPR standards limit for effluent discharge. Thus, the approach of bioremediation using NPK fertilizer to biostimulate the indigenous microbes in treating refinery effluent can be applied to a large-scale remedy, particularly using option C for optimal performance.

Keywords: Bioremediation; Total dissolved solids; Effluents; Bioaccumulation; Hydrocarbons

Introduction

The environment plays a very major role in the development of a nation. Besides the fact that it provides the physical environment for natural inhabitants, it is also the basis for industrial, commercial, agricultural, and tourism in a society [1]. Due to the increasing rate of urbanization and industrialization, Nigeria is expected to face a huge amount of massive industrial waste with little or no proper management. One such industrial waste is the refining of petroleum products as one of Nigeria's major sources of revenue.

The quests for oil exploration and exploitation have immensely added to the fiscal growth of Nigeria. It has however led to numerous incidents of environmental degradation and pollution [2]. In the production of petroleum products, large amounts of petroleum effluents are generated [3]. Researchers [4,5,3] have noted that these effluents contain very high amounts of crude oil products such as phenols, poly aromatic hydrocarbons (PAHs), naphthalene acids, sulfides, and heavy metals which are potentially harmful to the environment [6,4,5,3].

Globally, the effluents discharged into the environment represents a very large source of pollution. The compilation of unwanted or discharged industrial waste released into the environment without proper remedy is harmful [4,5,7]. These effluents when discharged in rivers and on land [8] leads to several toxic effects on living organisms in foods through biomagnification and bioaccumulation [9]. The World Health Organization (WHO) had noted that the rate of mortality of water-related diseases surpasses five million people annually with microbial intestinal infections which account for over 50% [10]. Other effects may comprise algal blooms, death to aquatic life, habitat destruction from sedimentation, debris, increased water flow, and other short-term and long-term toxicity effects from chemical contaminants; in combination with chemical accumulation and magnification at higher levels of the food chain [11].

Petroleum effluents have been previously treated with different techniques (chemical, physical, thermal, and electrochemical) that have been considered environmentally unfriendly and very costly. However, another technique that is both economically and environmentally friendly is the bioremediation technique. [12] opined that the treatment method is efficient and preferable to other treatment methods. This technique offers the use of organic or inorganic (biostimulants) nutrients to facilitate remediation [13,14,15,16,17,18,19]. There have been many studies on the use of biostimulants in plant productivity [20,21,22]. However, [23] noted that there has not been much about biostimulants and their role in petroleum effluent treatment.

This study aims to assess the effectiveness of using biostimulants to treat petroleum effluents from the Warri refinery and petrochemical company (WRPC).

Material And Methods

Study Area

Figure 1 shows the study area of WRPC. The oily effluent sample was collected from the effluent discharge point of Warri Refining and Petrochemical Company (WRPC), at Latitude: 005.71449°; Longitude: 05.56565°, under Uvwie Local Government Area, Effurun - Warri, Delta State, Nigeria. The sample was collected in Ambah glass bottles to avoid light penetration and in Sterile containers for Microbiological analysis. The sample was preserved in an ice chest and transported to TUDAKA Laboratory, Warri, Delta State for analysis.

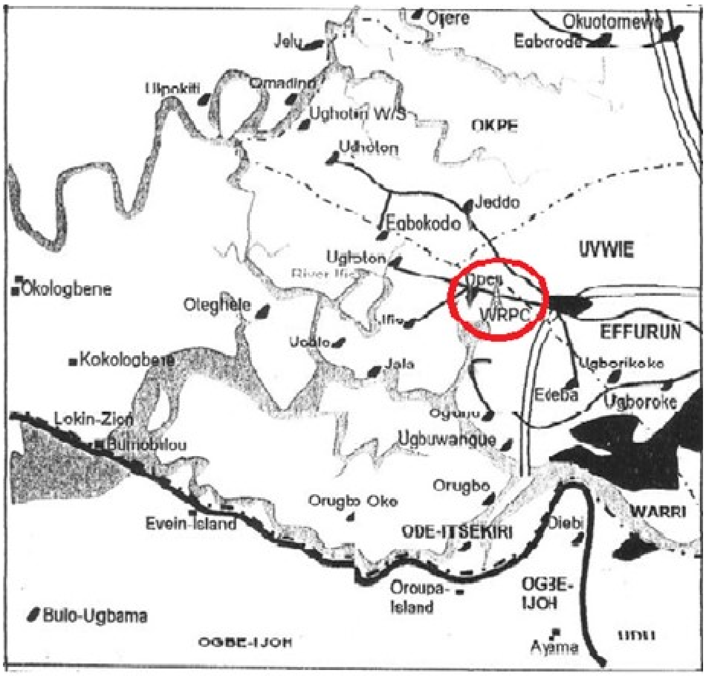

Figure 1: Administrative Map of Delta State showing the sampling location [24].

The biostimulant is an inorganic nutrient (NPK) that was purchased from a chemical company in Lagos (Metoxide Chemical Industries). The introduction of biostimulant was done at different concentrations (10mg/mL, 20 mg/L, and 30 mg/L) as described by [25].

Effluent Treatment using Biostimulant

The effluent was treated with different concentrations of biostimulants (NPK: 10 mg/L, 20 mg/L, and 30 mg/L) (table 1). A uniform sample volume of effluent (1000ml) was measured in three (3) different conical flasks, and different dosages of liquefied NPK (Nitrogen, potassium, and phosphorus), also known as biostimulant were added at different concentrations (10mg/L, 20 mg/L, and 30 mg/L). They were put in a mechanical shaker for thorough mixing at a speed of 200rpm for 30 minutes, after which they were left at room temperature for one (1) week for acclimatization. Thereafter, the samples were analyzed as treated samples. Physicochemical and microbial analyses were duly carried out on them, and the values were compared with the standard limits of the Department of Petroleum Resources (DPR), Nigeria's regulatory body for the petroleum industry.

Table 1: Treatment ratio of Effluent and Biostimulants

	Sample A	**Sample B**	**Sample C**
The volume of wastewater (ml)	1000	1000	1000
Biostimulants Concentration (mg/mL)	10	20	30

The Total Dissolved solids (TDS) and pH/Temperature determination was carried out with the

APHA standard 460 and 145 respectively. The Dissolved Oxygen (DO) was determined using Winkler's method. The Biochemical Oxygen Demand (BOD5) (BOD5 at 20°C; Part 5210 method B) was determined by the closed reflux method. Whereas the Chemical Oxygen Demand (COD) (COD; Part 5220 method C) was determined using the open reflux method. Both methods were determined with the APHA method. The Total Suspended Solids (TSS) and Total Hydrocarbon Content (THC) were determined using the filtration technique (APHA 208 method D) and solvent extraction method (ASTM D3921).

The biochemical test adopted for the identification and isolation of Hydrogen Utilizing Bacteria (HUB) was carried out using the Pour Plate method where the plates were incubated at 37^{o}C for 4 days following Bergey's Manual of Determinative Bacteriology.

Results And Discussion

Table 2 shows laboratory results for the untreated effluent. The untreated petroleum effluent was tested to be alkaline with a pH of 8.7 and a temperature of 30.4^{0}C. The electrical conductivity (EC) and total dissolved solids (TDS) were found to be 12570.00µS/cm and 9260.00mg/L respectively. Meanwhile, chemical oxygen demand (COD), biochemical oxygen demand (BOD), and total hydrogen content (THC) which serve as pollution indicators, with values of 36.38mg/L, 71.62mg/L, and 86.273mg/L respectively, were found to be far above the acceptable limit for effluent discharge, thus raising environmental concerns. The low Dissolved Oxygen (DO) further confirmed the high pollution level of the effluent. The turbidity value of 14.57NTU shows that the effluent was turbid. The presence of a low count of Hydrocarbon Utilizing Bacteria (HUB) 1.27×10^{4}cfu/ml showed that the petroleum effluent could not remediate itself unless fortified with biostimulants.

Table 2 shows the laboratory analysis result of untreated oil effluent indicating the presence of *Bacillus sp, Proteus sp, Pseudomonas sp, and micrococcus sp*

Table 2: Physicochemical and Microbial Characterization of Untreated Petroleum Effluent

Parameters	Units	Untreated Effluent (Raw sample)
pH		8.7
Temperature	°C	30.4
Total Dissolved Solid (TDS)	mg/L	9260.00
Electrical Conductivity (EC)	µS/cm	12570.00
Turbidity	N.T.U	14.57
Total Suspended Solid (TSS)	mg/L	40.00
Dissolved Oxygen (DO)	mg/L	0.80
Biochemical Oxygen demand (BOD)	mg/L	36.38
Chemical Oxygen Demand	mg/L	71.62
Total Hydrocarbon Content (THC)	mg/L	86.27
Hydrocarbon Utilizing Bacteria (HUB)	CFU/ml	0.89×10^{4}
Bacteria isolates		*Micrococus sp, Bacillus sp, Pseudomonas sp and Nocardia sp,*

Physicochemical and Microbiological Characterization of the Treated Effluent

Table 3 shows that the treated effluent with the maximum pollutant removal was sample C (sample treated with a concentration of 30mg/L).

Table3: Physicochemical and Microbial Results of Raw and Treated Effluents

Parameters	**Raw sample**	**Treated samples**					
	A	B	C	Mean	Std-Dev	DPR	Limits
pH	8.7	6.8	6.6	6.3	6.57	0.25	6.5-8.5
Temperature (°C)	30.4	32.7	32.7	32.5	32.63	0.12	25-35
Total Dissolved Solid (mg/L)	9260	5,780.00	6,630.00	7,410.00	6606.67	815.25	<2000
Electrical Conductivity (µS/cm)	12,570.00	8,530.00	9,760.00	10,940.00	9743.33	1205.09	N/A
Turbidity (NTU)	14.57	1.73	2.51	4.68	2.97	1.53	N/A
Total Suspended Solid (mg/L)	40	3.00	5.00	10.00	6	3.61	30
Dissolved Oxygen (mg/L)	0.8	4.10	4.80	5.60	4.83	0.75	>4.00
Dissolved Oxygen (mg/L)	0.8	4.10	4.80	5.60	4.83	0.75	>4.00
Biochemical Oxygen Demand (mg/L)	36.38	12.71	8.96	6.25	9.31	3.24	10
Chemical Oxygen Demand (mg/L)	71.62	22.35	15.81	10.06	16.07	6.15	40
Total Hydrocarbon Content (mg/l)	86.273	31.63	17.40	6.86	18.63	12.43	10
HUB (CFU/mL)	0.89×10^{4}	4.01×10^{4}	6.27×10^{4}	9.56×10^{4}	6.61	2.79	N/A
Bacteria Isolates	*Micrococcus sp.;*	*Bacillus sp; Staphylococcus; Pseudomonas sp.; Bacillus sp*					
Proteus sp.; Micrococcus sp.; Enterobacter Pseudomonas sp. sp.; Proteus sp.							

Figure 2 shows the graphical representation of the pH. The pH of the treated effluent was found to be slightly acidic, ranging from 6.3 to 6.8, while untreated effluent was 8.7. Meanwhile, the alkalinity in pH of the untreated effluent was a result of the organic composition of the effluent, being a refinery effluent. This is confirmed in a report by [26] whose study characterized refinery effluent to be alkaline pH. The gradual reduction of pH in the treated water was due to the biostimulants (NPK). However, the pH for treated effluents was below the allowable DPR range (6.0 – 8.5). The report by [26] further ascertained that microorganisms mostly bacteria grew optimally within a pH range of 6.0 - 7.5.

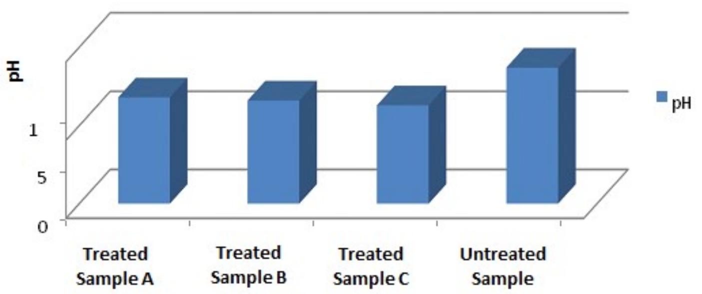

Figure 2: Graphical representation of pH of the samples

The temperature of the treated effluent was good for effluent discharge, ranging from 28.5^{O}C to 28.7 ^{O}C. The temperature for untreated effluent was found to be 30.4 ^{O}C. The recorded temperature was due to the climatic weather condition at the time of analysis, which complied with the regulatory limit of DPR (25 ^{O}C – 35 ^{O}C). It is noted that there is no single microorganism that can degrade oil or temperature that supports the metabolism of all microorganisms [27].

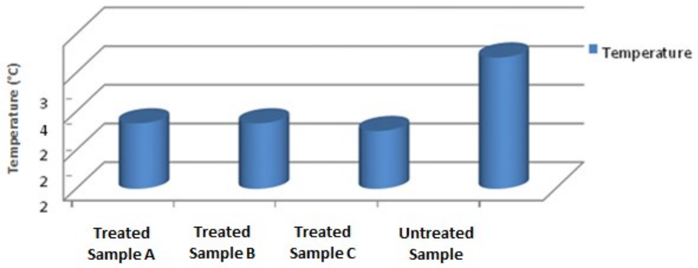

Figure 3: Graphical representation of the temperature of the samples

The TDS of the treated effluent was found to increase from 5780.00mg/L to 7410.00mg/L as the concentration increased from 20mg/L to 30mg/L for samples B and C respectively. This shows a corresponding increase of about 28.2% which may be a result of physicochemical reactions such as coagulation and sedimentation [28]. Also, the EC values followed the same trend as the TDS, which was found to be 12570.00µS/cm but increased from 8530.00 to 10940.00µS/cm for the treated samples. This trend could however be attributed to the high concentration of biostimulants (NPK) applied to the sample. This shows that the mineral salt dissolved in the untreated effluent was more than in the treated samples.

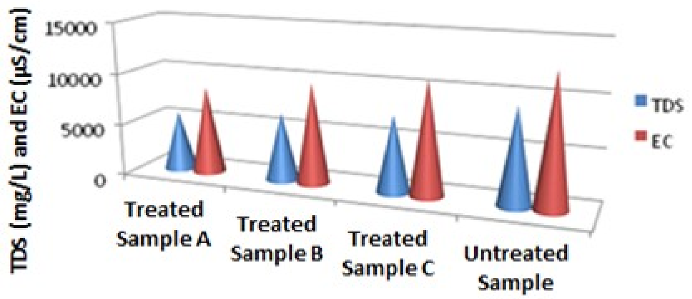

Figure 4: Graphical representation of TDS and EC of the samples

Turbidity is a significant factor in the mixing and transportation of nutrients and waste products in water. Numerous microorganisms depend entirely on this process for their survival. The values ranging from 1.73 to 4.68 were recorded for treated samples, while 14.57 for untreated effluent. The high value from the untreated sample was a pointer to the pollution level of the effluent [29]. Figure 5 shows the graphical representation of the turbidity and total suspended solid of the samples.

TSS is directly proportional to the turbidity levels of water samples. The TSS and TDS amount to Total Solid. The TSS for the treated samples ranged from 3.00 to 10.00mg/L, while a value of 40.00mg/L was recorded for untreated effluent, as shown in figure 6.

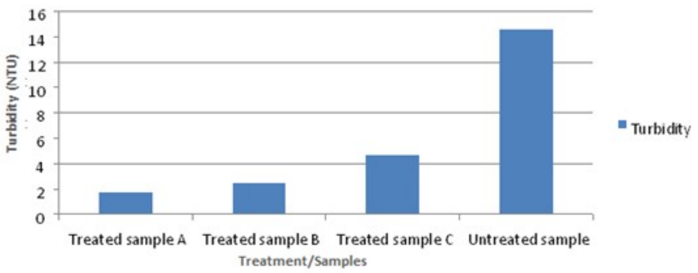

Figure 5: graphical representation of Turbidity of the samples

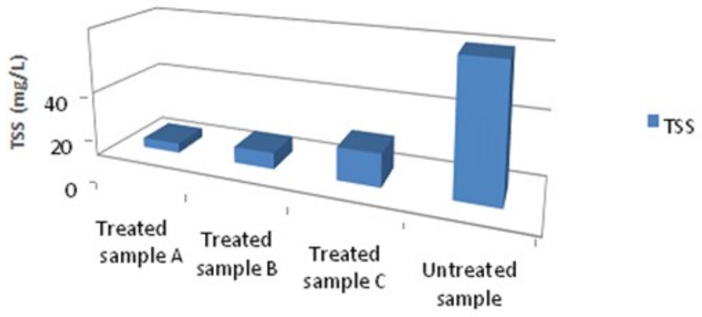

Figure 6: graphical representation of TSS of the samples

The Dissolved Oxygen (DO) content was found to be 0.8mg/L. This small amount of DO in the untreated effluent shows that the sample was polluted. An indication of the presence of biodegradable organic matter in the sample. After treatment, the DO was observed to have decreased from 4.1mg/L to 4.8mg/L showing a decrease of about 17.1%. this can be credited to the breaking down of aerobic microorganisms [28]. There was the presence of a reduced DO in the treated sample, the result nonetheless shows that the treatment was effective. The discharge of effluent with high organic content into an aquatic environment leads to a reduction of oxygen in water [30]. The treated effluent samples were moderately oxygenated as clearly shown in figure 4.6. However, the DO of the treated samples complied with the DPR standard limit of > 4.00 mg/l.

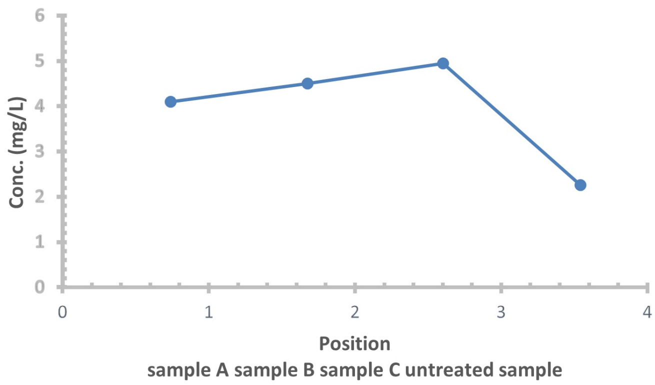

Figure 7: graphical presentation of DO of the samples

The BOD is a measure of the relative oxygen requirement of effluents in polluted water. This is a test for oxygen used in a specific incubation period, usually five (5) days for the biochemical degradation of organic materials and the amount of oxygen required to oxidize inorganic materials. However, the values ranging from 6.25 to 12.71 for treated samples indicate the effectiveness of the treatment, compared to the BOD value of 36.38 recorded for the untreated sample which was high. Figure 8 shows the graphical representation of the BOD of the samples. COD and BOD concentrations are markers of the level of organic compounds in the contaminated water [31]. The disproportionate level of COD and BOD in effluents released into water bodies will lower the level of dissolved oxygen which may induce fish kills and lower the rate of reproduction in aquatic life (Biswas, 2009).

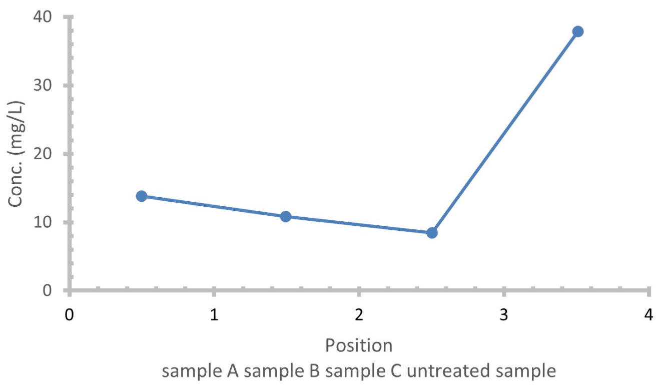

Figure 8: Graphical representation of BOD of the samples

COD is used to measure the oxygen corresponding to the organic matter content of water that is prone to oxidation by a strong chemical oxidant. This is useful for examining and controlling water effluents from industry or polluted sources. The COD values from this study range from 15.81 mg/L to 22.35 mg/L for treated samples, compared to the COD value of 71.62 mg/L for untreated samples, as shown in figure 9. This agrees with the report of [32] that organic pollutants generated through industrial activities like hydrocarbons can be gradually removed through bioremediation. The COD follows the same trend as the BOD with an increasing range of 10.06mg/L to 22.35mg/L.

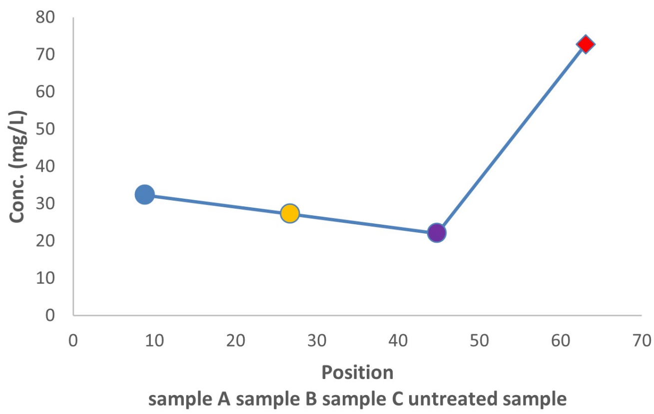

Figure 9: Graphical representation of COD of the samples

The THC which indicates petroleum-related pollution of the untreated sample as obtained was 86.273mg/L. However, the mean of the lowest and highest THC content of the treated samples ranged from 6.857 mg/L to 31.631mg/L. This implies that the treated samples still contain a certain amount of hydrocarbons even though there was a significant decrease in THC. The source of the hydrocarbon contamination as shown in table 2 is not unconnected to the activities of the petroleum refining companies. Figure 10 shows the graphical representation of THC. *[33]* noted that hydrocarbon in petroleum effluent can cause a reduction in dissolved oxygen and biodiversity in water bodies.

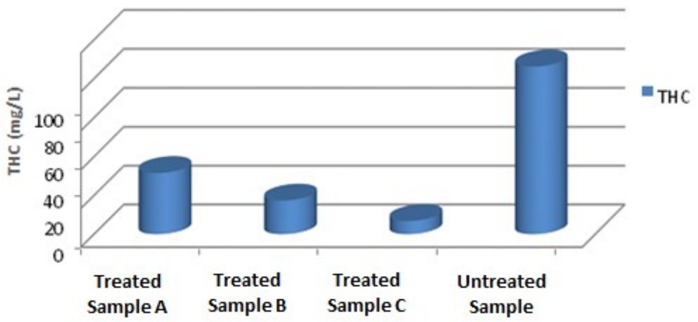

Figure 10: Graphical representation of THC of the samples

Biochemical Identification of Bacteria Species Using Pour Plate Method

Hydrocarbon-utilizing bacteria are a group of microorganisms that are known for feeding on hydrocarbons and using them as sources of energy for metabolic activities. As shown in table 2, the microbial load of HUB in this study for treated samples was systemically found to be increasing as treatment concentration increased, with the values ranging from 4.01 x 10^4 to 9.56 x 10^4, while the untreated sample was 0.89 x 10^4. Figure 11 shows the graphical representation of HUB in the samples. From the results of bacteria isolation, the microbial load of the originally isolated bacteria continued to increase with an indication of the presence of *Micrococcus sp Staphylococcus sp, Proteus sp Bacillus sp,* and *Pseudomonas sp*. Researchers [13,14,34,35,36] have reported on some of these bacterial isolates. [37] reported the ability of *Pseudomonas* species to decrease oil under laboratory conditions, while [38] proved *Pseudomonas* specie a very efficient hydrocarbon-reducing bacteria that are widespread in the Nigerian environment. Yet, the ability of these microorganisms to utilize oil has been credited to their enzymatic ability and their ability to survive tough environmental conditions [39]. The various changes in the parameters analyzed showed the effectiveness of the treatments. The continuous application and increase in the concentration of biostimulants (NPK) brought about the resultant performance observed. Thus, the effluent sample C with the highest dosage of biostimulants (30mg/L) was found to be the most effective treatment.

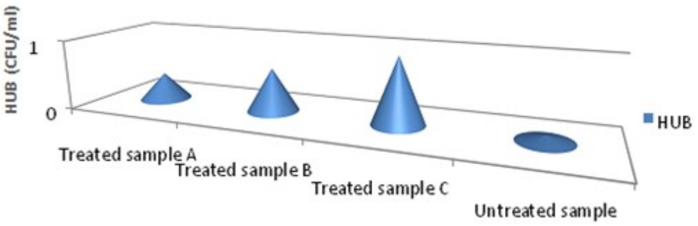

Figure 11: Graphical representation of Microbial Load (HUB) of the samples

Figure 12 presents the parameters analyzed showing the percentage effectiveness of the treatments. The continued increase in the concentration of biostimulants (NPK) brings about the resultant performance observed in the experiment. Virtually all the parameters analyzed complied with the compared DPR regulatory standards. However, the most effective treatment was observed in sample C, which had a biostimulant concentration of 30mg/L. At this concentration, the optimal performance was recorded, and therefore the effluent could be affirmed treated and can be discharged into the environment or used for agricultural purposes like irrigation. Table 4 shows the percentage efficiency of the treatment, particularly hydrocarbon reduction, with respect to different biostimulants concentration. From the results, it can be inferred that the removal of pollutants has been observed with the increase in concentration. In this study, the percentage treatment value of 87.94% for sample C shows the most effective treatment, where the sample was able to absorb the highest amount of pollutants from the effluent. Thus, parameters like BOD, COD, and THC, being the pollution indicators were used for the evaluation of the percentage treatment and remediation.

Parameters	Treated Sample A	Treated Sample B	Treated Sample C	Untreated Sample	% Treatment A	% Treatment B	% Treatment C
BOD	12.71	8.96	6.25	36.38	65.06	75.37	82.82
COD	22.35	15.81	10.06	71.62	68.79	77.93	85.95
THC	31.631	17.402	6.857	86.273	63.34	79.83	92.05
% Remediation					**65.73**	**77.71**	**87.94**

Table 4: Percentage treatment of samples

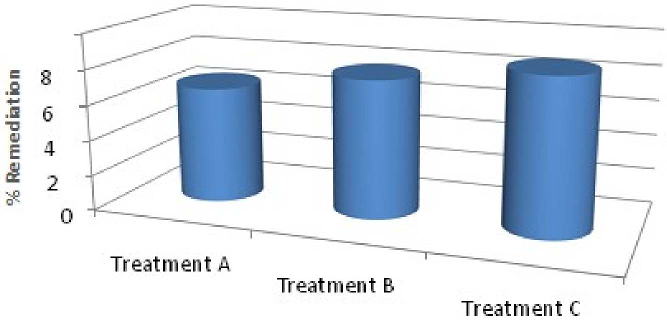

Figure 12: Graphical representation of the treatment/remediation percentage

Conclusion

Hydrocarbon-contaminated effluent from petroleum refineries, as well as other related pollution caused by the oil industry, have been determined to be hostile to health and ecosystem balance of various environments. Hence, the necessity to implement an eco-friendly approach in the remediation and recovery of petroleum effluent and other hydrocarbon polluted environments. This study has shown a massive potential in bioremediation for ensuring efficient petroleum effluent cleanup and other hydrocarbon related pollution on the environment. It could therefore be concluded that the bioremediation of petroleum refinery effluent through biostimulation of indigenous microbes was effective to an appreciable extent. Six microorganisms namely *Micrococcus sp, Enterobacter sp, Bacillus sp, Proteus sp, Staphylococcus sp.*, and *Pseudomonas sp.* were isolated from the treated petroleum refinery effluent. This study hereby establishes the degradative nature of indigenous microbes and also confirms its efficacy when the biostimulants are applied for the treatment of petroleum effluent and other hydrocarbons polluted environments. A full-scale remediation technique can therefore be adopted using treatment option C in this study. It has been discovered that the rate of bioremediation is usually slow and if the rate can be sped up, it will help to enhance the remediation process

Declaration of Interest

The authors hereby declare that there is no known financial interest that could affect the work as reported in this paper.

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