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Biodegradation of Premium Motor Spirit using surfactant-expressing bacteria from mechanic workshops in Malumfashi, Katsina State, Nigeria

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Abstract

Biosurfactant-expressing bacteria have been shown to have potential in many biotechnological applications including the biodegradation of petroleum fractions, such as premium motor spirit (PMS). This study was aimed at investigating the potential use of biosurfactant-expressing bacterial isolates in the biodegradation of premium motor spirit (PMS) at various concentrations (100-100,000ppm). The biosurfactant-expressing bacteria were isolated from mechanic workshop in Malumfashi, Katsina, Nigeria using standard techniques. The isolates identified belonged to the genera Acinetobacter, Bacillus, Micrococcus, Pseudomonas and Stenotrophomonas. These isolates were screened for biosurfactant expression using drop collapse, haemolysis, oil-water behavior assays and emulsification index test. Positives isolates were investigated for PMS degradation by growing the isolates on mineral salt media supplemented with (0.1ml) premium motor spirit (PMS) as sole source of carbon. Although, higher total hydrocarbon degrading bacterial counts were obtained from soils where isolates positive for biosurfactant expression are predominant, there was no statistically significant difference between isolate source using Kruskal-Wallis H test (p = 0.67). The isolates Bacillus velezensis and Stenotrophomonas maltophilia were positive for biosurfactant-production potential using drop-collapse, ß-haemolysis, oil spreading, and emulsification index and drop collapse tests with higher tolerance to PMS at concentrations up to 100,000 ppm. Statistical analysis using multiple-comparison analysis of variance (ANOVA) confirmed that the isolates exhibited varying PMS degradation response (p = 0.0066); furthermore, the tolerance of the bacteria to the PMS is dose-dependent (p = 0.00012). Post-hoc analysis using Tukey's test identified Bacillus velezensis as the most efficient biosurfactant-producing and hydrocarbon degrading isolate (p = 0.0264 and 0.0034); moreover, the threshold concentration for high PMS tolerance was found to be 1000ppm and above (p = 0.0174, 0.0008 and 0.0001). These isolates' ability to grow on mineral salt media supplemented with PMS as a sole source of carbon presents a veritable avenue for exploitation in biotechnology, towards biosurfactants-mediated bioremediation of hydrocarbon pollutants in oil contaminated soils.

Keywords: ***Biodegradation, Petroleum Hydrocarbons, Premium Motor Spirit***

INTRODUCTION

As population expansion significantly increase, the demand for crude oil products and their environmental pollution effects are experienced (Mahuta *et al.*, 2021). Such effects are more prevalent in places where crude oil products (petrol, diesel, engine oil and kerosene) are frequently used; such as mechanical workshops, which serve as hubs of petroleum-related pollution, including the spillage of such crude oil products, together with used and fresh engine oils, lubricants (Aboaba *et al.*, 2007).

The spilled wastes contain hazardous chemicals, such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals, which pose various threats to both humans, plants, animals and the environment. These chemicals alter microbial composition, destabilise biodiversity and can result to long-term toxicity effects as in the case of bioaccumulation and biomagnifications (Ron & Rosenberg 2002; Mahuta *et al.*, 2021; Umar *et al.*, 2020a).

It is therefore necessary to devise means of addressing the toxic effects of such contaminations in the area under study. This endeavor is often carried out using physico-chemical methods, such as dispersion, acid treatment, separation; solidification and stabilization, but these strategies are associated with inherent drawbacks, such as high cost, low efficiency, and exertion of adverse impacts on the surrounding environment (Umar *et al.*, 2020b).

Bioremediation is an alternative approach that involves the extensive use of naturally occurring microorganisms to transform and mineralize environmental pollutants, including petroleum products, such as complex mixtures of saturated and unsaturated polyaromatic hydrocarbon compounds; linear and branched alkanes (Zhang *et al.*, 2012).

According to Unimke *et al.* (2017), the efficiency of the process of microbial remediation of petroleum hydrocarbons is affected by certain hindrances including hydrophobic nature of petroleum hydrocarbons. This can slow down or even prevent the uptake and subsequent degradation of the petroleum hydrocarbons to utilizable products by microorganisms (Adams *et al.*, 2015). Therefore, improvements of the availability of the hydrophobic substrates through the use of surfactants, which can solubilize the hydrocarbons, are among the strategies employed to curtail the problem (Rocha *et al.*, 2011; Pekdemir *et al.*, 2005).Surfactants can be chemical or biological in nature. Biosurfactants are surface-active amphipathic compounds mainly produced by aerobically growing microbes (Zhang *et al.*, 2010).Surfactants produced by microbes are considered more effective than synthetic surfactants due to their low toxicity, biodegradability, high specificity, emulsification, pH- and temperature-tolerance as well as their ability to reduce interfacial tension between oil and water phases (Zhang and Xiang 2010; Ainon *et al.*, 2013; Sari *et al.*, 2019).

Many oil-degrading bacteria found in oil contaminated sites produce extracellular biosurfactants which help in enhancing the uptake of oil (Morikawa *et al.*, 2000; Ainon *et al.*, 2013).These include species belonging to the *Bacillus, Pseudomonas, Acinetobacter, Achromobacter, Arthrobacter, Brevibacterium* and *Corynebacterium* genera, as well as fungal species of *Candida* and *Rhodotorula* genera (Abalos *et al* 2004; Hua and Wang 2012).

Li *et al.* (2019) proposed a four-stepped model to explain how biosurfactants affect the effectiveness of microbial degradation of hydrocarbons. Firstly, surfactants mediate the emulsification of crude oil products; secondly, the microbe involved in the degradation carries out adsorption of the emulsified hydrocarbon to its cellular surface; thirdly, the microbe takes the emulsified crude oil product intracellularly via endocytosis, active or passive transport; and finally, enzyme-catalyzed degradation of the crude oil product occurs.

Therefore, one of the current areas of focus in effective microbial remediation is the identification of surfactant-expressing microbial strains that efficiently degrade petroleum hydrocarbons (Tanzadeh *et al.*, 2020). To this end, study was carried out to isolate biosurfactant-expressing bacterial isolates from mechanic workshop soil and investigate their potential for use in the biodegradation of premium motor spirit (PMS).

MATERIALS AND METHODS

Sampling

Soil samples were collected aseptically using an auger that was sterilized with 75% alcohol prior to use at the depths of 0-10 cm from Bakin Kasuwa, Bakin Stadium and Unguwar Sodangi automobile workshops. Control soil sample was collected from non- oil contaminated site 100 meters away from the mechanic workshops using the same procedure (Adebajo *et al.*, 2018; Adeyemo and Aliu, 2021). The samples were labeled and transported aseptically in the sterile polythene bags to the Microbiology Laboratory, Department of Microbiology, Umaru Musa Yar'adua University, Katsina, for further analyses (Saadoun *et al.*, 2008).

Bacteria isolation

One gram (1g) of each soil sample was measured and homogenized in 9ml of sterile distilled water by shaking at 180 rpm for 24 hours on an orbital shaker. The homogenized soil was used as diluent for bacteria isolation (Kabir2017), and serially dilutedto10^{-4}. The diluent was used to isolate bacteria using pour plate technique.

The colonies obtained were observed morphologically, counted and expressed a scolony forming unit per gram (CFU/g) of soil. Pure cultures of morphologically distinct colonies were prepared by sub culturing on nutrient agar, and used for identification and subsequent screening assays (Kabir, 2019).

From each sampling location, pure cultures of dominant bacterial species were obtained, and these were characterized on the basis of colonial morphology, gram's reaction and biochemical features, before comparison using Cowan and Steel and Bergy's Manuals for Identification of Bacteria (Barrowand Feltham, 2004; de Vos *et al.*, 2003; Darma *et al.*, 2019).

Screening of Biosurfactant expressing isolates.

Bacterial isolates were screened for biosurfactant expression using the rapid drop-collapse assay where a clean glass slide was coated with PMS and a drop of bacterial culture supernatant was put over the slide, a collapse of the drop indicates the presence of biosurfactant. The positive isolates were further subjected to haemolysis test by inoculating the bacterial culture on blood agar to confirm biosurfactant expression using protocols reported by Kabir (2017) and Kurniati *et al.* (2019).

Oil behavior assay

Biosurfactant expressing isolates were further subjected to different oil-water behavior assays including oil-spreading and emulsification assays. The oil spreading assay was carried out according to method described by Youssef *et al.* (2004). Ten ml of sterile distilled water was transferred into a plastic petri dish followed by addition of 15µl PMS to form a thin layer on the surface of water after which 10µl of supernatant from 24-hour old bacterial broth culture was then added to the oil surface. Presence of biosurfactant in the supernatant was indicated by the separation of the PMS, leading to the formation of a clear zone. The test was carried out using supernatants from non-biosurfactant expressing isolates as negative control, and no zone of displacement was observed.

Emulsification assays

Exactly 2ml of PMS were added to 2ml of the bacterial culture supernatant and the mixture vortexed at high speed for 2 mins in a test tube and left for 24 hrs. The height of the stable emulsion layer was measured and the emulsification index (EI_{24}) was calculated as the ratio of the height of the emulsified layer in millimeter and the total height of the liquid column in millimeter (Satpute *et al.*, 2010; Astuti *et al.*, 2019).

The following relationship was used to calculate the emulsification index:

$$EI_{24} = \frac{H_{emulsion}}{H_{total}} \times 100\%$$

Where:

EI_{24} is emulsification index after 24 hrs.

$H_{emulsion}$ is the height of emulsion layer.

H_{total} is the total height of the liquid (Kabir, 2017)

Biodegradation of PMS using Biosurfactant-expressing Bacteria

The medium used for the biodegradation assays was Mineral Salt Medium supplemented with varying amount of PMS (100; 1,000; 10,000 and 100,000 parts per million, PPM) as the sole carbon source, the media was prepared according to the protocol described by Kareem *et al.* (2017) and Mahuta *et al.* (2021).

RESULTS

Total Heterotrophic Bacterial Counts of the Soil Samples.

The results of the total heterotrophic bacterial counts from each location are presented in table 1. On average, the control soil sample had the highest heterotrophic bacterial count, followed by samples from Unguwar Sodangi, Bakin Stadium and Bakin Kasuwa mechanical garages in that order.

Table 1:Total heterotrophic bacterial counts of the soil samples collected from the various sampling areas

S/No	Sample Codes	Depth cm)	Location	THBC (× 10^5 CFU/g)
1	BK1	0-5	Bakin Kasuwa Mech. Garage	1.36 ± 0.2
2	BK2	5-10	Bakin Kasuwa Mech. Garage	1.80 ± 0.2
3	BS1	0-5	Bakin Stadium Mech. Garage	1.92 ± 0.2
4	BS2	5-10	Bakin Stadium Mech. Garage	1.52 ± 0.3
5	US1	0-5	Unguwar Sodangi Mech. Garage	3.08 ± 0.5
6	US2	5-10	Unguwar Sodangi Mech. Garage	1.68 ± 0.1
7	C1	0-5	Non-PMS contaminated soil (Control)	8.00 ± 2.0
8	C2	5-10	Non-PMS contaminated soil (Control)	2.00 ± 0.5

Legend: THBC = Total Heterotrophic Bacterial Count

Bacteria characterization

Pseudomonas species were isolated only from control soils (C1 and C2) while *Acinetobacter* spp., *Bacillus* spp., *Bacillus velezensis*, *Micrococcus* spp., *Stenotrophomonas* spp. and *Stenotrophomonas maltophilia* were isolated from either top or bottom soil samples obtained from the three mechanic workshops (US, BS and BK) (Table 2).

Table 2: Identification of dominant bacteria from the collected samples

S/No	Isolate ID	Colonial Morphology	Gram's stain		Biochemical characteristics							Probable Identity of the Bacterium
			Gram's Reaction	Cell morphology	Catalase	Oxidase	Urease	Citrate	Indole	Methyl Red	Motility	
1	US 1	Erose, circular, rough, shiny and creamy colonies	Gram +ve	Rods	+	+	+	+	-	+	+	*Bacillus velezensis*
2	US 2	Entire, circular, smooth, shiny, whitish colonies	Gram -ve	Rods	+	-	+	+	-	+	-	*Acinetobacter* spp.
3	BS 1	Erose, irregular, smooth, shiny, yellowish colonies	Gram -ve	Rods	+	-	-	+	-	+	+	*Stenotrophomonas* spp.
4	BS 2	Erose, circular, rough, shiny, yellowish colonies	Gram -ve	Rods	+	-	-	+	-	+	+	*Stenotrophomonas maltophilia*
5	BK 1	Entire, circular, smooth, shiny, creamy colonies	Gram +ve	Rods	+	+	+	+	-	+	-	*Bacillus* spp.
6	BK 2	Entire, irregular, rough, shiny, transparent colonies	Gram +ve	Cocci	+	+	-	+	-	+	-	*Micrococcus* spp.
7	C 1	Undulate, irregular, rough, shiny, greenish colonies	Gram -ve	Rods	+	+	+	+	-	+	+	*Pseudomonas* spp.
8	C2	Entire, circular, smooth, dull, greenish colonies	Gram -ve	Rods	+	+	-	+	-	+	+	*Pseudomonas* spp.

Legend: +ve = positive, -ve = negative, + = positive, - = negative. Colonial morphology results are presented in this order: margin, form, texture, appearance and then color, respectively.

Screening of the Identified Isolates

Results of isolates screening for biosurfactant expression is presented in table 3.*Pseudomonas spp*. isolated from control soil samples were negative for oil displacement and drop collapse tests, and positive for haemolysis (ß- haemolysis on blood agar after 24 hrs incubation). However, *Bacillus velezensis* and *Stenotrophomonas maltophilia* were found to be positive for drop collapse, haemolysis and oil displacement assays respectively.

Table 3:Screening of obtained isolates for biosurfactant expression

S/No	Samples	Bacteria Isolate	Drop Collapse	Haemolysis	Oil Displacement
1	US1	*Bacillus velezensis*	+	+	+
2	US2	*Acinetobacter* spp.	+	+	-
3	BK1	*Stenotrophomonas* spp.	NC	+	+
4	BK2	*Stenotrophomonas maltophilia*	+	+	+
5	BS1	*Bacillus* spp.	-	+	+
6	BS2	*Micrococcus* spp.	-	+	+
7	C1	*Pseudomonas* spp.	-	+	-
8	C2	*Pseudomonas* spp.	-	+	-

Legend: + Positive, - Negative, NC Not clear

The results showed that bacteria belonging to the *Bacillus, Acinetobacter, Micrococcus* genera recorded the same value for EI (39%) despite being isolated from different mechanic workshops while all the strains of *Stenotrophomonas maltophilia* have the same EI value of 40%. Meanwhile, *Bacillus velezensis* isolated from the top soil surface of Bakin Stadium mechanic workshop has the highest value of emulsification index (42%).

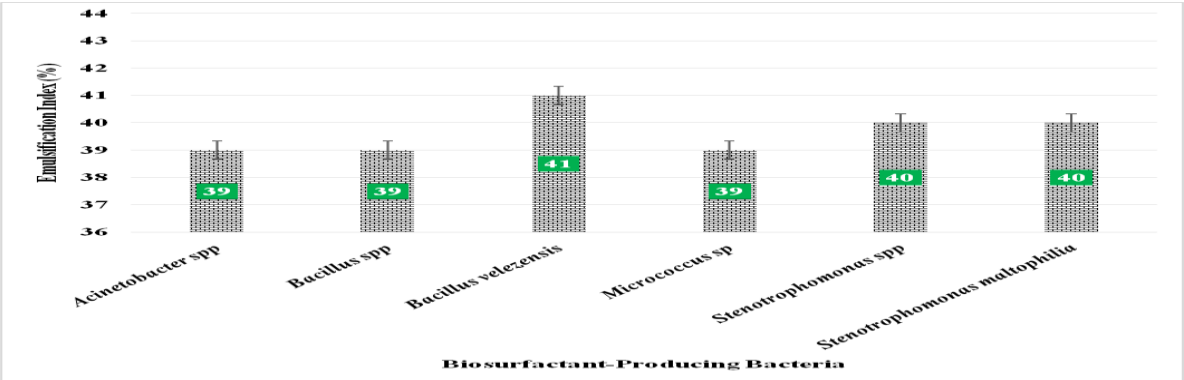

Figure 1: Emulsification index for biosurfactant expressing bacteria isolated from mechanic workshops.

Biodegradation Assays

Characterized surfactant expressing bacteria when subjected to biodegradation assay showed relatively higher THDBC in soil samples obtained at Bakin Stadium mechanic workshop, followed by Unguwar Sodangi mechanic workshops.The lowest THDBC were obtained from soil samples at Bakin Kasuwa. However, samples with the highest hydrocarbon degrading bacteria counts were found to be those in which either *Bacillus velezensis* or *Stenotrophomonas maltophilia* were the predominant isolates.

Table 4: Total Heterotrophic and Hydrocarbon Degrading Bacterial Counts.

S/No	Sample Code	Predominant Bacterial Isolate	Total Hydrocarbon Degrading Bacterial Count ($\times 10^5$ CFU/g)
1	BK1	*Bacillus velezensis*	5.30±1.0
2	BK2	*Acinetobacter* spp.	1.80±0.2
3	BS1	*Bacillus* spp.	2.30±0.2
4	BS2	*Micrococcus* spp.	1.46±0.3
5	US1	*Stenotrophomonas* spp.	1.84±0.1
6	US2	*Stenotrophomonas maltophilia*	3.19±0.1

Source or locations of soil samples (mechanic workshops) was found to have no significant effect on hydrocarbon degrading bacteria counts when compared using Kruskal-Wallis H test ($p = 0.65$, calculated H value = 0.86, x^2 value = 5.99).

The tolerance ability of identified isolates at concentrations (100-100,000 ppm) of PMS is shown in figure 2. Generally, higher CFU/ml values were obtained with decrease in concentrations of PMS. Similar growth responses were observed for *Acinetobacter* spp. and *Stenotrophomonas maltophilia*. However, for *Micrococcus* spp., a very low growth response was observed at 100 and 1000 ppm, while no growth was observed at higher concentrations between 10,000 and100,000 parts per million (PPM). Highest counts (CFU/ml)were obtained with *Bacillus velezensis* and *Stenotrophomonas maltophilia*.

Multiple comparison analysis of variance (ANOVA) revealed statistically significant differences between tolerance ability of individual bacteria to varying concentration of PMS ($p = 0.0066$, $F_{cal} = 5.02$, $F_{crit} = 2.90$).Furthermore, post-hoc analysis using Tukey's test shows a significant difference between *Stenotrophomonas spp.* and *Bacillus velezensis* ($p = 0.0264$) and *Micrococcus sp.* and *Bacillus velezensis* ($p = 0.0034$) in terms of ~~the~~ bacterial isolates involved in the degradation of PMS.

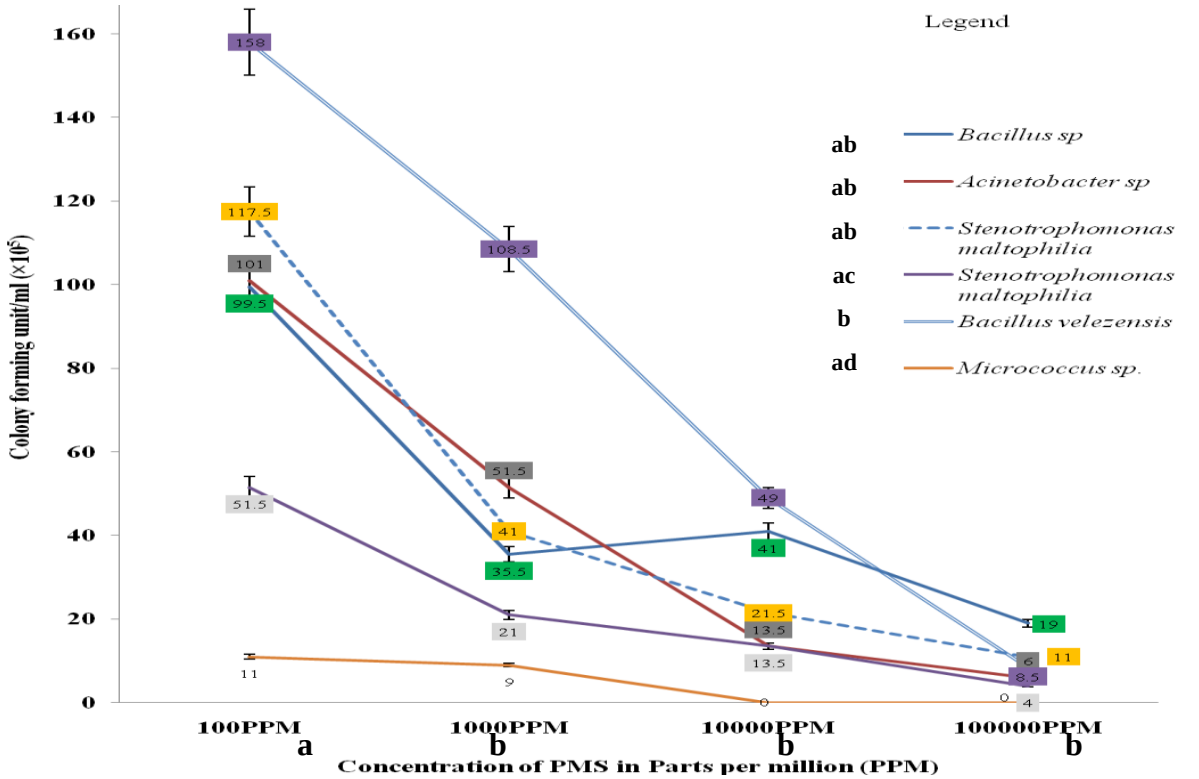

Figure 2: Tolerance ability of biosurfactant producing bacteria to different concentrations of PMS on Mineral Salt Medium

DISCUSSION

The higher bacteria counts obtained from the mechanic workshops indicate active hydrocarbons degrading strains as hypothesized by Zekri and Chaalal (2005). The authors demonstrated concomitant decrease in asphaltenes (an oil component) with increase in time and bacterial growth.

Similarly, higher bacterial counts were obtained from the top soil of some sampled areas compared to the subsurface soil stratum which could be attributed to the abundance of organic constituents favoring the microbial growth in the upper soil surfaces as reported by Aislabie and Deslippe (2013). This result of the present study is also buttressed by the findings of Collins (2010) and Saadoun *et al.* (2008) who reported that, both numbers and diversity of microbes are higher at the top 10 cm of the soil and decline with increasing depth.

The hydrocarbon degrading bacteria isolated from the mechanic sites highlighted the point made by Fahad (2019), who posited that the ability to degrade petroleum hydrocarbons is dependent on the capability of indigenous microorganisms to transform organic contaminant.

The majority (62.5%) of the identified bacteria in this study are gram negative which have been reported to be good degraders of hydrocarbons (Umar *et al.*, 2020b; Chikere *et al.*, 2018; Mwamura, 2017).

Furthermore, Batista *et al.* (2016); Kabir, (2019); Noor *et al.* (2017); Udgire *et al.* (2015) highlighted in their studies that Gram negative bacteria are associated with biosurfactant production. Similarly, Fetchner *et al.* (2017) reported that 84% of the bacteria isolates producing biosurfactant from petroleum contaminated sites were Gram-negative.

The validity and reliability of screening tests depend on the use of appropriate technique and skilled technician. In this study different screening techniques (drop collapse test, haemolysis test, oil spreading assay, and emulsification index test) were employed to determine biosurfactant expression ability of the isolates. This is in line with Satpute *et al.* (2010) who recommended the use of more than one screening method for the primary screening of potential biosurfactant producers.

The response of isolates where only three out of the six identified isolates were positive for drop collapse assay is in line with previous reports. Batista *et al.* (2016) in a related study reported that out of 17 isolates screened for biosurfactant production using the qualitative drop-collapse test, only 2 were positive, one each from terrestrial and marine environments.

However, clear zones of inhibition around the inoculated area were observed in all the isolates tested from both mechanic workshops and control sites indicating positive reaction for haemolysis test. Liu *et al.* (2018) and Grayyna *et al.* (2005) in their finding reported the isolation of biosurfactant producing strains using both hemolytic assay and oil-displacement test.

For oil-displacement test, all the five positive isolates were from the oil contaminated sites and none were reactive from the control soil samples, this could be related to the sensitivity of the test as it can detect low amount of biosurfactant produced (Youssef *et al.*, 2004). This is in accordance with the findings of Jaysree *et al.* (2011) who reported that Oil displacement was higher in the pollutant exposed (soil) isolates than that in the uncontaminated isolates. Furthermore, Plaza *et al.* (2006) in their findings concluded that oil spreading technique is reliable in detecting biosurfactant production as all their tested isolates were positive for the test.

Stated by Femi-Ola *et al.*(2015) that emulsification activity of 30% or more is considered positive for biosurfactant production, hence the bacteria isolated in this research are biosurfactant producers based on the baseline range values (39 - 41%) obtained from the isolates in both mechanic workshops and control site.

The values obtained were also in agreement with those obtained by Krepsky *et al.* (2007), where they recorded E_{24}values of 46.51%. Similarly, Jaysree *et al.* (2011) reported that the biosurfactants produced by *B. subtilis* had an emulsification capacity (E_{24}) of 20% and 15%, and those produced by *B. cereus* had emulsification indexes of 30% and 20% for diesel and engine oil, respectively.

CONCLUSION

This study reported the characterization of hydrocarbon degrading bacteria belonging to the genera *Acinetobacter*, *Bacillus*, *Micrococcus* and *Stenotrophomonas*, from three mechanic workshops in Malumfashi, Katsina state. Higher Hydrocarbon Degrading Bacteriacounts were recorded from all the sampled sites, even though there was no significant difference between individual mechanic garages ($p > 0.05$).The isolates found to be positive for biosurfactant-production using drop-collapse, β-haemolysis, oil spreading, and emulsification index tests were *Bacillus velezensis* and *Stenotrophomonas maltophilia*. These isolates also showed higher tolerance to PMS concentrations of100,000 ppm. The isolates' ability to grow on mineral salt media supplemented with PMS as a sole source of carbon may be a promising potential for the bioremediation of hydrocarbon pollutants in oil contaminated soil through expression of biosurfactants.

REFERENCES

Abalos, A., Vinas, M., Sabate, J., Manresa, M. and Solanas, A. (2004). Enhanced biodegradation of Casablanca crude oil by a microbial consortium in presence of a rhamnolipid produced by *Pseudomonas aeruginosa* AT10, *Biodegradation,* 15, 249–260.

Aboaba, O.A., Aboaba, O.O., Nwachukwu, N.C., Chukwu, E. E. and Nwachukwu, C.U. (2007). Evaluation of bioremediation of agricultural soil polluted with crude oil by planting beans seeds, *Phaseolu svulgaris*. *Nature and Science, 5*(4), 53-60.

Adams, D. D., Maikaje, D. B. and Umar, Y.A. (2015). Characterization and

determination of bioremediation potential of some Microbes isolated from oil contaminated soil in Kaduna metropolis Mechanic Workshops. A Ph.D research thesis submitted to the Department of Biological Science, Nigerian Defence Academy, Kaduna Nigeria. 57-118.

Adebajo, S.O., Akintokun, A. K. and Bolaji, S. F (2018). Biosurfactants producing bacteria from oil-polluted soil in Abeokuta, Ogun State. *Ife Journal of Science, 20*(2), 287-297.

Adeyemo, I. A. and Aliu, O. (2021). Effect of oil spillage on soil bacteriological and physicochemical properties in Awoye Community, Ilaje, Ondo State, Nigeria. *East African Scholars Journal of Agricultural & Life Sciences*,*4*(1), 1-5.

Ainon, H., Noramiza, S. and Shahidan, R. (2013). Screening and optimization of biosurfactant production by the hydrocarbon-degrading bacteria. *Sains Malaysiana*, *42*(5), 615–623.

Aislabie, J. and Deslippe, J. R. (2013). Soil microbes and their contribution to soil services. InJ.R. Dymond (ed). *Ecosystem services in New Zealand – conditions and trends* (pp. 143-161). Manaaki Whenua Press.

Astuti, D. I., Isty, A. P., Ratna, E. P., Maghfirotul, A. and Yuichi, S. (2019). Screening and characterization of biosurfactant produced by *Pseudoxanthomonas* sp. G3 and its applicability for enhanced oil recovery. *Journal of Petroleum Exploration and Production Technology*, *9*, 2279-2289.

Barrow, G. I. and Feltham, R. K. A. (2004). *Cowan and Steel's manual for the identification of medical bacteria*. (3rd ed.). Cambridge University Press.67-80

Batista, S., Mounteer, A. and Amorim, F. (2016). Isolation and characterization of biosurfactant/bioemulsifier-producing bacteria from petroleum contaminated sites. *Bioresource Technology, 97*(6), 868–875.

Chikere, C.B., Fenibo, E.O. and Akaranta, O. (2018). Comparative effectiveness of activated soil in bioremediation of a farmland polluted soil by polyaromatic hydrocarbon in the Niger Delta. *Journal of Bioremediation and Biodegradation,6*(9), 456.

Collins, H. (2010). *Impacts of fumigation and crop rotation on soil microbial populations*. USDA-ARS Irrigated Research Center

Darma, U. Z., Mansir, A. Z. and Riko, Y. Y. (2019). Compatibility and formulation of diesel degrading consortia using bacteria isolated from contaminated soil. *Bayero Journal of Pure and Applied Sciences, 12*(1), 199-208.

de Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.H. and Whitman, W.B. (Eds). (2003). *Bergy's manual of systematic bacteriology (Volume III, The Firmicutes)*. (2nd ed.). Springer.

Fahad, A. A. (2019). Morphological, biochemical and molecular identification of petroleum hydrocarbons biodegradation bacteria isolated from oil polluted soil in Dhahran, Saudi Arabia. *Saudi Journal of Biological Sciences*, *26*, 1247–1252.

Femi-Ola, T. O., Oluwole, O. A., Olowomofe, T. O. and Yakubu, H. (2015). Isolation and screening of biosurfactant- producing bacteria from soil contaminated with domestic waste water. *British Journal of Environmental Sciences*, *3*(1), 58-63.

Fetchner, J., Scott, S., Deeni, Y.Y.,Hapca, S.M.,Kabir, K., Mohammed, I.U. and Spiers A.J. (2017).Limitation of biosurfactant strength produced by bacteria. In R. Upton (Ed.). *Biosurfactants: Occurrences, Applications and Research*, NOVA Science Publishers.

Grayyna, A. P., Ireneusz, Z. and Ibrahim, M. B. (2005). Use of different methods for detection of thermophilic biosurfactant producing bacteria from hydrocarbon-contaminated and bioremediated soils. *Journal of Petroleum Science and Engineering,50*, 71– 77.

Hua, F. and Wang, H. (2012). Uptake modes of octadecane by *Pseudomonas* sp. DG17 and synthesis of biosurfactant. *Journal of Applied Microbiology,112*, 25–37.

Jaysree, R.C., Basu, S., Priyanka, P. S., Twinkle, G., Pragya, A. Patra, Y. K. and Rajendran N. (2011). Isolation of biosurfactant producing bacteria from environmental samples. *Pharmacology Online, 3*, 1427-1433.

Kabir, K. (2017). Bioprospecting surfactants produced by *Pseudomonas* spp. isolated from soil for potential application in biotechnology. Unpublished thesis submitted for the degree of Doctor of Philosophy (PhD). Abertay University.

Kabir, K. (2019). Isolation and characterisation of biosurfactant-producing *Pseudomonas* specie from soil. *UMYU Journal of Microbiology Research, 4*(2), 1–6.

Kareem, S. O., Adegoke, O. O., Balogun, S. A., Afolabi, A. T. and Akinde, S. B. (2017). Biodegradation of premium motor spirit (PMS) by lipase from *Bacillus thuringiensis* and *Lysinibacillus sphaericus*. *Nigerian Journal of Biotechnology*, *33*, 34-40.

Krepsky, N., Da Silva, F.S., Fontana, L. F. and Crapez, M. A. C. (2007). Alternative methodology for isolation of biosurfactant-producing bacteria. *Brazilian Journal of Biology, 67*(1), 117-124.

Kurniati, T. H., Rahayu, S., Sukmawati D. and Maharani, W. (2019). Screening of biosurfactant producing bacteria from hydrocarbon contaminated soil (4th Annual Applied Science and Engineering Conference). *Journal of Physics*: *Conference Series*,*1402,* 055026.

Li, X., Li, H. and Qu, C. (2019). A review of the mechanism of microbial degradation of petroleum pollution. *IOP Conference Series: Materials Science and Engineering*, *484,* 012060.

Liu, W. J., Duan, X. D., Wu, L. P., & Masakorala, K. (2018). Biosurfactant Production by Pseudomonas aeruginosa SNP0614 and its Effect on Biodegradation of Petroleum. *Applied Biochemistry and Microbiology*, *54*(2), 155–162. https://doi.org/10.1134/S0003683818020060

Mahuta, A. U., Wagini, N. H., Bello, A., Kabir, K., Riko, Y. Y. and Mannir, K. (2021). Characterization of hydrocarbon-degrading, heavy metal tolerant and antibiotic resistant bacteria isolated from oil contaminated soil and organic wastes within Katsina Metropolis. *IOSR Journal of Biotechnology and Biochemistry, 7*(4), 48-60.

Morikawa, M., Hirata, Y.andImanaka, T. (2000). A study on the structure–function relationship of the lipopeptide biosurfactants. *Biochimica et BiophysicaActa, 1488*, 211-218.

Mwamura, A. (2017). *Screening, isolation and characterization of hydrocarbonoclastic bacteria from oil contaminated soils*. MSc. (Biochemistry) Thesis submitted to the University of Nairobi, Unpublished.

Noor, S. L., Mohamad, Z., Suhaila, M. O. and Mardiana, M. A. (2017). Isolation and characterization of biosurfactant-producing bacteria isolated from petroleum contaminated sites with the potential to be used in bioremediation. *Science Heritage Journal*, *1*(2), 11-15.

Pekdemir, T., Copur, M. andUrum, K. (2005). Emulsification of crude oil–water systems using biosurfactants. *Process Safety and Environmental Protection,83*(B1), 38-46.

Płaza, G. A., Zjawiony, I. and Banat, I. M. (2006). Use of different methods for detection of thermophilicbiosurfactant-producing bacteria from hydrocarbon-contaminated and bioremediated soils. *Journal of Petroleum Science and Engineering*,50(1), 71–77.

Rocha, C. A., Pedregosa, A.M. and Laborda, F. (2011). Biosurfactant-mediated biodegradation of straight and methyl-branched alkanes by *Pseudomonas aeruginosa* ATCC 55925. *AMB Express*,*1*(1), 1–10.

Ron, E. Z. and Rosenberg, E. (2002). Biosurfactants and oil bioremediation. *Current Opinion in Biotechnology*, *13*, 249–252.

Saadoun, I., Munir, J. M., Khalid, M. H. and Mo'ayyad, S. (2008). Microbial populations of crude oil spill polluted soils at the Jordan-Iraq desert (the Badia region). *Brazilian Journal of Microbiology, 39*, 453-456.

Sari, C. N., Hertadi, R., Gozan, M. and Roslan, A. M. (2019). Factors affecting the production of biosurfactants and their applications in Enhanced Oil Recovery (EOR): A review *Earth and Environmental Science, 353,* 012048.

Satpute, S. K., Banpurkar, A.G., Dhakephalkar, P.K., Banat, I.M. and & Chopade, B.A. (2010). Methods for investigating biosurfactants and bioemulsifiers: a review. *Critical Reviews in Biotechnology,30*, 127–144.

Tanzadeh, J., Mohammad, F. G., Masumeh, A. and Khosro, I. (2020). Biological removal of crude oil with the use of native bacterial consortia isolated from the shorelines of the Caspian Sea, *Biotechnology & Biotechnological Equipment*, **34**(1):361-374, DOI: 10.1080/13102818.2020.1756408

Udgire, M., Shah, N. and Jadhav, M. (2015). Enrichment, isolation and identification of hydrocarbon degrading bacteria.

International Journal of Current Microbiology & AppliedSciences,*4*(6), 708–713.

Umar, Z. D., Aminu, M. and Yahaya, Y.R. (2020a). Optimization of diesel biodegrading conditions using Response Surface Methodology based on Central Composite Design. *Polycyclic Aromatic Compounds, 40*(4), 1-11.

Umar, Z. D., Aminu, M. and Yahaya, Y. R. (2020b). Survival response of consortium isolates from diesel contaminated soil within Katsina State, Nigeria. *International Journal of Environment, 9*(1), 51-66.

Unimke, A. A., Mmuoegbulam, A. O., Bassey, I. U. and Obot, S. E. (2017). Assessment of the Microbial Diversity of Spent-Oil Contaminated Soil in Calabar, Nigeria. *JAMB. 4(4): 1-9, Article no. 34847ISSN: 2456-7116*

Youssef, N. H., Duncan, K. E., Nagle, D. P., Savage, K. N., Knapp, R. M. and Mclnerney, M. J. (2004). Comparison of methods to detect biosurfactant production by diverse microorganism. *Journal of Microbiology Methods*, *56*(3), 339–347.

Zekri, A.Y. and Chaalal, O. (2005). Effect of temperature on biodegradation of crude oil. *Energy Sources*, *27*, 233–244.

Zhang, X.and Xiang, T. (2010). Review on microbial enhanced oil recovery technology and development in China. *International Journal of Petroleum Science & Technology*,*4*, 61–80.

Zhang, X., Dejun,X., Chunyan, Z., Tserennyam, L. and Kerstin, E. S. (2012). Isolation and identification of biosurfactant producing and crude oil degrading *Pseudomonas aeruginosa* strains. *Chemical Engineering Journal*, *209*, 138–146.

Zhang, X., Li, M. and Xiang, T. (2010). Genetic modification of MEOR bacterium *Bacillus licheniformis* H strain by low energy ion beam irradiation. *Open Biotechnology Journal, 4*, 14–17.