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Original Research Paper

Heterotrophic activity and hydrocarbon degradation in crude oil-contaminated coastal soil augmented with indigenous biosurfactant producing *Pseudomonas* sp.

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Abstract

The enhanced bioremediation of the petroleum-contaminated soil from the Ibeno coastal area was investigated using standard microbiological and biotechnological methods. Soil samples were simulated with 200ml, 400ml, and 800ml of Bonny Light crude oil representing 5%, 10%, and 20 % contamination levels respectively, and allowed to mimic natural crude oil degradation for 48 hours. The contaminated soil was bioaugmented with 15 ml of 24-hour culture of potent biosurfactant-producing bacteria – *Pseudomonas aeruginosa* with a viable cell count of 2.6 x 10^3 CFU/ml and monitored for 12 weeks. Analysis of the fate of the hydrocarbon contamination in soil augmented with the biosurfactant-producing pseudomonad revealed that the degradation of total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbons (PAHs) was faster in augmented soils. At the end of the degradation, the augmentation process induced a reduction in the TPH content and PAH levels of soil exposed to 20% contamination from 32.85 mg/kg to 15.14 mg/kg and from 16.34 mg/kg to 5.35 mg/kg respectively. These represent 45.9% and 32.7% remediation rates of TPH and PAH, respectively. The findings of this study also indicate that bioaugmentation of crude oil-contaminated soil with a culture of *P. aeruginosa*, does not only influence the density of heterotrophic and hydrocarbon-degrading bacteria in the soil but increases the natural attenuation potentials of the contaminated soil.

Keywords: Bioaugmentation; Biosurfactant; Pseudomonas; Petroleum hydrocarbon; Polycyclic aromatic hydrocarbons

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1. Introduction

Crude oil spills continue to be a serious environmental problem in many nations especially where the economy is based mostly on oil exploration (Okafor et al., 2021; Dominguez-Rodriguez et al., 2020). Crude oil is a brownish black naturally occurring liquid and a complex mixture of hydrocarbon compounds. Total Petroleum Hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbon (PAHs) are two important hydrocarbon families that are frequently employed for the analysis and environmental monitoring of crude oil. TPH generally describes petroleum-based hydrocarbons in the environment. The term refers to a mixture of several hundreds of hydrocarbon compounds (ranging from C5 – C40) which include petrol, kerosene, diesel, and aviation fuel. TPH is a common group of persistent organic environmental pollutants (Vane et al., 2017) that may enter the environment through accidental spills, industrial releases, or as by-products of commercial or private uses (Kuppusamy et al., 2020). PAHs are aromatic hydrocarbons with more than one benzene ring. PAHs exist as

colourless, white/pale yellow solids with low solubility in water, high melting and boiling points, and low vapor pressure (Obi, 2017). They are encountered either naturally in the environment or from anthropogenic activity. Some examples of natural sources are forest fires and volcanic eruptions, while examples of anthropogenic sources are vehicles and industrial emissions (Kumar et al., 2016).

Bioremediation is the most cost-effective and sustainable treatment strategy for the degradation of crude oil after spillage (Dey et al., 2024). During microbial remediation, microorganisms utilize organic pollutants such as crude oil converting them into environmentally friendly compounds such as CO_2 and H_2O (Ron & Rosenberg, 2014). This approach has been widely used to degrade hydrocarbons and restore the oil-spilled site (Abraham & Essien, 2016; Varjani & Upasani, 2017; Abraham et al., 2021; Udofia et al., 2021; Okafor et al., 2021; Shahid et al., 2023).

Despite the various advantages of bioremediation, their efficiency in remedying crude oil contaminated sites is most times limited by various factors. One of the major factors is the limited availability of the pollutant to microbes due to their low solubility and strong and/or irreversible sorption to soil. Surfactants (biological and synthetic) are often used to overcome this setback as it has the potential to increase desorption and apparent solubility in the aqueous phase (Abraham & Essien, 2016; Jayasena & Perera, 2021). Surfactants increase the apparent solubility of hydrocarbons and enhance their bioavailability by adsorption and emulsification, which helps hydrocarbon-utilizing bacteria and fungi in the contaminated environment to break down hydrocarbons (Jayasena & Perera, 2021; Aruotu et al., 2023). Biosurfactants are less toxic and more biodegradable than chemical surfactants; this makes them more preferred for the bioremediation of petroleum hydrocarbon-contaminated environments (Fenibo et al., 2019; Patowary et al., 2018). This study is designed to evaluate the effect of bioaugmentation using biosurfactant-producing bacterial strain on the heterotrophic activity and hydrocarbon degradation in crude oil-contaminated Niger Delta coastal soil.

2. Materials and Methods

2.1 *Study Area and Sample Collection*

Ibeno Local Government area is a coastal area located in the southern part of Akwa Ibom State, a petroleum-rich Niger Delta region of Nigeria. The region has a shoreline with the Atlantic Ocean that runs beside the Bight of Bonny (Udoh & Amadi, 2020). The shoreline is approximately 56.7 km in length and spans from a point at Atabarikang village on latitude 4˚31'23''N and longitude 7˚49'16.0114''E to Okposo village on latitude 4˚34'09.7667''N and longitude 8˚17'52.6643''E. The area has two distinct seasons namely the wet or rainy and the dry season. The climate characteristics correspond to Koppen's climate classification. It is characterized by very high rainfall (annual totals >4000 mm), high-temperature values of about 27˚C, and high values of relative humidity with a mean value of 80.3% (Ekong, 2017). A bulk quantity of the coastal soil was transferred to the Experimental Garden of the Department of Microbiology, University of Uyo for investigation.

2.2 *Isolation of Indigenous Biosurfactant Producing Bacterial Isolate*

One gram of the soil sample was serially diluted using 10-fold (v/v) serial and plated on Centrmide-Nalidixic (CP) selective agar (01-609), a selective solid medium was used for the detection of Pseudomonas aeruginosa according to the EN12780 and 15016266 standard. Cultures obtained were purified by repeated subculturing and preserved in agar slant at 4°C.

2.3 *Screening for Biosurfactant Producing Potentials of the Isolate*

The biosurfactant-producing potential of the isolates was determined based on their potential to produce hemolysis on blood agar, emulsify oil, their oil displacement activity as well as the characteristics of culture suspension in oil. For the hemolytic test, the isolates were streaked on blood agar. The inoculated plates were incubated at 37°C for 24-48 hours and the plates were observed for hemolysis. In the oil spread technique, precisely 10 ml of crude oil was added to the surface of 40 ml of distilled water in a petri dish to form a thin oil layer. Then, 10 ml of the supernatant culture of the test isolate was gently placed on the centre of the oil layer (Abraham & Essien, 2016). The formation of a clear zone was indicative of the presence of biosurfactants. The

drop-collapse test was performed according to Plaza et al. (2006). Supernatant from the sample culture broth was pipetted onto a microplate lid (12.7 × 8.6 cm^2) previously coated with Tapis crude oil. When the drop of the supernatant became flat 1 minute after adding, the result was taken to be positive and when the drops remained beaded, the result was recorded as negative. For the emulsification index, the same volume of a 72-hour-old test culture supernatant and kerosene in a ratio of 1:1 were mixed in a glass test tube (125 mm × 15 mm). Then, the mixture was vortexed for 2 min and left to stand for 24 h. The %EC is given as the percentage yielded by dividing the height of the emulsified layer (mm) by the total height of the liquid in the glass test tube (mm), then multiplying by 100 (Abraham & Essien, 2016).

2.4 Molecular Identification of Best Biosurfactant Producer

The bacterial isolates were subjected to molecular analysis to confirm their identity as described by Liu et al. (2014). Sequence analysis of the 16S rRNA gene colony polymerase chain reaction (PCR) was used to amplify the target 16S rRNA region of the DNA in bacterial cells. The process was performed by picking a single colony of bacteria isolates from the nutrient agar medium using the tip of a sterile pipette and placing it in 100 µl of sterile distilled water in a 1.5 ml microcentrifuge tube. The tube was incubated at between 94 and 95 °C for 10 min using a digital dry bath (Bio-Rad). A volume of 2 µl was used as a DNA template for the amplification reaction. The 16S rRNA region was amplified by PCR using the forward primer, 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and reverse primer 1492R (5′-CGG CTA CCT TGT TAC GAC TT-3′) (Abellan-Schneyder et al., 2021, Prashanthi et al., 2021, Sadiqi et al., 2022). The amplification reaction was prepared using 10 µl of 2× PCR Master Mix (Thermo Scientific Phusion Flash High-Fidelity), 1 µl of each forward and reverse primer (10 µM), 2 µl of the DNA template, and 6 µl of sterile distilled water resulting in a 20 µl reaction volume. The negative control was set up without genomic DNA. The amplification reaction was performed in a thermal cycler (Bio-Rad T100™) as follows: one cycle at 98 °C for 10 s, followed by 34 cycles at 98 °C for one second, 53 °C for 1 min and 72 °C for 15 s. A final extension step at 72 °C for 1 min was performed for 1 cycle. The reaction was held at 4 °C until the amplicons were removed from the thermal cycler (Sambrook & Russell, 2001; Liu et al., 2014; Prashanthi et al., 2021; Sadiqi et al., 2022). The amplicons were then assessed by running 1% agarose gel electrophoresis and viewed in the Gel Doc imager (Bio-Rad). PCR products were sent to Inqaba Biotechnological Industries for purification and sequencing. The amplified 16S rRNA gene sequences were aligned using the Bioedit and CLUSTALW software. The Basic Local Alignment Search Tool (BLAST) program of the National Centre for Biotechnology Information (NCBI) was used to search and identify the closest species.

2.5 Experimental Design

Precisely 4 kg of the coastal soil was placed in six wooden boxes (A-1 to B-3) with a dimension of 2ft by 2ft and 2ft high. The soil samples were simulated (contaminated) with 200ml, 400ml and 800ml of Bonny Light crude oil representing 5%, 10% and 20 % contamination levels respectively. The mixture was allowed to stand for 48 hours. After 48 hours, 15 ml of a 24-hour culture broth of the best biosurfactant-producing bacteria (BPB) was added to all the boxes labeled A-1 to A-3. The viable cell density of the BPB used was 2.6 x 10^3 CFU/ml and the density applied in the experimental setup was 3.9 x 10^4 CFU/15 ml. A summary of the experimental setup is presented in **Figure 1**. The set-up was allowed to stand for 12 weeks with intermittent monitoring of the heterotrophic status and attenuation rate of the hydrocarbons.

2.5.1 Evaluation of the Heterotrophic Status and Hydrocarbon Attenuating Potential of Microbes in Bio-augmented Soil

The effects of bioaugmentation with biosurfactant-producing strain of *P. aeruginosa* on the heterotrophic status and crude oil degradability of indigenous microbial population as well as its effect on hydrocarbon attenuating index or recovery index (HUB/THB ratio) of the contaminated soil were determined. The total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) counts were determined by the pour plate and vapour phase transfer methods respectively as described by Oboh et al. (2006) as well as Okpokwasili & Amanchukwu (1998). The derived values were used in calculating the HUB/THB ratio.

2.5.2 *Determination of Residual Hydrocarbon Concentrations*

The effect of bioaugmentation with biosurfactant-producing strain of *P. aeruginosa* on the degradation of Bonny Light- crude oil in coastal soil was measured in terms of reduction in the amount of total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbons (PAHs) in the polluted soil. This was monitored at 6-week intervals for 12 weeks using standard analytical methods. TPH was determined using Gas chromatography equipped with a flame ionization detector (GC-FID) while extractable organic PAHs in treated soil were quantified using a gas chromatograph (GC, Hewlett-Packard HP 6890 series) coupled (to) a mass spectrometer MS, model 5971, Hewlett-Packard). The analytical procedures followed the method used by Offiong et al. (2022), Inam et al. (2016) and Offiong et al. (2020).

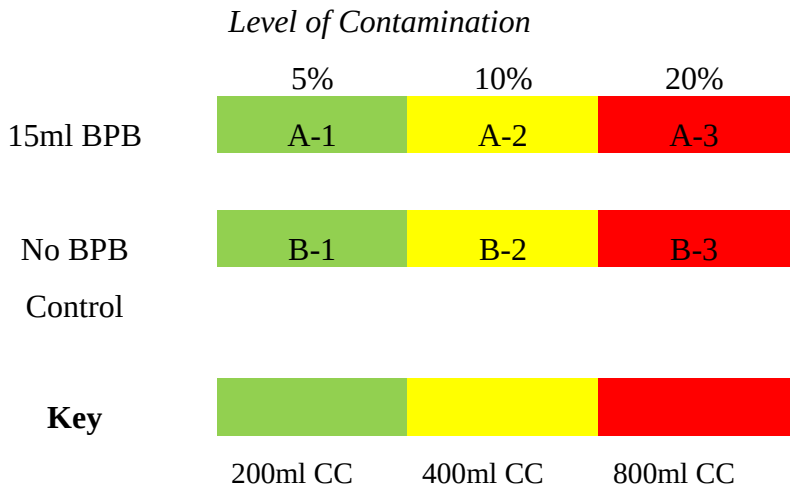

Figure 1. The Experimental Arrangement for the Simulated Soils

3. Results and Discussion

Microorganisms are distributed in the biosphere based on natural selection (Abraham et al., 2021). In the aquatic ecosystem, these microorganisms play a vast number of important roles in the decomposition of organic matter, mineralization, element recycling and transformation due to their versatile metabolic abilities. These metabolic potentials enable the organism to adapt and survive even in the presence of environmental pollutants. One of the indices of microbial adaptation in any ecosystem is its biomass. The mean density of Pseudomonads associated with the soil samples was 5.8 x 10^3 CFU/g. Six distinct (IS-1, IS-2, IS-3, IS-4, IS-5, IS-6) bacterial species were obtained and screened for their biosurfactant-producing potentials.

Table 1. Biosurfactant Producing Potentials of the Isolates

Assay	IS-1	IS-2	IS-3	IS-4	IS-5	IS-6
Hemolytic activity	+	+	+	+	+	+
Emulsification capacity (mm)	8.2	10.2	15.3	10.3	11.2	9.2
Drop collapse	+	+	+	+	+	+
Oil Spread	+	+	+	+	+	+

Key: + = Positive, - = Negative, mm – Millimeter

Screening for the biosurfactant-producing potentials of the isolates revealed that all the isolates were able to produce hemolysin on blood agar. Their culture supernatant was also able to displace oil on a coated surface and become flat. The quantity of biosurfactant produced by the sample isolate was determined based on the

emulsification capacity of their culture supernatant. The result revealed that IS-3 elaborated the highest quantity of biosurfactant producers with an emulsification index of 15.3% compared to the other Pseudomonads. Characterization of IS-3 revealed its identity to be *Pseudomonas aeruginosa*, with 99% similarities to *Pseudomonas aeruginosa* ATCC 27853. This finding agrees with several studies that have reported the biosurfactant-producing potentials of various *Pseudomonas* species (Karlapudi et al., 2018; Patel et al., 2015). Rhamnolipids, a surface-active compound, belonging to the class of glycolipid biosurfactants have been reported to be produced by *P. chlororaphis* (Gunther et al., 2005), *P. putida* (Wittgens et al., 2011; Nanganuru and Korropati, 2012), *P. fluorescens* (Abouseoud et al., 2008; El-Amine Bendaha et al., 2012), *P. nitroreducens* (Onwosi & Odibo, 2012), and *P. alcaligenes* (Oliveira, 2009).

Crude oil pollution is a worldwide problem that leads to the uptake and accumulation of toxic chemicals/pollutants along the food chain and harm to the flora and fauna of the affected habitat as well as distortion of the ecological balance of the affected and interrelated ecosystem. Microorganisms are known to be the most effective tools for naturally degrading crude oil after a spillage episode. Bioremediation is a process in which microorganisms are utilized to convert dangerous organic pollutants from crude oil into environmentally friendly compounds such as CO_2 and H_2O (Ron & Rosenberg, 2014; Varjani & Gnansounou, 2017).

Analysis of the effect of the bioaugmentation with a culture of BPB on the heterotrophic and hydrocarbonoclastic bacterial density in crude oil-contaminated soil was determined by measuring the changes in the density of heterotrophic and hydrocarbonoclastic bacteria in the contaminated and uncontaminated soil over a period of 12 weeks.

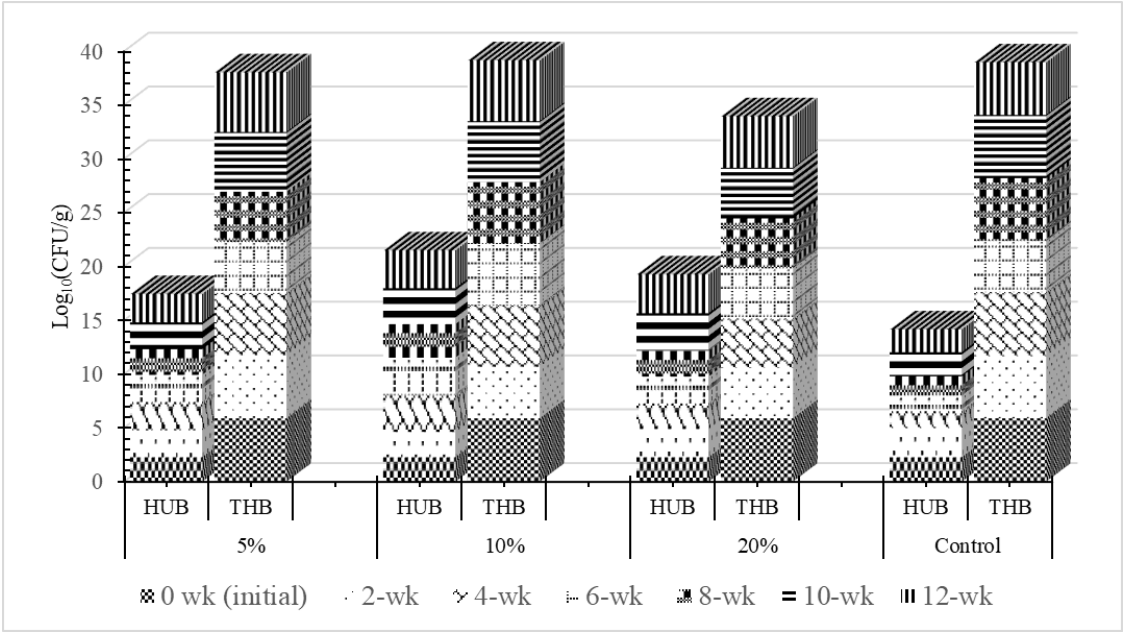

Figure 2. Populations of heterotrophic and hydrocarbon-utilizing bacteria in contaminated and uncontaminated soils not augmented with biosurfactant-producing strain of *Pseudomonas aeruginosa*. (Control Experiments)

Figure 2 shows that the un-contaminated soil was characterized by heterotrophic bacteria density of between 4.75 Log_{10}CFU/g and 5.94 Log_{10}CFU/g while the density of hydrocarbon utilizing bacteria range of 1.45 Log_{10}CFU/g to 2.71 Log_{10}CFU/g. Contamination of the soil samples with crude oil led to an increase in the density of hydrocarbon-utilizing bacteria in coastal soil. This was concentration-dependent and occurred mostly at the beginning of the process (1-14 days). On the other hand, densities of the indigenous population of heterotrophs in crude oil-polluted soil were retarded by immediate exposure and the effect was also concentration dependent. The relationship between the density of hydrocarbon degraders and heterotrophic bacteria (HUB/THBC ratio) in the crude oil-contaminated soil samples not augmented with the BPB throughout the 12-week study was below 0.1 (**Figure 3**) indicating a poor recovery potential of the soil.

On the other hand, a rapid increase in the density of hydrocarbon-degrading bacteria was observed in all the contamination levels for the experimental set-up bioaugmented with a culture of BPB (**Figure 4**). The density of the HUB in contamination levels 5%, 10% and 20% increased from 2.28 Log_{10}CFU/g to 4.54 Log_{10}CFU/g, 2.28 Log_{10}CFU/g to 4.28 Log_{10}CFU/g and 2.28 Log_{10}CFU/g to 4.48 Log_{10}CFU/g respectively. However, the density of heterotrophic bacteria was observed to decrease slightly from 5.90 Log_{10}CFU/g to 4.86 Log_{10}CFU/g, 5.90 Log_{10}CFU/g to 4.63 Log_{10}CFU/g and 5.90 Log_{10}CFU/g to 4.72 Log_{10}CFU/g for 5%, 10% and 20% contamination levels respectively (**Figure 4**).

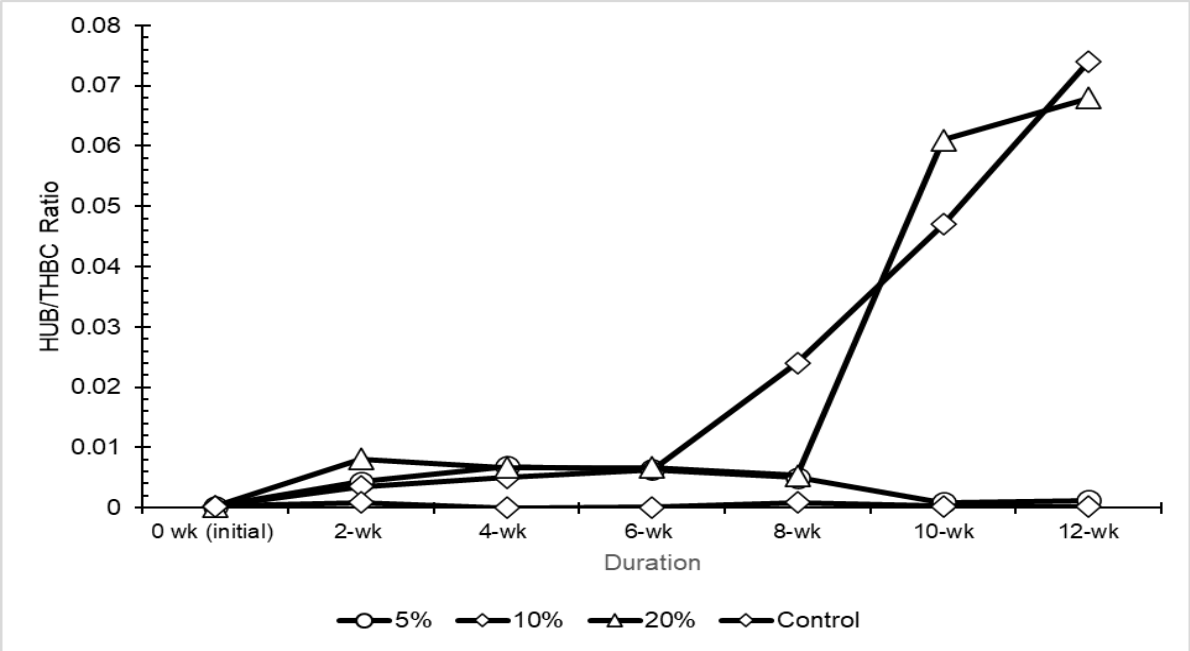

Figure 3. HUB/THBC Ratio of the Crude oil contaminated soil not bioaugmented with BPB

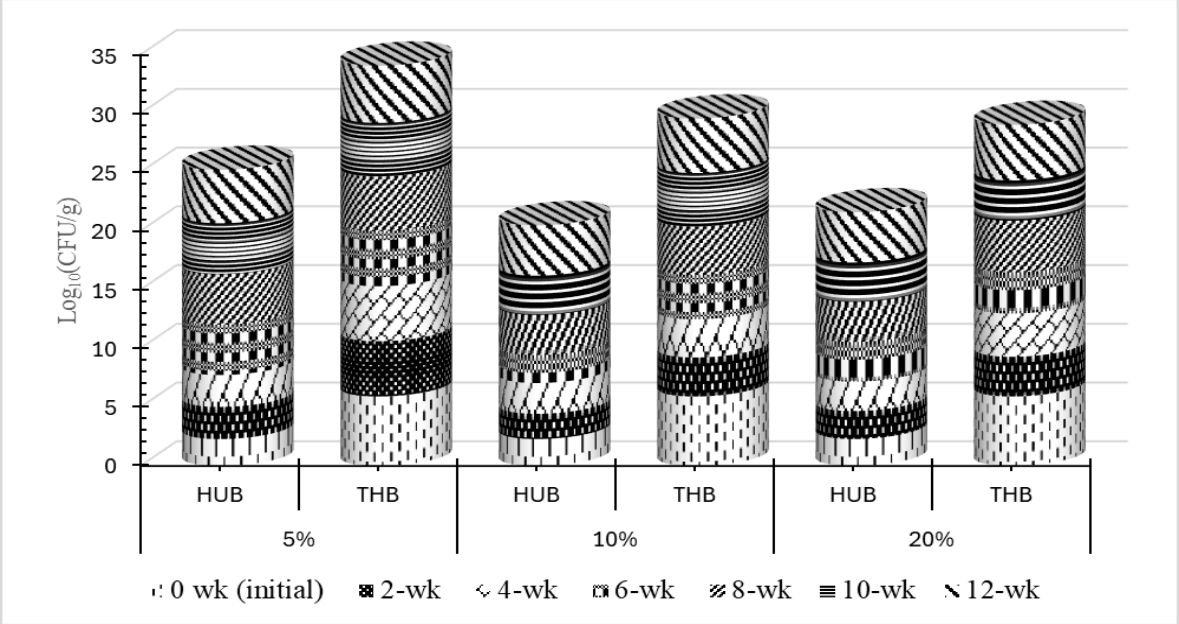

Figure 4. Level of bacterial heterotrophism (Growth) in crude oil contaminated soil augmented with 15 ml (2.6 x 10^3 CFU/ml) broth culture of biosurfactant-producing strain of *P. aeruginosa*.

The potentials for the increased density of HUB in the bioaugmented soil samples to lead to remediation/natural attenuation of the crude oil in the soil samples were also measured by the relationship between the density of hydrocarbon degraders and heterotrophic bacteria (HUB/THBC ratio) throughout the 12 weeks study (**Figure 5**). The findings revealed a gradual increase in the HUB/THBC ratio from 0.39 to a peak of 0.95 in week 10 for the 5% contamination level, 0.92 for 10 % in week 12, and 0.97 in week 10 for 20 % contamination level.

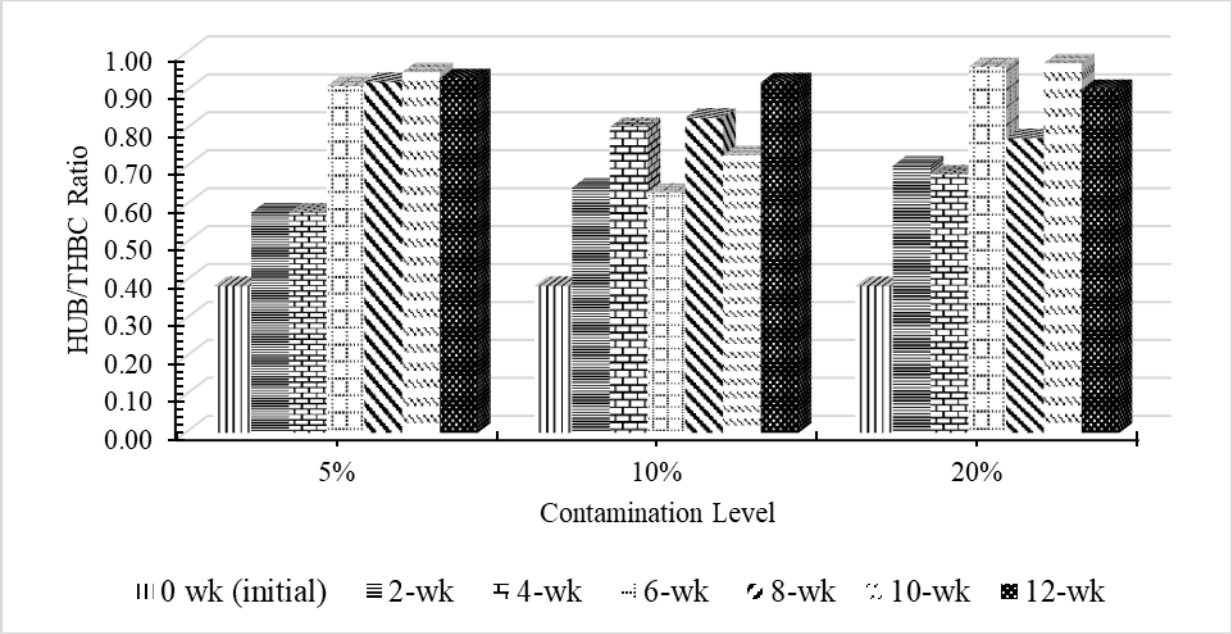

Figure 5. HUB/THBC Ratio of the Crude oil contaminated soil bioaugmented with BPB

The enhanced heterotrophic effects of augmentation with biosurfactant-producing *Pseudomonas aeruginosa* reduced the HUB/THB ratios of the amended soils and generally improved the hydrocarbon attenuating level of the impacted soils. Species of *Pseudomonas* have been reported to possess the ability to decrease oil apart from being isolated from hydrocarbon-polluted sites. *Pseudomonas sp* have been revealed to exhibit a considerable numerical increase in hydrocarbon sites and act as a petroleum hydrocarbon degrader. The degradation of petroleum hydrocarbon by microorganisms is majorly due to the catalyses of intracellular enzymes which is posed by Pseudomonas (Pereira et al., 2013; Xiaokang, et al., 2019).

The present study has shown that the simulation of coastal soil with Bonny Light crude oil raised the total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbons (PAHs) levels of the soil. Hydrocarbon elevation in contaminated soil varied with the level of contamination. More precisely, the TPH level of the coastal soil was raised from 4.14 mg/kg to 20.34 mg/kg, 24.23 mg/kg and 32.85 mg/kg in soils loaded with 5%, 10% and 20% of crude oil respectively (**Table 2**). Similarly, the PAH level was elevated from 0.89 mg/kg to 5.58 mg/kg, 9.92 mg/kg and 16.34 mg/kg respectively (**Table 3**).

Table 2. Concentrations of Residual Total Petroleum Hydrocarbon (TPH) (mg/kg) in coastal soil augmented BPB

Parameter	Control	0-week			6-weeks			12 – weeks		
		5% cont.	10% cont.	20% cont.	5% cont.	10% cont.	20% cont.	5% cont.	10% cont.	20% cont.
C_8-C_{11}	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
C_{12}	BDL	BDL	BDL	2.86	BDL	BDL	1.04	BDL	BDL	1.78
C_{13}	0.12	BDL	1.12	1.12	BDL	1.45	1.76	BDL	1.07	1.04
C_{14}	0.05	BDL	0.86	1.65	BDL	BDL	2.00	BDL	1.18	1.60

Table 2. (Cont'd)

C_{15}	BDL	BDL	0.72	2.23	BDL	1.43	1.98	BDL	0.67	1.31
C_{16}	BDL	BDL	0.25	1.56	BDL	BDL	1.60	BDL	BDL	1.03
C_{17}	BDL	0.86	0.36	2.21	1.21	1.63	0.43	0.77	0.86	0.76
C_{18}	0.01	0.57	0.12	1.56	1.11	1.32	1.25	1.24	1.06	0.58
C_{19}	BDL	3.12	4.03	4.03	1.46	1.35	1.33	1.00	0.99	0.69
C_{20}	0.28	1.26	1.10	1.90	BDL	0.35	1.05	BDL	0.57	0.95
C_{21}	0.31	1.13	1.11	0.31	1.11	1.23	1.41	BDL	1.01	1.06
C_{22}	0.05	0.06	BDL	1.34	BDL	BDL	1.46	BDL	0.35	1.00
C_{23}	1.10	1.56	1.12	1.10	0.56	0.96	1.06	0.20	0.79	0.80
C_{24}	0.34	1.45	1.25	2.34	BDL	1.02	1.34	BDL	0.23	0.71
C_{25}	BDL	1.99	0.14	BDL	1.09	1.23	0.36	0.92	0.09	BDL
C_{26}	0.51	0.26	1.02	1.51	1.26	1.35	0.43	0.39	BDL	0.07
C_{27}	0.07	2.01	1.20	1.69	1.01	1.23	0.09	BDL	0.87	BDL
C_{28}	0.59	1.03	1.07	2.59	1.02	1.12	BDL	1.28	0.62	BDL
C_{29}	0.36	1.07	1.03	1.37	1.08	1.09	1.30	0.08	0.48	0.83
C_{30}	0.20	0.99	0.85	1.30	0.31	0.20	BDL	BDL	0.30	BDL
C_{31}	0.10	0.62	2.24	0.10	BDL	0.12	BDL	BDL	BDL	BDL
C_{32}	BDL	0.52	BDL	BDL	0.32	0.89	BDL	0.40	0.07	BDL
C_{33}	BDL	0.46	1.14	BDL	0.05	0.57	0.34	BDL	0.05	BDL
C_{34}	BDL	0.51	2.44	BDL	BDL	BDL	BDL	BDL	BDL	0.08
C_{35}	BDL	0.41	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
C_{36}	BDL	0.32	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
C_{37}	BDL	BDL	BDL	BDL	BDL	1.34	1.02	BDL	BDL	BDL
C_{38}	BDL	0.15	BDL	BDL	BDL	BDL	BDL	0.98	0.78	0.84
C_{39} - C_{40}	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Total	**4.14**	**20.34**	**24.23**	**32.85**	**11.58**	**19.98**	**21.25**	**6.31**	**12.05**	**15.14**

BDL=below detection limit

The fate of the hydrocarbon contaminants in coastal soil augmented the BPB – *P. aeruginosa* have shown that the degradation of TPH and PAHs were faster in soils augmented with *P. aeruginosa*. The enhanced remediation rate also varied with the level of contamination and duration of the remediation course. At the end of the 12-week degradation course, the augmentation process induced a reduction in the Total petroleum hydrocarbon (TPH) content of crude oil in soil exposed to 5% contamination from 20.34 mg/kg to 6.3130 mg/kg. For 10% and 20% levels of contamination, the TPH levels were reduced from 24.2305 mg/kg and 32.8546 mg/kg to 12.0463 mg/kg and 15.1462 mg/kg respectively. PAH levels in 5%, 10% and 20% contaminated soil were also reduced from 5.5789 mg/kg, 9.9159 mg/kg and 16.3427 mg/kg to 1.8000 mg/kg, 3.0687 mg/kg and 5.3501 mg/kg respectively, after the 12-week remediation course.

A relationship exists between the hydrocarbons' biodegradation percentage and the contaminant concentration, and this is consistent with Boldu-Prenafeta et al. (2004), Ambrazaitiene et al. (2013), Abioye et al. (2012) and Rahman et al. (2002). High concentrations of contaminants may be responsible for the decrease in biodegradation percentage since high concentrations can be inhibitory of microorganisms by toxic effects (Abioye et al., 2012; Rahman et al., 2002; Ijah & Antai, 2003; Ławniczak et al., 2020). Thus, it has been reported that bioremediation is a useful method of soil remediation if contaminant concentrations are moderate. Also, the results show there are differences between the biodegradation percent of hydrocarbons and these results agreed with (Ambrazaitiene et al., 2013; Boldu-Prenafeta et al., 2004) who concluded the rate of biodegradation depends on the microbial population, the type, structure, and level of contamination. This result is consistent with Wang et al. (1998), Li et al. (2020) and Rahmati et al. (2022) who observed that the biodegradation rate of n-alkane is inversely proportional to chain length and branched alkanes have less ability to biodegradation.

Table 3. Levels of Residual PAH Suites (mg/kg) in coastal soil during 0 to 12-week biodegradation period

Parameter	Control	0-week			6-weeks			12 - weeks		
		5% cont.	10% cont.	20% cont.	5% cont.	10% cont.	20% cont.	5% cont.	10% cont.	20% cont.
Naphthalene	BDL	0.36	1.37	1.70	0.27	0.07	0.50	0.05	0.02	0.08
2-methylnapthalene	BDL	0.24	0.34	1.08	0.23	1.94	0.48	0.02	0.08	0.05
Acenapthene	BDL	0.25	0.66	0.99	0.33	0.99	2.76	0.68	0.04	0.36
Acenapthylene	BDL	0.18	0.54	0.96	0.58	0.23	0.74	0.03	0.50	0.84
Fluorene	BDL	0.44	0.57	0.89	0.44	0.57	1.31	0.20	0.02	0.05
Phenanthrene	BDL	0.21	1.12	1.39	0.21	0.29	0.98	0.16	0.40	0.67
Anthracene	BDL	0.59	1.00	0.85	1.17	1.12	0.10	0.04	0.81	0.57
Fluoranthene	BDL	0.32	0.40	0.95	0.13	0.06	0.10	0.07	0.61	1.32
Pyrene	BDL	0.40	0.51	0.87	0.13	0.52	0.08	0.09	0.04	0.22
Benz(a)anthracene	BDL	0.14	0.64	0.86	0.14	0.05	0.12	0.05	0.03	0.13
Benzo(b)fluoranthene	0.11	1.01	0.03	0.64	0.01	0.09	0.04	0.07	0.11	0.07
Chrysene	0.09	0.06	0.24	0.97	0.16	0.04	0.10	0.04	0.05	0.07
Benzo(k)fluoranthene	BDL	0.24	0.47	0.70	0.06	0.05	0.12	0.06	0.05	0.09
Benzo(a)pyrene	0.01	0.35	0.48	0.86	0.34	0.09	0.09	0.08	0.06	0.06
Dibenz(a,h)anthracene	0.30	0.28	0.68	0.80	1.22	0.04	0.04	0.03	0.10	0.05
Benzo(g,h,i)perylene	0.23	0.17	0.31	0.91	0.17	0.16	0.16	0.07	0.10	0.11
Indeno(1,2,3-cd) pyrene	0.12	0.34	0.57	0.95	0.34	0.08	0.07	0.06	0.06	0.01
Total	**0.90**	**5.58**	**9.92**	**16.34**	**5.98**	**6.40**	**8.72**	**1.80**	**3.07**	**5.35**

BDL=below detection limit; cont=contamination

4. Conclusion

The study revealed that the isolate of indigenous *Pseudomonas aeruginosa* was able to produce biosurfactant that would not only affect the heterotrophic activity in crude oil-contaminated coastal soil but also positively influence the density of hydrocarbon degraders in the contaminated soil. Bioaugmentation of the crude oil-contaminated soil with a culture of *P. aeruginosa* was able to increase the natural attenuation potential of the contaminated soil.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abellan-Schneyder, I., Matchado, M. S., Reitmeier, S., Sommer, A., Sewald, Z., Baumbach, J., List, M. & Neuhaus, K. (2021). Primer, pipelines, parameters: issues in 16S rRNA gene sequencing. *Msphere*, 6(1), 10-1128. https://doi.org/10.1128/msphere.01202-20

Abioye, O. P., Agamuthu, P., & Abdul Aziz, A. R. (2012). Biodegradation of used motor oil in soil using organic waste amendments. *Biotechnology Research International*, 2012(1), 587041. https://doi.org/10.1155/2012/587041

Abouseoud, M., Maachi, R., Amrane, A., Boudergua, S., & Nabi, A. (2008). Evaluation of different carbon and nitrogen sources in production of biosurfactant by Pseudomonas fluorescens. *Desalination*, 223(1-3), 143-151. https://doi.org/10.1016/j.desal.2007.01.198

Abraham, N. A. & Essien, J. P. (2016). *Enhanced Bioremediation.* Germany: LAP Lambert Academic Publishing, pp. 1 – 133.

Abraham, N. A., Odokuma, L. O., & Okpokwasili, G. C. (2021). Oily sludge degrading potentials of single and consortium of autochthonous bacterial species. *GSC Advanced Research and Reviews*, 8(3), 093-101. https://doi.org/10.30574/gscarr.2021.8.3.0192

Ambrazaitienė, D., Žukauskaitė, A., Jakubauskaitė, V., Reikaitė, R., Zubrickaitė, M., & Karčauskienė, D. (2013). Biodegradation activity in the soil contaminated with oil products. *Žemdirbystė*, 100(3), 235-242. http://dx.doi.org/10.13080/z-a.2013.100.030

Aruotu, J. O., Chikere, C. B., Okafor, C. P., & Edamkue, I. (2023). Microbial consortium for polycyclic aromatic hydrocarbons degradation from petroleum hydrocarbon polluted soils in rivers state, Nigeria. *Applied Sciences*, *13*(16), 9335.https://doi.org/10.3390/app13169335

Dey, S., Das, A., Mallick, K., Sahu, A., & Das, A. P. (2024). Environmental Petroleum Waste: Pollution, Toxicity, Sustainable Remediation. In: Behera, I.D., Das, A.P. (eds). *Impact of Petroleum Waste on Environmental Pollution and its Sustainable Management Through Circular Economy*. Cham: Springer Nature Switzerland, pp. 159-175. http://dx.doi.org/10.1007/978-3-031-48220-5_7

Domínguez-Rodríguez, V. I., Adams, R. H., Vargas-Almeida, M., Zavala-Cruz, J., & Romero-Frasca, E. (2020). Fertility deterioration in a remediated petroleum-contaminated soil. *International Journal of Environmental Research and Public Health*, 17(2), 382. http://dx.doi.org/10.3390/ijerph17020382

Ekong, F. U. (2017). GIS based analysis of shoreline change in Ibeno, Akwa Ibom State, Nigeria. *Journal of Environmental Protection*, 8(5), 637-649. https://doi.org/10.4236/jep.2017.85041

EL-Amine Bendaha, M., Mebrek, S., Naimi, M., Tifrit, A., & Belaouni, H. A. (2012). Isolation and Comparison of Rhamnolipids Production in. *Pseudomonas aeruginosa*, 2. http://dx.doi.org/10.4172/scientificreports.544

Fenibo, E. O., Ijoma, G. N., Selvarajan, R., & Chikere, C. B. (2019). Microbial surfactants: the next generation multifunctional biomolecules for applications in the petroleum industry and its associated environmental remediation. *Microorganisms*, 7(11), 581. https://doi.org/10.3390/microorganisms7110581

Gunther IV, N. W., Nunez, A., Fett, W., & Solaiman, D. K. (2005). Production of rhamnolipids by Pseudomonas chlororaphis, a nonpathogenic bacterium. *Applied and Environmental Microbiology*, 71(5), 2288-2293. https://doi.org/10.1128/aem.71.5.2288-2293.2005

Ijah, U. J. J., & Antai, S. P. (2003). The potential use of chicken-drop micro-organisms for oil spill remediation. *Environmentalist*, 23, 89-95. https://doi.org/10.1023/A:1022947727324

Inam, E., Offiong, N. A., Essien, J., Kang, S., Kang, S. Y., & Antia, B. (2016). Polycyclic aromatic hydrocarbons loads and potential risks in freshwater ecosystem of the Ikpa River Basin, Niger Delta—Nigeria. *Environmental Monitoring and Assessment*, 188, 1-16.

Jayasena, S., & Perera, M. (2021). Microbial Bioremediation of petroleum hydrocarbons. *Microbial Rejuvenation of Polluted Environment*, 1, 263-291. http://dx.doi.org/10.1007/978-981-15-7447-4_11

Karlapudi, A. P., Venkateswarulu, T. C., Tammineedi, J., Kanumuri, L., Ravuru, B. K., Ramu Dirisala, V., & Kodali, V. P. (2018). Role of biosurfactants in bioremediation of oil pollution-a review. *Petroleum*, 4(3), 241-249.http://dx.doi.org/10.1016/j.petlm.2018.03.007

Kumar, V., Kothiyal, N. C., Saruchi, Vikas, P., & Sharma, R. (2016). Sources, distribution, and health effect of carcinogenic polycyclic aromatic hydrocarbons (PAHs)–current knowledge and future directions. J*ournal of the Chinese Advanced Materials Society*, 4(4), 302-321. http://dx.doi.org/10.1080/22243682.2016.1230475

Kuppusamy, S., Maddela, N. R., Megharaj, M., & Venkateswarlu, K. (2020). An Overview of Total Petroleum Hydrocarbons. In: *Total Petroleum Hydrocarbons: Environmental Fate Toxicity and Remediation*. Switzerland: Springer Nature, pp. 1-27. http://dx.doi.org/10.1007/978-3-030-24035-6

Ławniczak, Ł., Woźniak-Karczewska, M., Loibner, A. P., Heipieper, H. J., & Chrzanowski, Ł. (2020). Microbial degradation of hydrocarbons—basic principles for bioremediation: a review. *Molecules*, 25(4), 856. https://doi.org/10.3390/molecules25040856

Li, H., Lai, R., Jin, Y., Fang, X., Cui, K., Sun, S., Gong, Y., Li, H., Zhang, Z., Zhang, G. and Zhang, Z. (2020). Directional culture of petroleum hydrocarbon degrading bacteria for enhancing crude oil recovery. *Journal of Hazardous Materials*, 390, 122160. https://doi.org/10.1016/j.jhazmat.2020.122160

Liu, H., Yao, J., Yuan, Z., Shang, Y., Chen, H., Wang, F., ... & Choi, M. M. (2014). Isolation and characterization of crude-oil-degrading bacteria from oil-water mixture in Dagang oilfield, China. International Biodeterioration & Biodegradation, 87, 52-59. https://doi.org/10.1016/j.ibiod.2013.11.005

Nanganuru, H. Y, Korrapati N. (2012). Studies on the production of rhamnolipids by *Pseudomonas putida. International Journal of Research in Computer Science*, 2(4): 19-21. http://dx.doi.org/10.7815/ijorcs.24.2012.035

Obi, C. C. (2017). Bacterial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs) in Polluted Estuarine Sediments of the Lagos Lagoon, Nigeria (Doctoral dissertation, University of Lagos (Nigeria). https://ir.unilag.edu.ng:8080/xmlui/handle/123456789/3268

Oboh, B. O., Ilori, M. O., Akinyemi, J. O., & Adebusoye, S. A. (2006). Hydrocarbon degrading potentials of bacteria isolated from a Nigerian bitumen (Tarsand) deposit. *Nature and Science*, 4(3), 51-57. https://www.sciepub.com/reference/38588

Offiong, N. A. O., Inam, E. J., Etuk, H. S., Essien, J. P., Ofon, U. A., & Una, C. C. (2020). Biochar and humus sediment mixture attenuates crude oil-derived PAHs in a simulated tropical ultisol. *SN Applied Sciences*, 2, 1-14. https://doi.org/10.1007/s42452-020-03744-5

Offiong, N. A. O., Inam, E. J., Fatunla, O. K., Ofon, U. A., Abraham, N. A., & Essien, J. P. (2022). Metagenomic signature and total petroleum hydrocarbons distribution in a crude oil contaminated ultisol remediated with biochar–humus sediment slurry. *Remediation Journal*, 32(4), 299-308. https://doi.org/10.1002/rem.21734

Okafor, C. P., Udemang, N. L., Chikere, C. B., Akaranta, O., & Ntushelo, K. (2021). Indigenous microbial strains as bioresource for remediation of chronically polluted Niger Delta soils. *Scientific African*, 11, e00682. https://doi.org/10.1016/j.sciaf.2020.e00682

Okpokwasili, G. C., & Amanchukwu, S. C. (1988). Petroleum hydrocarbon degradation by Candida species. *Environment International*, 14(3), 243-247. https://doi.org/10.1016/0160-4120%2888%2990145-6

Oliveira, F. J. S., Vazquez, L., De Campos, N. P., & De Franca, F. P. (2009). Production of rhamnolipids by a Pseudomonas alcaligenes strain. *Process Biochemistry*, 44(4), 383-389. http://dx.doi.org/10.1016/j.procbio.2008.11.014

Onwosi, C. O., & Odibo, F. J. C. (2012). Effects of carbon and nitrogen sources on rhamnolipid biosurfactant production by Pseudomonas nitroreducens isolated from soil. *World Journal of Microbiology and Biotechnology*, 28, 937-942. https://doi.org/10.1007/s11274-011-0891-3

Patel, J., Borgohain, S., Kumar, M., Rangarajan, V., Somasundaran, P., Sen, R. (2015). Recent developments in microbial enhanced oil recovery. *Renewable and Sustainable Energy Reviews*, 52, 1539-1558. https://doi.org/10.1016/j.rser.2015.07.135

Patowary, R., Patowary, K., Kalita, M. C., & Deka, S. (2018). Application of biosurfactant for enhancement of bioremediation process of crude oil contaminated soil. *International Biodeterioration & Biodegradation*, 129, 50-60. http://dx.doi.org/10.1016/j.ibiod.2018.01.004

Pereira, J. F., Gudiña, E. J., Costa, R., Vitorino, R., Teixeira, J. A., Coutinho, J. A., & Rodrigues, L. R. (2013). Optimization and characterization of biosurfactant production by *Bacillus subtilis* isolates towards microbial enhanced oil recovery applications. *Fuel*, 111, 259-268. https://doi.org/10.1016/j.fuel.2013.04.040

Plaza, G. A., Zjawiony, I., & Banat, I. M. (2006). Use of different methods for detection of thermophilic biosurfactant-producing bacteria from hydrocarbon-contaminated and bioremediated soils. *Journal of Petroleum Science and Engineering*, 50(1), 71-77. http://dx.doi.org/10.1016/j.petrol.2005.10.005

Prashanthi, R., Shreevatsa, G. K., Krupalini, S., & Manoj, L. (2021). Isolation, characterization, and molecular identification of soil bacteria showing antibacterial activity against human pathogenic bacteria. Journal of Genetic Engineering and Biotechnology, 19(1), 120. https://doi.org/10.1186/s43141-021-00219-x

Prenafeta-Boldú, F. X., Ballerstedt, H., Gerritse, J., & Grotenhuis, J. T. C. (2004). Bioremediation of BTEX hydrocarbons: effect of soil inoculation with the toluene-growing fungus *Cladophialophora* sp. strain T1. *Biodegradation,* 15(1), 59–65. https://doi.org/10.1023/B%3ABIOD.0000009973.53531.96

Rahman, K. S. M., Rahman, T. J., McClean, S., Marchant, R., & Banat, I. M. (2002). Rhamnolipid biosurfactant production by strains of *Pseudomonas aeruginosa* using low-cost raw materials. *Biotechnology Progress*, 18(6), 1277-1281. https://doi.org/10.1021/bp020071x

Rahmati, F., Asgari Lajayer, B., Shadfar, N., van Bodegom, P. M., & van Hullebusch, E. D. (2022). A review on biotechnological approaches applied for marine hydrocarbon spills remediation. *Microorganisms*, 10(7), 1289. https://doi.org/10.3390%2Fmicroorganisms10071289

Ron, E. Z., & Rosenberg, E. (2014). Enhanced bioremediation of oil spills in the sea. *Current Opinion in Biotechnology*, 27, 191-194. https://doi.org/10.1016/j.copbio.2014.02.004

Sadiqi, S., Hamza, M., Ali, F., Alam, S., Shakeela, Q., Ahmed, S., ... & Zaman, W. (2022). Molecular characterization of bacterial isolates from soil samples and evaluation of their antibacterial potential against MDRS. Molecules, 27(19), 6281. https://doi.org/10.3390/molecules27196281

Sambrook, J., & Russell, D. W. (2001). Molecular Cloning: A Laboratory Manual, The third edition.

Shahid, M., Khan, M. S., & Singh, U. B. (2023). Pesticide-tolerant microbial consortia: Potential candidates for remediation/clean-up of pesticide-contaminated agricultural soil. *Environmental Research*, 116724. http://dx.doi.org/10.1016/j.envres.2023.116724

Udofia, E. G., Abraham, N., Fatunla, O. K., Ntino, E. S., Obot, U. R., Akan, O. D. & Essien, J. P. (2021). Petroleum Hydrocarbon Degrading Potentials of Indigenous Bacterial Strains from "Blackwater" Ecosystem of Eniong River – Nigeria. *World Journal of Applied Science and Technology,* 13(1): 56 – 62. https://www.ajol.info/index.php/wojast/article/view/227430

Udoh, B. O. & Amadi, A. N. (2020). Evaluation of Heavy Metal Pollution Level in Soils and Plants around Ibeno Area, Akwa-Ibom State, Niger Delta, Nigeria. http://repository.futminna.edu.ng:8080/jspui/handle/123456789/5803

Vane, C. H., dos Santos, R. A. L., Kim, A. W., Moss-Hayes, V., Fordyce, F. M., & Bearcock, J. M. (2017). Persistent organic pollutants (PAH, PCB, TPH) in freshwater, urban tributary and estuarine surface sediments of the River Clyde, Scotland, UK. *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*, 108(2-3), 299-313. http://dx.doi.org/10.1017/S1755691018000294

Varjani, S. J., & Gnansounou, E. (2017). Microbial dynamics in petroleum oilfields and their relationship with physiological properties of petroleum oil reservoirs. *Bioresource Technology*, 245, 1258-1265. https://doi.org/10.1016/j.biortech.2017.08.028

Wang, Z., Fingas, M., Blenkinsopp, S., Sergy, G., Landriault, M., Sigouin, L., Foght, J., Semple, K. & Westlake, D. W. S. (1998). Comparison of oil composition changes due to biodegradation and physical weathering in different oils. *Journal of Chromatography A*, 809,(1-2):89-107. https://doi.org/10.1016/S0021-9673(98)00166-6

Wittgens, A., Tiso, T., Arndt, T.T., Wenk, P., Hemmerich, J. & Müller, C. (2011). Growth-independent rhamnolipid production from glucose using the non-pathogenic *Pseudomonas putida* KT2440. *Microbial Cell Factories*, 10: 80. https://doi.org/10.1186/1475-2859-10-80

Xiaokang, L., Li, H. & Qu, C. (2019). A review of the mechanism of microbial degradation of petroleum pollution. *IOP Conf. Series: Materials Science and Engineering* 484, 012060. http://dx.doi.org/10.1088/1757-899X/484/1/012060