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Evaluation of Bioremediation Potential of Biosurfactant Producing Bacteria Isolated from Crude Oil Polluted Soil

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ABSTRACT

Bioremediation technique depends on the ability of microorganisms to break down pollutants that are released into the environment. Evaluation of bioremediation potential of biosurfactant-producing bacteria isolated from hydrocarbon contaminated soil was investigated. Hydrocarbon contaminated soil was collected at different points in OB/OB flow station in Ogba/Egbema/Ndoni L.G.A, River State using hand auger. Each experimental set up of contaminated soil and control set up contained 500g of soil. The set up was augmented with 25ml of the selected biosurfactant producing bacteria (*Pseudomonas koreensis* (Pseu), *Bacillius mycoides* (Bac), and biosurfactant (rhamnolipid) except the two (2) controls. Bioremediation process monitoring lasted for 70 days, at 14 days' interval. Physicochemical properties and bacterial load of treated soil were analyzed using standard methods. Percentage bioremediation was estimated from amount of total petroleum hydrocarbon (TPH) reduction. ANOVA was used to ascertain significant difference of various treatments. The highest amount of hydrocarbon removed and % remediation efficiency after 70 days with treatment was Cs+Bac+Pseu+bios 5287.62mg/kg (78.07%) while controls were Cs (69.1%) and Uc (0%). The highest count for total heterotrophic bacteria for the set ups was recorded on day28 Cs+Bac+Pseu+Bio (9.20×10^{8}cfu/g). The highest count of hydrocarbon utilizing bacteria (HUB) was on day42 by CS + Bac + Pseu + Bio (9.40×10^{6}cfu/g). Results from the study revealed that the *Bacillus mycoides, Pseudomonas koreensis* and rhamnolipids and their consortium are capable of remediating TPH in crude oil contaminated soil. There was a faster removal of hydrocarbon by mixed cultures than individual strains. It is recommended that, these known Biosurfactant producing and hydrocarbon degrading isolates like *Pseudomonas koreensis, Bacillus mycoides* should be harnessed, improved and applied in the bioremediation of petroleum hydrocarbon impacted ecosystem since bioremediation via effective microorganisms and biosurfactants remains an effective, viable as well as a promising strategy for the cleanup of crude oil contaminated environment.

Keywords: Biosurfactant (rhamnolipid), hydrocarbon contaminated soil, hydrocarbon utilizing bacteria, bioremediation.

Introduction

Microorganisms are stimulated to grow by altering the environmental conditions of the soil so they can degrade specific pollutants (Narong and James, 2016). Bioventilation, biostimulation and bioaugmentation are generally the three mechanisms used in bioremediation (Nelson-Smith, 2018).

Bioaugmentation involves the introduction of genetically engineered bacteria in the contaminated soil to enhance the degradation process while Biostimulation involves the adjustment of environmental conditions of soil in which the bacteria have to grow by limiting or injecting nutrients to stimulate the growth of microorganisms and increase the biodegradation process.

On the other hand, Bioventilation involves the injection of oxygen into the voids of soil to enhance growth of the microbes (Prince *et al*., 2013). The rates of uptake and mineralization of many organic compounds by microbial populations in the aquatic environment are proportional to the concentration of the compound, generally conforming to Michaelis-Menten kinetics (Lau *et al*., (2010). Hydrocarbons are susceptible to microbial degradation in different ways as follows in decreasing susceptibility: n-alkanes > branched alkanes > low-molecular-weight aromatics > cyclic alkanes (Lau *et al*., (2010). Degradation is usually highest in saturates, followed by light aromatics, then high molecular aromatics and polar compounds exhibit very low degradation rates (Nelson-Smith, 2018).

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Bioremediation, the use of microorganism or microbial process to detoxify and degrade environmental contaminants is among these new technologies. The goal of bioremediation is to at least, reduce pollutant levels to undetectable, non-toxic or acceptable levels (Pointing, 2001) or ideally completely mineralize oregano-pollutants to carbon dioxide. From environmental point of view this total mineralization is desirable as it represents complete detoxification (Gros *et al.*, 2014; Hua *et al*., 2003). Regardless of the exact nature of the treatment technology, all bioremediation techniques depend on having the right microbes in the right place with the right environmental conditions for degradation to occur (Atlas and Bartha, 2009). Efforts to achieve biodegradation of oil products have involved bacteria and fungi, since they are the only biological species which have the metabolic capability of utilizing petroleum carbon for cell synthesis (Jinlan, 2012; Batista *et al.,* 2016).

The use of biosurfactants for different purposes including oil recovery has taken a center stage in the recent years because they are renewable, biodegradable and environmentally friendly (Gudina *et al.,* 2015). Biosurfactants are applied for different purposes in the oil industry but the commonest applications include recovery of oil from oil wells, emulsion facilitated transport, cleaning of oil tanks, emulsion-based fuel production and environmental remediation Das, and Mukherjee 2007, Batista *et al.*, 2016). Nelson-Smith (2018) argues that *Bacillus licheniformis* can be used for in-situ MEOR because of its halo-tolerant properties. A wide range of different hydrophobic substrates, such as aliphatic or aromatic hydrocarbons can be emulsified efficiently to 65 ± 5 % by the lipopeptide surfactant produced by *Bacillus licheniformis*. With the halo-thermal properties of the genus bacillus, its strains accompanied with nutrients can be inoculated in the oil well for the in situ MEOR (Godleads *et al.*, 2015).

The in-situ growth of microorganisms in MEOR can be hindered by the lack of sulphur, phosphorus and nitrogen in petroleum hydrocarbons much as there is presence of carbon and hydrogen. Biosurfactants have great surface and interfacial reduction capabilities which make them a high priority in enhancement of oil recovery and demulsification processes (Nelson-Smith, 2018).

Indeed, when lipopeptide biosurfactant produced by the strain *Bacillus subtilis* will be introduced to motor oil contaminated soil, it recovered 85 % of the oil in 24 hr. (Nelson-Smith, 2018). When Gudina *et al*. (2015) used rhamnolipids in comparison to enordet and petrostep (synthetic surfactants) to recover crude oil from sand, the observed that rhamnolipids recovered 55 % of the crude oil while enordet and petrostep recovered 54.4 % and 30.5 % respectively.

Petroleum products are released indiscriminately into free land space, drainage systems which carry them into coastal waters and inland (Batista *et al*., 2016). Crude oil polluted land is unhealthy for plant proliferation, microbial survival and unpleasant to sight. Hence this study was aim at evaluating the bioremediation potential of biosurfactant producing bacteria and make recommendations that can enhance bioremediation of hydrocarbon polluted soil.

Materials and Methods

Description of the Study Area

The study area was OB/OB flow station in Obrikom community, Ogba/Egbema/Ndoni L.G.A, River State. The OB/OB flow station is situated between latitude 5.3998°N and longitude 6.6211°E, and 11 meters elevation above the sea level.

The distance from this OB/OB flow station to the next village Egbema is 224.25 meters and the activities here include the building and repair of oil pipelines, fishing, wastewater irrigation, agricultural, sand dredging activities and gas flaring.

Sample Collection and Preparation

Crude oil contaminated soil was collected at different points in OB/OB flow station using a sterile hand auger. While the uncontaminated soil which served as control was collected from Rivers State University farm.

Collected soil samples were transferred into sterile containers with icepack and were immediately transported to Rivers State University, Microbiology Laboratory for analysis.

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Table 1: Map Coordinates of the Different Points of Crude Oil Impacted Area and None Impacted Study Area

Sample locations	Latitude/northing(N)	Longitude/easting (E)
Obrikom ONELGA	Lat:5.38874 N5^{0}23'19.61016"	Log:6.65733 E6^0.39'26.37504
Obrikom ONELGA	Lat:5.238869 N5^{0}23'19.29696"	Log6.65703 E6^{0}39'25.29324
Obrikom ONELGA	Lat:5.38755 N5^{0}23'15.16211"	Log:6.65851 E6^{0}39'30.65333
Obio/Akpor Rivers State University	Lat:4.80711 N4^{0}48'25.57872"	Log:6.97674 E6^{0}5836.25104

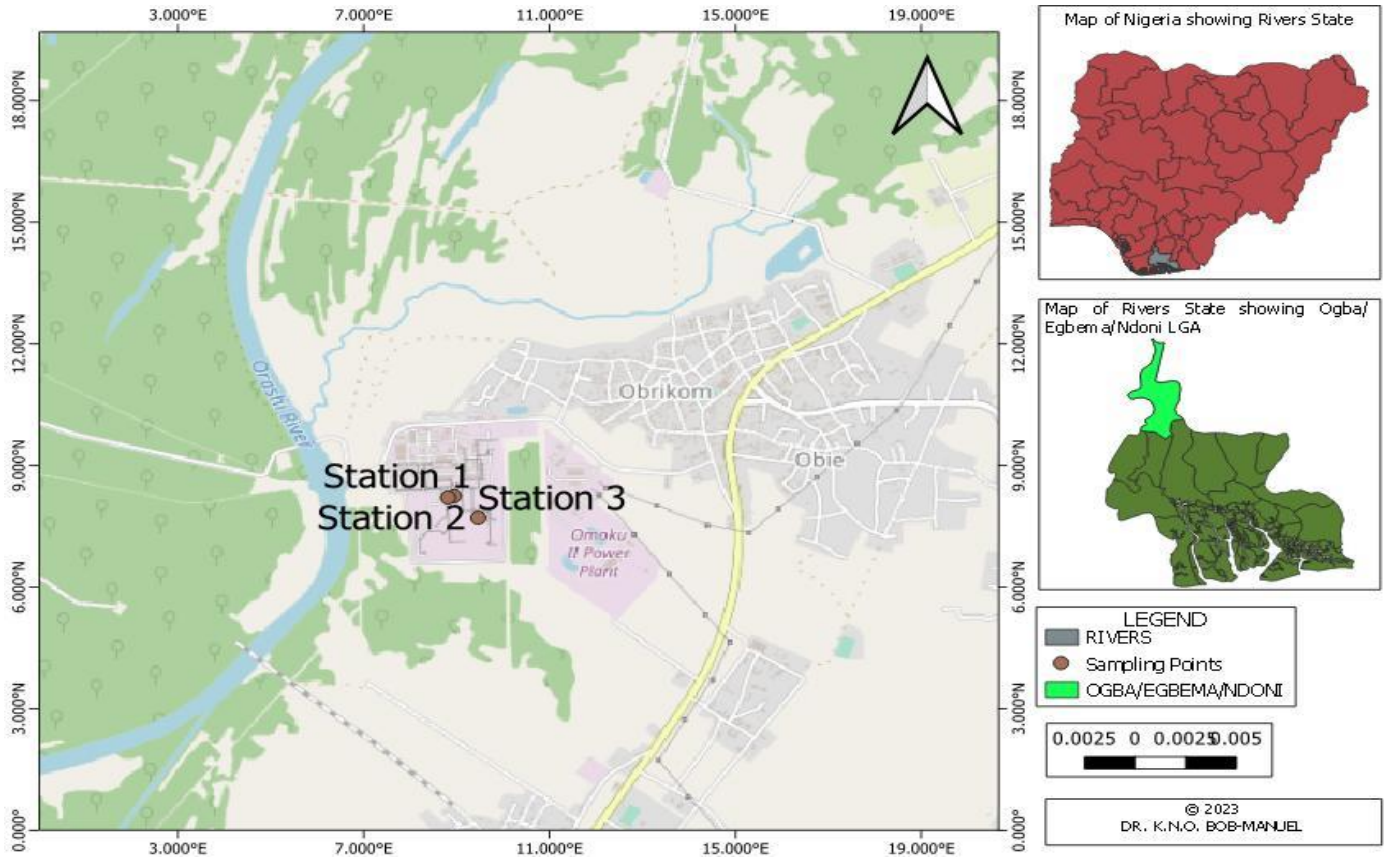

Fig. 1: Map of Obrikom Onelga Local Government Area showing the Sample Location

Baseline Physicochemical and Microbiological Analysis of the Soil before Treatment

The soil was analyzed for physicochemical and microbiological before treatment. The parameters determined were pH, temperature, electrical conductivity, phosphorus, moisture content, total organic carbon (TOC), Nitrite, nitrogen, chromium, Cadmium, lead, zinc, nickel total polycyclic aromatic hydrocarbons (PAHs), and total petroleum hydrocarbon (TPH) and microbiological parameters following standard procedures by APHA (1998).

The microbial properties determined were Total Heterotrophic Bacteria (THB), Hydrocarbon Utilizing Bacteria (HUB).

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Enumeration of Total Heterotrophic Bacterial Count (THBC)

Total heterotrophic bacterial count was carried out on both the contaminated and uncontaminated soil by weighing 1g of sample which was serially diluted in 9ml normal saline up to ten-fold. Using spread plate method, an aliquot of 0.1ml from 10^{-6} test tube was inoculated into solidified nutrient agar in duplicates. The culture plates were incubated at 30^{o}C for 24hrs for count. Discrete colonies that developed were counted and expressed in CFU/g (Chikere *et al*., 2009; Ibiene *et al*., 2011).

Enumeration and Isolation of Hydrocarbon Utilizing Bacteria (HUB)

The vapour phase transfer technique according to Ibiene *et al*. (2011) and Ezekoye *et al*. (2015) was used to enumerate hydrocarbon-utilizing bacteria (HUB). The culture medium used for the isolation of hydrocarbon utilizing bacteria was mineral salt agar (MSA). Using a spread plate method, an aliquot of 0.1ml of the 10^{-4} dilution was plated into a solidified MSA plates medium supplemented with a nystatin (fungosol) to inhibits the growth of fungi in duplicates and the plates were inverted and filter papers were placed inside the inverted plates covers. The filter papers was flooded with 1ml of sterile crude oil as the sole source of carbon and energy and incubated at 30^{0}C for 5 to 7 days. Discrete colonies that developed were counted and expressed in colony forming unit per gram (CFU/g) soil. To obtained a pure culture, the bacterial isolates was sub-cultured using the accepted techniques, the pure cultures were characterized and identified to ascertain the bacterial species.

Preparation of Bacterial Suspension for Inoculants for the Bioremediation Setup

Suspension of *Pseudomonas* was prepared from 24hr pure culture Petri dish (Hadibarata, and Tachibana, 2009). Two hundred milliliter (200ml) of nutrient media broth was transferred into 250ml conical flask and was sterilized using autoclave at 121^{o}C for 15minutes at 15psi, and allowed to cool at room temperature. Zero-point eight gram (0.8g) of Nystatin was supplemented to the broth to suppress the growth of fungi.

Pseudomonas sp were scraped out from the surface of bacterial colonies and then transferred to the 250ml flask containing the 200ml nutrient broth until a turbid appearance was formed. The flask was cap with cotton wool (Balakrishnan *et al.*, 2013). This was incubated at room temperature (28^{o}C) for 24hrs. This process was repeated for *Bacillus* sp isolate.

Experimental Design/ Bioremediation Setup

The experimental design/ bioremediation set up is as presented in Table 2 below. This bioremediation set up was monitored for selected bacteriological and TPH for 6 weeks, for three months interval from week 0, 14, 28, 42, 56, and 70 days. The parameters include HUB, THB and TPH, at 14 days' interval.

Eighty milliliter (80ml) of sterilized water was added to the set up three times weekly and agitated for proper aeration and adequate distribution of microorganism. The set up was covered with a net to prevent aerospores.

Table 2: Experimental Design/Bioremediation Setup

Experimental Setup code	Amount of Soil (g)	Volume Inoculum in Broth		Volume of Biosurfactant
		Bac	Pseu	
US	500	-	-	-
CS	500	-	-	-
CS+Bio	500	-	-	25
CS+ Bac	500	25	-	-
CS+ Pseu	500	-	25	-
CS+ Bac+Bio	500	12.5	-	12.5
CS+Pseu+Bio	500	-	12.5	12.5
CS+Bac+Pseu+Bio	500	8.3	8.3	8.3

Key: CS= Contaminated, **US** = Uncontaminated, **Bio**= Biosurfactant, **Bac** = *Bacillus*, **Pseu** = *Pseudomonas.*

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Determination of the Total Petroleum Hydrocarbon

The soil samples were taken for Total Petroleum Hydrocarbon (TPH) analysis at the various time interval of the bioremediation. Extraction of residual crude oil was done by adding an equal amount of n-hexane in relation to the amount of medium. The mixture was agitated on a magnetic stirrer for 15 minutes and centrifuged at 2000g for 5 minutes. The lower organic phase was collected with a micropipette and condensed at 28rpm and 400C until the n-hexane was evaporated. The dried crude oil soil was collected using n-hexane as solvent and analyzed for TPH using GC-FID (TPI, 2007; Ibiene *et al.*, 2011). The amount of crude oil removes in TPH and the % remediation of TPH in the experiment were determined using the approach of Nrior and Mene (2017).

For the amount of pollutant remediated:
Pollutant remediated amounts are equal to Initial Pollutant Concentration (day 1) minus Final Pollutant Concentration (day70). i.e, Ba = Ic – Fc

Where:
Ba= Amount of pollutant remediated
Ic = Initial Concentration of pollutant (day1)
Fc = Final Concentration of pollutant in plot x (day70)

For percentage remediation:
The percentage (%) remediation equals Amount of pollutant remediated divided by the Initial Concentration of pollutant (day1), multiplied by 100
% remediation = (Bc/Ic) x 100

Statistical Analysis

Data obtained in this study was analyzed using IBM SPSS. Statistical analysis was carried out for microbiological and physicochemical parameters when treated using Statistical Package for Social Science (SPSS) from the data obtained. Analysis of variance (ANOVA), P-values test of significance was carried out at 95% level of confidence to ascertain significant difference of mean value between various treatment and data that was obtained during the study.

Results

The physicochemical and bacteriological properties of the soil before the application of various bioremediation treatments are shown in Table 3 and 4.

The results revealed that the pH was slightly acidic with value of 5.06 for uncontaminated soil and 6.0 for contaminated soil, temperature was observed to be 28.1ºC for uncontaminated while the contaminated was 27.9ºC, electrical conductivity was 25µS/cm for uncontaminated soil and 3µS/cm for contaminated soil.

Moisture content was 17.0% and 10.4% respectively, TOC was 0.83% and 0.549% for contaminated, SOM was 0.95% and 1.43%. For uncontaminated and contaminated, PAH was < 0.001mg/kg and 42.18mg/kg and TPH was < 0.001mg/kg and contaminated revealed value of 10545mg/kg.

The results analysis for bacteriological parameters for THB and HUB for uncontaminated and contaminated showed the average counts of 8.36±0.03cfu/g and 8.75±0.06cfu/g for THB, 5.32±0.08cfu/g and 5.50±0.06cfu/g for HUB, respectively.

Microbial dynamics obtained during the bioremediation of crude oil contaminated soil are shown in Figures 2 and 3. Counts for Total Heterotrophic Bacteria (THB), Hydrocarbon Utilizing Bacteria (HUB) during bioremediation of crude oil polluted soil were all determined.

There was no particular trend in the microbial counts during the period of monitoring.

Total heterotrophic bacteria counts for day 1 of the bioremediation ranged from $3.3\pm0.70 \times 10^8$ CFU/g (Cs+Bio) to $8.5\pm0.84 \times 10^8$ CFU/g (Cs+Bac) while day 70 ranged from 3.4 ± 0.49 CFU/g $\times 10^8$(Us) to $8.6\pm0.57 \times 10^8$ CFU/g (Cs+Bac+Pseu+Bio).

Changes in the hydrocarbon utilizing bacteria ranged $1.3\pm0.21 \times 10^6$ CFU/g (Cs+Bac) to $6.9\pm0.21 \times 10^6$ CFU/g (Cs+Bac+Pseu+Bio) for day 1 and $3.3\pm0.78 \times 10^6$CFU/g (Cs+Pseu) to $7.8\pm0.12 \times 10^6$CFU/g (Cs+Bac+Pseu+Bio) for day 70.

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Table 3: Baseline Data of Physicochemical Characteristics of Crude Oil Contaminated and Uncontaminated Soil

Parameter	Uncontaminated Soil (Control)	Crude Oil Contaminated Soil
Magnesium (mg/kg)	37.25	49.66
Phosphorous (mg/kg)	18	15
Temperature (°C)	28.1	27.9
pH unit	5.06	6.0
Moisture content (%)	28.8	30.4
Electrical conductivity	25	3
Soil organic matter (%)	1,43	0.95
Total organic carbon (%)	0.83	0.549
Nitrogen (mg/kg)	25.33	2.068
Chromium (mg/kg)	2.71	4.68
Cadmium (mg/kg)	0.36	1.55
Lead (mg/kg)	3.76	10.33
Zinc (mg/kg)	41.83	50.32
Nickel (mg/kg)	<0.001	5.28
Nitrite (mg/kg)	0.418	0.379
TPH (mg/kg)	<0.001	10545
PAH (mg/kg)	<0.001	42.180

Key: PAH: polycyclic Aromatic Hydrocarbons, TPH: Total Petroleum Hydrocarbons

Table 4: Baseline Data of Microbial Counts of Crude Oil Contaminated and Uncontaminated Soil

Microbiological	Microbial counts (CFU/g) Uncontaminated soil	Crude oil Contaminated Soil
Total heterotrophic bacteria	$8.36\pm0.03 \times 10^6$	$8.75\pm0.06\times 10^6$
Hydrocarbon utilizing bacteria	$5.32\pm0.08 \times 10^3$	$5.50\pm0.06\times 10^3$

Key: THB= Total heterotrophic bacteria, HUB= Hydrocarbon utilizing bacteria

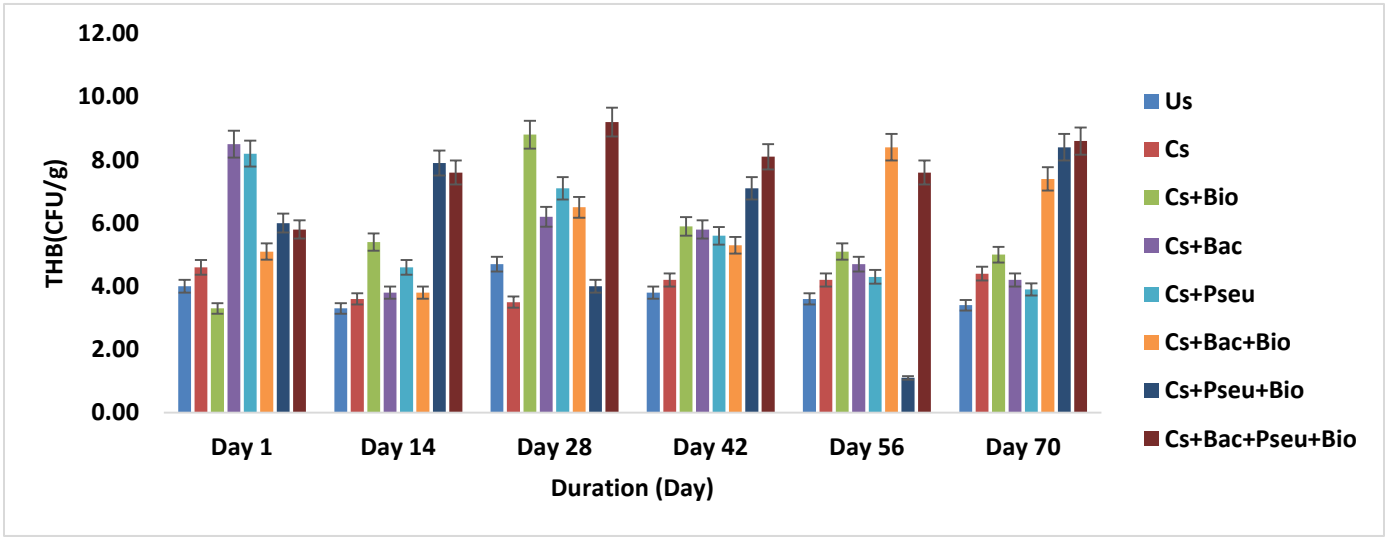

Fig. 2: Mean Changes in THB (Cfu/g) during Bioremediation of Crude Oil Contaminated Soil

Key: Us = Uncontaminated soil, Cs = Contaminated soil, Bios = Biosurfactant, Bac = *Bacillus*, Pseu = *Pseudomonas*

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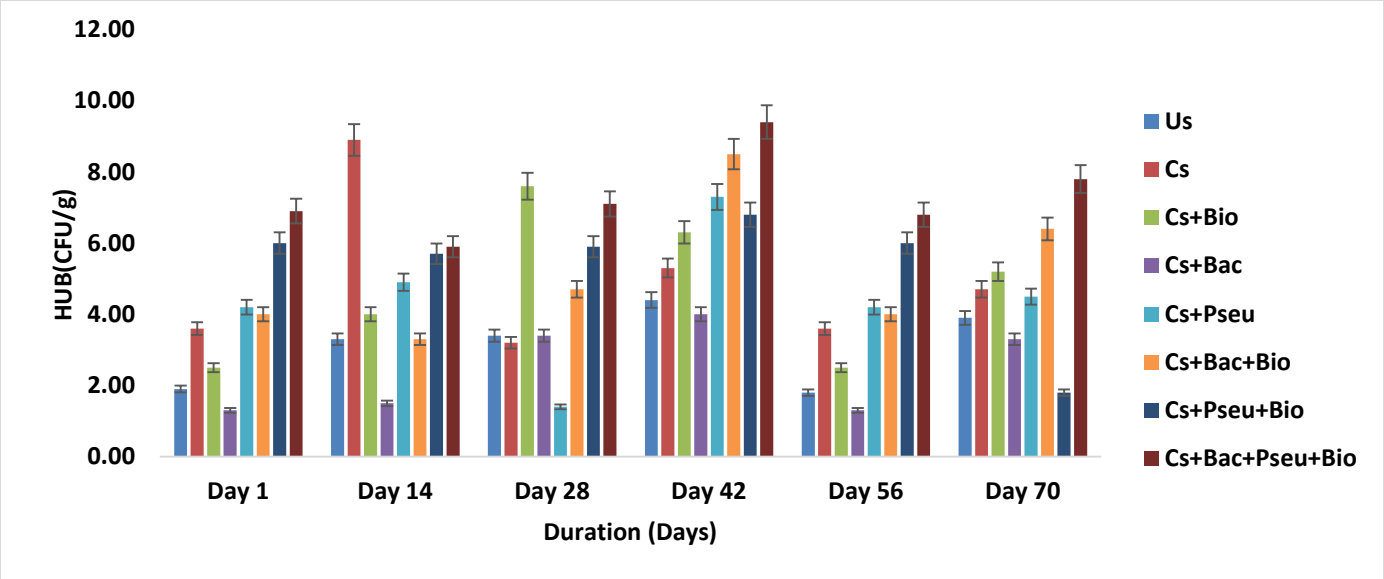

Fig. 3: Mean Changes in HUB (Cfu/g) during Bioremediation of Crude Oil Contaminated Soil

Key: Us = Uncontaminated soil, Cs = Contaminated soil, Bios = Biosurfactant, Bac = *Bacillus*, Pseu = *Pseudomonas*,

The amount of TPH removed and percentage remediation efficiency after 70 days of bioremediation monitoring with different treatment on the set up is given in a decreasing order as follows: Cs+Bac+Pseu+bios - 5287.62mg/kg (78.07%); Cs+Pseu+bios - 4819.02mg/kg (77.64%); Cs+Bac+bios - 3649.09mg/kg (75.82%); Cs+Pseu - 5115.9mg/kg (75.4%); Cs+Bio - 3263.1 mg/kg (72.5%); Cs+Bac - 3131.7mg/kg (71.2%); Cs - 2791.1mg/kg (69.1); and Uc – (0%) as presented in Figures 4 and 5.

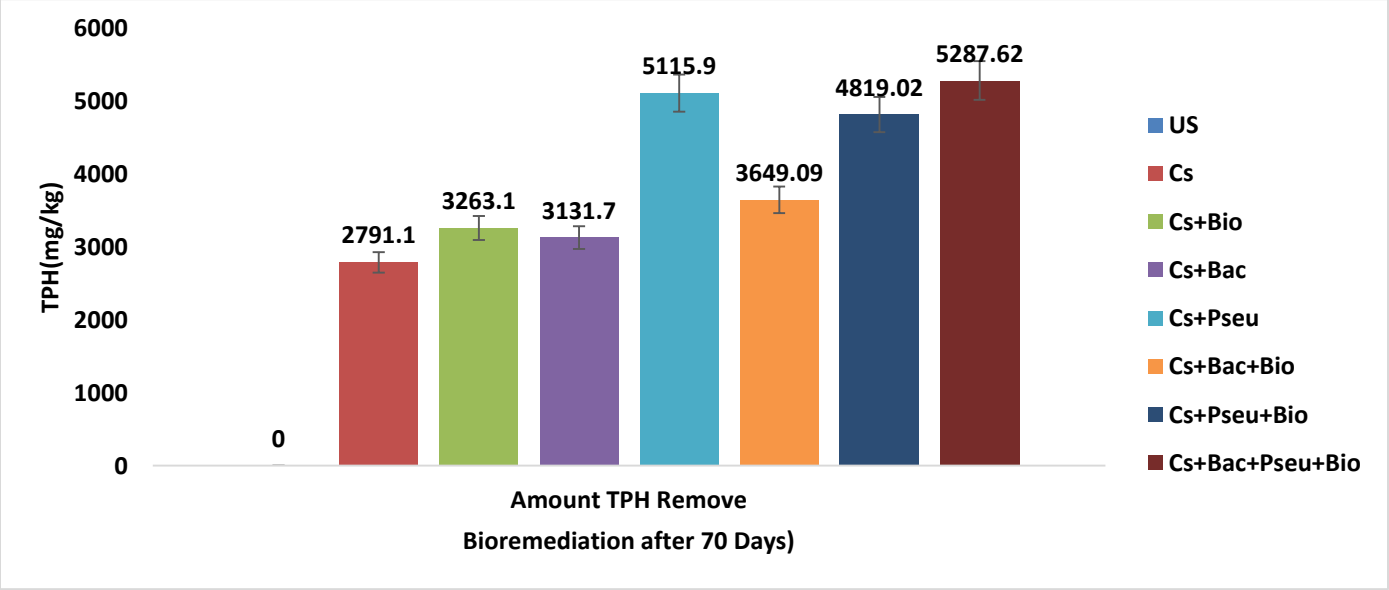

Fig 4: Amount of TPH (mg/kg) Removed from Crude Oil Contaminated Soil after 70 days of Bioremediation
Key: Us= Uncontaminated soil, Cs= Contaminated soil, Bio= Biosurfactant, Bac= *Bacillus*, Pseu= *Pseudomonas*

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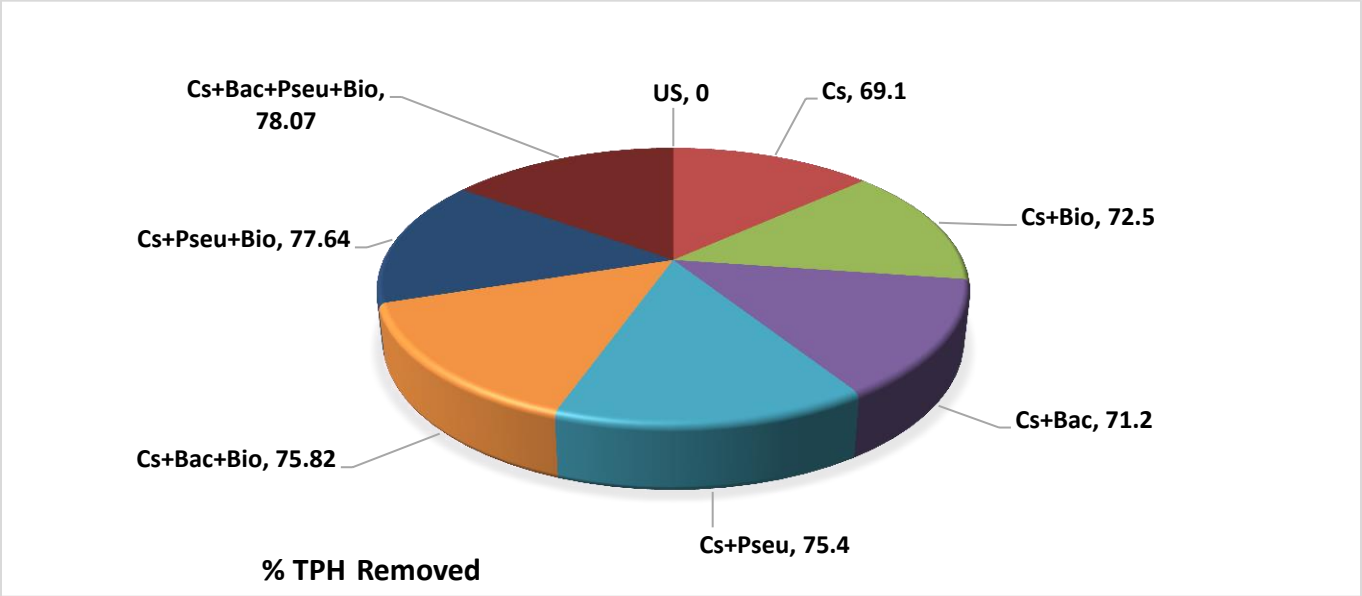

Fig. 5: Biosurfactant Remediation of TPH (%) (mg/kg) during Remediated of Crude Oil Contaminated Soil

Discussion

The baseline values of pH prior to treatment for bioremediation were 5.06 and 6.0 for crude oil uncontaminated and contaminate soil respectively, pH levels were shown to be tending toward acidity. According to Boonchan (2000), the ideal pH range for bioremediation is between 6.0 and 8.9.

Generally, alkaline or slightly acidic soil pH enhances bioremediation, while acidic environment pose limitation to biodegradation (Pawar, 2015); also said that optimum pH for bioremediation of hydrocarbons is around 6-8 pH. Temperature range were relatively same between uncontaminated and contaminated soil with 28.1 and 27.9 respectively, but were higher in the Uncontaminated soil.

The results of temperature before treatment shows that temperature range favored the acclimatization process for the hydrocarbon utilizing microbial growth Mentzer and Ebere (1996). Bioremediation rate, to an extent rises with increasing temperature and slows with decreasing temperature. Moisture content of the study samples were found to contain 28.8 and 30.4% moisture content for both uncontaminated and contaminated soil. Literature data indicate that moisture has a strong effect on biochemical processes in contaminated soils (Myazin *et al*., 2021).

The moisture content of the study sample before bioremediation was within the limits that are optimal for bioremediation. Gupta *et al*. (2021) stated that optimal moisture content for bioremediation is within 30 to 60%.

Nitrogen, and phosphorous were shown to be low prior to treatment. Nitrogen before treatment was 25.33mg/kg and 20.068, phosphorous was 18mg/kg and 15mg/kg while magnesium was 37.25mg/kg and 49.66mg/kg respectively. Nutrient availability is essential for the growth and metabolism of microorganisms which directly affects the rate of degradation of petroleum hydrocarbon. Nitrogen and phosphorus have been identified as the most growth limiting factors for organism mediated hydrocarbon degradation and potassium availability can affect bioremediation rates (Evans *et al*. 2004).

Hence are required by microorganisms for metabolism and other cellular functions. In the absence of one or more of these substrates, microbial growth will be limited. All biological forms must have nitrogen in order to survive. It is a part of the amino acids needed to make proteins. Proteins make up the majority of enzymes, bacteria, hormones, human and animal tissues. A key barrier to microorganism's capacity to actively breakdown petroleum hydrocarbons is a shortage in nutrient supplies (Jain *et al*. 2001).

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Total petroleum hydrocarbon (TPH) content in the soil sample before treatment for bioremediation was <0.001mg/kg for uncontaminated soil and 10545mg/kg for contaminated sample which exceeded the DPR 2018 intervention limits of 5000mg/kg. This shows the presence of high hydrocarbon content that is yet to be degraded in that environment. After bioremediation, the TPH content decreased to 78.07%. The substantial decrease in TPH content in the soil after bioremediation is consistent with findings reported in the literature (Rondon-Afanador *et al*., 2023).

The count obtained for total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) during the period of monitoring show no significant difference between the treatments. This proves the ability of the soil to harbor diverse and high population of bacteria despite the presence of physical forces or changes in chemical composition as a result of crude oil-contamination.

The bacterial counts (population densities) in both contaminated and uncontaminated soils were generally observed, to be higher than fungal counts (population densities) in both soils. The higher bacterial population densities observed may be as a result of the nutrient status of the soils (Onifade, 2007) and the presence of some toxic components which do not favor fungal growth. The versatility of bacteria is seen to have influenced the result obtained. This attests to the fact which has been established that hydrocarbon contaminated soil harbor a vast array of microflora that is capable of utilizing the hydrocarbon, and the discharge of hydrocarbon to ecosystem may result in selective increase or decrease in the microbial population (Eze and Eze, 2010).

The population densities of the hydrocarbon utilizing bacteria (HUB) in this study were observed to be higher in the contaminated soils than in the uncontaminated soils. The observations are in agreement with the works of Ijah and Abioye, (2003), in which higher counts for HUB were observed in contaminated soil samples. These results are also in agreement with the observed trend in the microbial activities in the soil where the contaminated samples have higher activities than the uncontaminated samples (Obire and Anyanwu, 2009). There was no particular trend in microbial counts during the period of monitoring.

The total Petroleum hydrocarbon content (TPH) of the treated setup decreased from day zero (0) value at the start of bioremediation to Cs+bac+pseu+bio (78.07%) Cs+pseu+bio (77.64%) > Cs+Bac+Bio (75.82%) > Cs+Pseu (75.4%) > Cs+Bio (72.5%) > Cs+bac (71.2%) > control (Cs) contaminated without amendment of organisms and biosurfactant (69.1%).at the end of experiment. (TPH initial contamination value of 10545mg/kg which exceeded the DPR intervention limits of 5000mg/kg), this shows the presence of high hydrocarbon content that is yet to be degraded in that environment. The experimental setup with higher percentage TPH remediation performed better showing the setup have positive effect on the crude oil contaminated soil. As shown, there was a significant decrease in TPH concentration of the different set ups compared to the control.

This can be attributed to the fact that the microorganisms in the soil had efficient ability in utilizing the crude as a source of carbon and energy. Set up with Cs+bac+pseu+bio showed a better remediation percentage response from 10545mg/kg to 5287.62mg/kg; (78.07%) followed by set up with Cs+pseu 5115.9mg/kg:75.4% while set up with >Cs had a lower percentage response with 2791.1mg/kg: 69.12%. The high concentration of TPH present in soil shows a high degree of crude oil contamination which exceeds the DPR intervention limit for soil. This high concentration is therefore unfriendly to the ecosystem, and is capable of altering soil fertility and productivity. A research by Edwin and Albert (2011) has shown that crude oil interferes with the pH and oxygen concentration in the soil, which could also cause a shift in the microbial species composition of the soil.

According to Okafor *et al.* (2021), the mixed culture removed the hydrocarbons better than the individual strains as showed in the result. In this study, it was observed that a higher remediation rate in TPH was in set up with mixed cultures of *Bacillus mycoides*+ *Pseudomonas koreensis*, biosurfactant (rhamnolipid) compared to other set up. The present result is not in agreement with Fatuyi *et al.* (2012) and Al-Jahwahri, (2014) who demonstrated that single isolates had a higher percentage of petroleum hydrocarbon degradation than mixed culture. But in agreement with the study of Sunita *et al*. (2013) who reported faster utilization of hydrocarbon by mixed cultures and biosurfactant (rhamnolipid) than individual strain.

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The phenomenon was explained by Vanesa and Zhu (2003), who assumed that there is no single strain with the metabolic ability to degrade all the components found within crude oil. It also agrees with the study conducted by Adebusoye *et al*. (2007) who demonstrated that a consortium of microorganisms is required to complete biodegradation of oil pollutants because the necessary enzymes needed for biodegradation cannot be found in a single organism because of differences in volatility, solubility and susceptibility of hydrocarbons.

In conclusion this study demonstrated that, the rhamnolipids and the consortium of, *Bacillus mycoides, Pseudomonas koreensis* and rhamnolipids rhamnolipids are capable of remediating total petroleum hydrocarbon in the crude oil contaminated soil. There was a faster removal of hydrocarbon by mixed cultures than individual strains. This study highlights the potential of biosurfactant-producing bacteria as effective agents for bioremediation applications, particularly in the remediation of hydrocarbon-contaminated environments. It is recommended that, these known Biosurfactant producing and hydrocarbon degrading species such as *Pseudomonas koreensis*, and *Bacillus mycoides* isolated in this study should be harnessed, improved and applied in the bioremediation of Petroleum hydrocarbon impacted ecosystem since such bioremediation technique remains an effective, viable as well as a promising strategy for the cleanup of crude oil contaminated environment.

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