Assessment of the Biosurfactant-Producing Potentials of Hydrocarbon-Utilizing Bacteria Isolated from Spent Engine oil Contaminated Soil

[1]Imade, O. S. , [1]Okwu, M. U., [2]Yerima, Y., [3]Josiah, S. J., [1]Izevbuwa O. E. [4]and Onuguh, I. C.

[1]Department of Biological Sciences, College of Natural and Applied Sciences, Igbinedion University, Okada, Edo State, Nigeria

[2]Department of Petroleum Engineering, College of Engineering, Igbinedion University, Okada, Edo State, Nigeria

[3]Department of Biochemistry, College of Natural and Applied Sciences, Igbinedion University, Okada, Edo State, Nigeria

[4]Department of Chemistry, College of Natural and Applied Sciences, Igbinedion University, Okada, Edo State, Nigeria

Correspondence: imade.stanley@iuokada.edu.ng

Abstract

Microbial surfactants that are largely produced by bacteria and yeasts are preferentially used in relation to the synthetic surfactants. Hence there is a need to carry out investigations that will isolate efficient biosurfactant-producing microorganisms. The present study isolated potential biosurfactant-producing bacteria from soil that were polluted by spent engine oil from some automobile workshops situated at Okada, Edo State, Nigeria. The counts and isolation of hydrocarbon-utilizing bacteria (HUC) in the spent engine oil-polluted soil samples were carried out using the spread plate technique. The identification of the hydrocarbon-utilizing bacteria was carried out with standard phenotypic and 16S rRNA gene sequencing techniques. Hydrocarbon-utilizing bacterial isolates were screened for biosurfactant-producing potentials by using their culture supernatants to perform haemolytic, oil displacement and emulsification assays. The mean HUC in the engine oil-polluted soil samples ranged from 4.47±0.03 log_{10}CFU/g to 4.84±0.05 log_{10}CFU/g. Staphylococcus anginosus KT 1, Micrococcus luteus KT 2, Bacillus subtilis KT 3 and Staphylococcus aureus KT 4 were the bacterial strains that were isolated from the engine oil-polluted soil samples, and have been respectively deposited in the United States GenBank under accession numbers OP602363, OP602365,

OP602411 and OP602687. Bacillus subtilis KT 3 (OP602411) had the highest biosurfactant-producing potential since it exhibited the highest β-haemolytic activities (63.64±15.21%), oil displacement activities (39.82±2.43 mm) and emulsification activities (49.84±1.01%).

Keywords: Biosurfactants, spent engine oil, hydrocarbon-utilizing bacteria

Introduction

Biosurfactants are valuable, surface active and biologically efficient microbial amphiphilic molecules for different industries or processes (Bhadra *et al.,* 2022; Sarubbo *et al.,* 2022). Microbes synthesize them and offer an alternative to chemically prepared conventional surfactants, especially when growing on water immiscible substrates. These molecules can be widely used in cosmetics, pharmaceutical and alimentary processes such as emulsifiers, humectants, preserving agents and detergents because of their structural diversity, their low toxicity and biodegradability (Barbosa *et al.,* 2022). Examples of biosurfactants include glycolipids, lipopeptides, fatty acids, etc. In the fields of bioremediation and waste treatment, they are also ecologically safe. They can be made from different substrates, mainly from renewables, such as vegetable oils, distillery and milk waste. Microbes synthesize surface active compounds known as biosurfactants (Pinto *et al.,* 2022) that have several distinct properties compared to other synthetic surfactants. These compounds are primarily biosynthesized as secondary metabolites and play important roles in the growth and localization of their microorganisms. Based on the chemical structure of their hydrophobic component, biosurfactants are classified into four types (Gayathiri *et al.,* 2022; Ingsel *et al.,* 2022): (1) glycolipid type, (2) fatty acid type, (3) lipopeptide type, and (4) polymer type. Due to their surface tension-reducing, emulsion-stabilizing, foam-promoting and biodegrading properties; use of the biosurfactants in place of chemical surfactants is a highly demanded area of interest. Thus, in hydrocarbon-contaminated sites, biosurfactant-producing microorganisms accelerate bioremediation. Bacteria and yeasts are

the dominant microbes that synthesize most of the biosurfactants. Biosurfactants such as glycolipid, phospholipid, rhamnolipid, etc., are the biochemicals with great surface activity synthesized by these organisms. The hydrocarbon substrate is emulsified by the production of biosurfactants for the facilitation of transportation into cells. A mechanism known as swarming motility is identified in the biosurfactants mechanism of action.

The structure of a biosurfactant is composed of a hydrophobic tail and a hydrophilic head (Georgiou *et al.*, 1992; Banerjee *et al.*, 2022) otherwise composed of amphophilic or hydrophobic peptides. Hydrocarbon uptake is highly related to the spontaneous release and function of biosurfactants. Thus, the maximum production is seen in the hydrocarbon-degrading microorganisms. Water-soluble compounds like glucose also seemed to produce biosurfactants in rare cases (Lang, 2002). These biosurfactants exhibit improved properties compared to chemically synthesized surfactants, which enable them to be used in the process of oil recovery in an environmentally safer way. Their tolerance in extreme conditions, ease of culturing, high-scale production, eco-friendly nature and diversified nature make biosurfactants efficient enough in the implementation of various fields, including microbial degradation. In light of changing climate circumstances and a growing global population, it is critical to investigate creative, efficient, and cost-effective natural products for the betterment of people. Biosurfactants have seen a tremendous increase in research and development, as well as commercialization of biological agents in recent years (Sarubbo *et al.*, 2022). Microorganisms create a wide variety of amphiphilic metabolites, many of which are unique in their structure. The present study aimed to assess the biosurfactant-producing potentials of hydrocarbon-degrading bacteria isolated from spent engine oil-contaminated soil in Okada Metropolis, Edo State, Nigeria.

Materials and Methods

Experimental design and sample collection

Sample collection was carried out with the benchmark sampling design. Spent engine oil-polluted sites in three automobile

workshops located at Okada in Ovia-North East Local Government Area of Edo State, Nigeria were used as sampling locations. The three sampling locations were visited weekly in the month of August 2022, and on each visit a representative site (benchmark) was demarcated in each of the sampling locations followed by random collection of soil samples up to a depth of 15 cm from ten sampling points within the benchmark of each of the sampling locations. The ten soil samples from each automobile workshop (sampling location) were subsequently composited and sieved with a 2 mm pore-size sieve to obtain a single composite soil sample. Overall, the three sampling locations were each visited five times in the month of August 2022 and a total of fifteen composite soil samples were collected from the three automobile workshops. These samples were sent to the Microbiology Laboratory at the Department of Biological Sciences, Igbinedion University, Okada, Edo State, Nigeria where bacteriological analysis and other biochemical assays were performed. The main treatments that were performed on the samples included counts of engine oil hydrocarbon-utilizing bacteria (HUB), as well as assays of oil displacement, drop collapse and haemolytic activities. The discrete counts of HUB were transformed into continuous variables. Statistical significance in the variability of the value of mean datasets of treatment variables was estimated with the one-way analysis of variance (ANOVA) based on the outcomes of normality and homoscedasticity tests. Normality of the datasets was performed with Shapiro-Wilk test while homoscedasticity of treatment groups was performed with the Levene test of homogeneity.

Isolation of hydrocarbon-utilizing bacteria

The isolation of bacteria that can degrade petroleum hydrocarbons from spent engine oil-contaminated soil samples was performed according to previously described methods (Udeani *et al*., 2009). The soil samples were serially diluted up to up to 10^{-7}, with the first dilution of the soil samples made by mixing 25 grams of soil with

225 ml of sterile buffered saline water. One hundred microlitres (100 µl) of each of the serially-diluted soil samples was spread-plated onto two different duplicate mineral salt agar (MSM) Petri dishes amended with 2% sterile spent engine oil. A one liter MSM agar medium consisted of 3.00 grams ammonium nitrate, 2.5 grams monopotassium phosphate, 2 grams dipotassium phosphate, 0.2 gram magnesium sulphate, 0.1 gram ferrous sulphate and 20 grams agar. The MSM Petri dishes were then incubated for 24 hours at a temperature of 37ºC. After incubation, visible hydrocarbon-utilizing bacterial colonies were isolated on the MSM Petri dishes.

Hydrocarbon-utilizing bacteria count

The hydrocarbon-utilizing bacterial colonies on the MSM Petri dishes were counted with the colony counter and counts on MSM Petri plates were then reported as total hydrocarbon-utilizing bacteria count (HUC). The HUC was expressed as colony-forming units per gram (CFU/g) of the soil sample.

Characterization and identification of hydrocarbon-utilizing bacteria

Bacterial colonies were selected from the Petri plates based on their colonial morphology. The bacterial isolates were identified and characterized by previously established phenotypic tests (Krieg and Holt, 1984) and molecular tests.

Phenotypic tests

The phenotypic tests that were performed include Gram staining, urease, citrate, methyl red, coagulase, catalase, oxidase and sugar fermentation tests (Sanger *et al*. 1977; Lane, 1991).

Molecular tests

Molecular characterization employed techniques that involved polymerase chain reaction (PCR) of DNA templates extracted from the bacterial isolates and sequencing of the partial 16S rRNA gene amplicons. Zymo-Spin column was used to extract the DNA templates as prescribed by the manufacturer (Zymo Research Corporation, USA). The ultra-pure DNA templates were used to

perform the polymerase chain reaction (PCR) as previously described (Lane, 1991) and derived amplicons subsequently sequenced with the dideoxy-chain termination method (Sanger *et al*. 1977). Upon confirmation of the taxonomic identity of the bacterial strains by algorithm of the United States National Center for Biotechnology Information (NCBI) GenBank, the identified bacterial strains were deposited in the GenBank database under specific accession numbers.

Screening of bacterial isolates for biosurfactant production

Screening for biosurfactant production was performed with haemolysis, oil displacement and emulsification assays according to previously described techniques (Sarubbo, 2006; Thavasi, 2011; Ibrahim *et al.*, 2013). The bacterial isolates were subcultured into a nutrient broth medium and incubated for 20 hours at room temperature in an orbital shaker set at 150 revolutions per minute (rpm). After incubation, the turbidity of the broth culture was adjusted to optical density of 1.0 (OD_{600nm} = 1.0), and 2.5% (v/v) of the broth culture was used as inoculum to screen for biosurfactant production. To screen for biosurfactant production, 2.5 ml inoculum was mixed with 97.5 ml mineral salt broth (MSB) medium amended with 2% spent engine oil in a 250 ml flask. The flask was incubated for 72 hours at room temperature in an orbital shaker set at 150 rpm. After incubation, crude culture supernatants of the bacterial isolates were centrifuged at 5000 rpm for 15 minutes to pellet out the bacterial cells before they were used to screen for the production of biosurfactants.

Oil displacement activity

Twenty microlitres (20 µl) crude petroleum oil was carefully layered over 20 ml distilled water on Petri dish forming a thin film on the water surface. A drop of culture supernatants was carefully pipetted onto the center of the oil film. The oil would be displaced with an oil free clearing zone in the presence of biosurfactants. The diameter of the clear zone on the surface of the oil film was subsequently measured and compared to the negative control

(distilled water) to determine the oil displacement activity of the biosurfactant.

Haemolytic activity

Blood agar plates were prepared by adding 5% (v/v) blood to a molten nutrient agar medium.

Aliquots (100 µl) of culture supernatants of each of the bacterial isolates were loaded into each well (6.5 mm in diameter) made by a cork borer in the blood agar Petri dishes. The inoculated Petri dishes were then incubated at 30ºC for 24–48 hours. Biosurfactant biosynthesis was confirmed by hemolysis activity as indicated by the presence of clearing zones around the wells. The diameter of the lysis zone is scored as no haemolysis, complete hemolysis (beta) and incomplete (alpha) haemolysis.

Emulsification activity

Four milliliters of culture supernatants and 4 ml of commercially-packed engine oil were mixed for 3 minutes and left to stand for 24 hours at 25ºC prior to measurement. The emulsification index (E_{24}) was expressed as the percentage of the height of the emulsified layer divided by the total height of the liquid column as given by the following formula.

$$E_{24}\ (\%) = \frac{\text{Height of emulsion layer}}{\text{Total height of liquid column}} \times 100$$

Statistical analysis

Descriptive statistics of HUC counts was done with NCSS ver. 12 data analysis software. Levene test of homogeneity, Shapiro–Wilk test, Fisher one-way ANOVA test for normally distributed datasets were also performed with NCSS ver. 12 data analysis software. The test of the hypothesis was considered statistically significant if the achieved level of significance (p) was less than 0.05.

Results

Counts of hydrocarbon-utilizing bacteria (HUC)

Table 1 shows the HUC in the engine oil-polluted soil samples. The mean HUC in the engine oil-polluted soil samples ranged from 4.47 ± 0.03 $\log_{10}$ CFU/g to 4.84 ± 0.05 $\log_{10}$ CFU/g. Mean HUC was highest in sampling location 2 and lowest in sampling location 1. The HUC datasets obtained from all the sampling locations were normally distributed ($p > 0.05$). The HUC datasets from all the sampling locations also had equal variances ($p > 0.05$). Fisher one-way ANOVA test indicated a significant difference ($p < 0.05$) in the mean HUC of all the soil samples obtained from the different sampling locations.

Table 1: Bacterial counts in spent engine oil-contaminated soil samples

Weekly visitations	Hydrocarbon-Utilizing Bacteria Count (HUC)					
	Sampling location 1		Sampling location 2		Sampling location 3	
	Composite Samples	Counts (Log_{10} CFU/g)	Composite samples	Counts (Log_{10} CFU/g)	Composite samples	Counts (Log_{10} CFU/g)
1	1	4.42	1	4.93	1	4.56
2	2	4.52	2	4.67	2	4.47
3	3	4.48	3	4.88	3	4.53
4	4	4.54	4	4.77	4	4.62
5	5	4.41	5	4.94	5	4.54
	Mean (N =5)	4.47 ± 0.03	Mean (N =5)	4.84 ± 0.05	Mean (N =5)	4.54 ± 0.02
	95% CI of mean	4.42 – 4.52	95% CI of mean	4.74 – 4.95	95% CI of mean	4.50 – 4.58

Mean values are presented as mean ± standard error of mean; N: total number of composite soil samples collected from each of the sampling locations; CI: confidence interval

Characterization of the hydrocarbon-utilizing bacterial isolates

Staphylococcus aureus, *Micrococcus* species, as well as *Bacillus* species, were the main hydrocarbon-utilizing bacteria isolated the engine oil-polluted soil samples (Table 2). Representative bacterial isolates such as *S. anginosus* KT 1, *M. luteus* KT 2, *B. subtilis* KT 3 and *S. aureus* KT 4, as revealed by 16S rRNA gene sequence analysis, have been respectively deposited in the United States GenBank under accession numbers OP602363, OP602365, OP602411 and OP602687. Gel electrophoresis of amplified 16S rRNA genes obtained from the identified bacterial isolates is shown in Figure 1.

Screening for biosurfactant productions

S. aureus isolates across the sampling locations exhibited mean beta (β)-haemolysis (complete haemolysis) that ranged from 44.44 ± 17.57% to 57.14 ± 13.73% (Table 2). The highest β-haemolytic activity was seen in culture supernatants of *S. aureus* isolates collected from sampling location 2 while lowest the activity occurred in sampling location 3. Mean β-haemolysis for culture supernatants of the *Staphylococcus* species across all sampling locations ranged from 50.00 ± 16.67% to 52.94 ± 12.48%, while it ranged from 56.25 ± 12.81% to 63.64 ± 15.21% for culture supernatants of the *Bacillus* species. Amongst all culture supernatants of the hydrocarbon-utilizing bacteria isolated from all the sampling locations, those of the *Bacillus* species exhibited the highest β-haemolytic activities. However, the highest γ-haemolytic (partial haemolysis) activities were exhibited by culture supernatants of *S. aureus* across all sampling locations. Culture supernatants that exhibited no haemolysis (γ-haemolysis) were also more prevalent with those collected from *S. aureus* across all sampling locations.

The oil displacement activity of *S. aureus*, as indicated by the mean diameter of the displaced circle, ranged from 24.54 ± 3.50 mm to 27.50 ± 3.64 mm across all sampling locations, while it ranged from 12.90 ± 2.95 mm to 18.59 ± 2.45 mm and from 37.06

± 1.86 mm to 39.82 ± 2.43 mm for culture supernatants collected from *Staphylococcus* and *Bacillus* species, respectively. *Bacillus* species exhibited the highest oil displacement activities.

The emulsification activity (E_{24}) of *S. aureus* ranged from 26.81 ± 1.74% to 31.07 ± 1.36% across all sampling locations, while it ranged from 12.50 ± 0.76% to 14.00 ± 1.12% and from 46.85 ± 0.88% to 49.84 ± 1.01% for culture supernatants collected from *Staphylococcus* and *Bacillus* species, respectively. *Bacillus* species again exhibited the highest emulsification activities.

Table 2: Characterization of the bacterial isolates

Sampling locations	Representative bacterial isolates	Colonial and morphological characteristics		Biochemical characteristics of bacterial isolates								Molecular tests	Identified bacteria	Frequency of bacteria	
		Growth on Petri plates	Gram staining	CA	CO	OX	CI	UR	MR	LA	MA	16S similarity		P	%
1	1	Mucoid colony on MSA plates	Positive cocci	+	+	-	-	-	+	v	+	96 - 99%	*Staphylococcus aureus* (OP602687)	8/30	26.67
	2	Mucoid colony on MSA plates	Positive cocci	+	+	-	-	-	+	v	+	98 - 100%	*Staphylococcus anginosus*	5/30	16.67
	3	Mucoid colony on MSA plates	Positive cocci	+	-	-	-	-	+	v	v	95 - 99%	*Micrococcus luteus* (OP602365)	17/30	56.67
2	1	Mucoid colony on MSA plates	Positive cocci	+	+	-	-	-	+	v	+	96 - 100%	*Staphylococcus aureus*	14/30	46.67
	2	Mucoid colony on MSA plates	Positive rods	+	-	+	v	v	+	v	-	99 - 100%	*Bacillus subtilis* (OP602411)	16/30	53.33
3	1	Mucoid colony on MSA plates	Positive rods	+	-	+	v	v	+	v	-	95 - 99%	*Bacillus subtilis*	11/30	36.67
	2	Mucoid colony on MSA plates	Positive cocci	+	+	-	-	-	+	v	+	96 - 99%	*Staphylococcus aureus*	7/30	23.33
	3	Mucoid colony on MSA plates	Positive cocci	+	+	-	-	-	+	v	+	97 - 100%	*Staphylococcus anginosus* (OP602363)	2/30	6.67
	4	Mucoid colony on MSA plates	Positive cocci	+	-	-	-	-	+	v	v	99 - 100%	*Micrococcus luteus*	10/30	33.33

Co: Coagulase test; Ca: Catalase test; Ur: Urease test; Mr: Methyl red test; CI: Citrate test; OX: Oxidase test; LA: lactose fermentation test; MA: mannitol fermentation test; -: negative reaction; +: positive reaction; v: variable reaction; MSA: Mineral salt agar

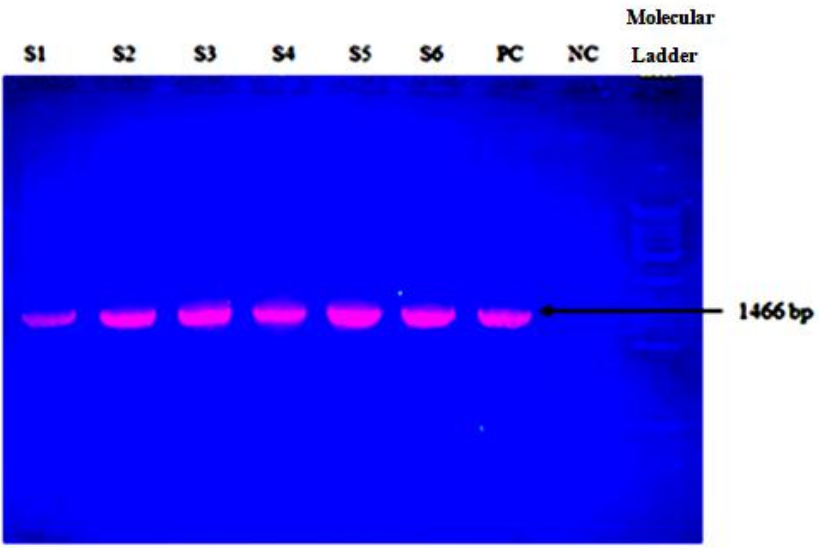

Figure 1: Gel electrophoresis of amplified 16S rRNA genes obtained from hydrocarbon-utilizing bacteria present in the samples.

Lanes S1 to S6 were positive samples for 16S rRNA genes. Lane PC is the positive control (*S. aureus* ATCC 25923) while lane NC is the negative control (distilled water). The molecular ladder is a 100 base ladder. Forward primer is 27F (AGAGTTTGATCMTGGCTCAG) and Reverse primer is 1492R (GGTTACCTTGTTACGACTT). Gel electrophoresis was performed with 2% agarose.

Table 3: Biosurfactant properties of the bacterial isolates

Sampling Location	Identified Bacteria	H	Biosurfactant properties tested				
			Haemolytic activity			Oil displacement activity (mm)	Emulsification activity (E_{24}) (%)
			β (%)	α (%)	γ (%)		
1	*Staphylococcus aureus*	13	53.85 ± 14.39	23.08 ± 12.16	23.08 ± 12.16	24.54 ± 3.50	28.65 ± 1.49
	Staphylococcus spp.	17	52.94 ± 12.48	29.41 ± 11.39	17.65 ± 9.53	18.59 ± 2.45	12.50 ± 0.76
2	*Staphylococcus aureus*	14	57.14 ± 13.73	28.57 ± 12.53	14.29 ± 9.71	27.50 ± 3.64	31.07 ± 1.36
	Bacillus spp.	16	56.25 ± 12.81	25.00 ± 11.18	18.75 ± 10.08	37.06 ± 1.86	49.84 ± 1.01
3	*Bacillus* spp.	11	63.64 ± 15.21	18.18 ± 12.20	18.18 ± 12.20	39.82 ± 2.43	46.85 ± 0.88
	Staphylococcus aureus	9	44.44 ± 17.57	33.33 ± 16.67	22.22 ± 14.70	25.22 ± 3.74	26.81 ± 1.74
	Staphylococcus spp.	10	50.00 ± 16.67	30.00 ± 15.28	20.00 ± 13.33	12.90 ± 2.95	14.00 ± 1.12

β: beta-haemolytic; α: alpha-haemolytic; γ: gamma-haemolytic; H: relative number of the identified bacterial isolates in each of the sampling locations

Discussion

Microbes are known to synthesize biosurfactants which offer an alternative to chemically prepared conventional surfactants, especially when grown on water immiscible substrates. Biosurfactants are primarily biosynthesized as secondary metabolites and play important roles in the growth and localization of their microorganisms. Bacteria and yeasts are the dominant microbes that synthesize most of the biosurfactants. Biosurfactants have seen a tremendous increase in research and development. Thus, the present research assessed the biosurfactant-producing potentials of hydrocarbon-degrading bacteria isolated from spent engine oil-contaminated soil in Okada Metropolis, Edo State, Nigeria.

The mean HUC in the engine oil-polluted soil samples ranged from 4.47 ± 0.03 $\log_{10}$ CFU/g to 4.84 ± 0.05 $\log_{10}$ CFU/g. The high counts of hydrocarbon-utilizing bacteria seen in the spent engine oil-polluted soil collected from the different automobile workshop may be due to the high petroleum hydrocarbon concentration in these polluted environments, as well as other geoecological factors, such as particle size distribution and moisture content, that may affect the survival of soil microbial inhabitants that play vital roles in the degradation and transformation of organic matter into nutrients (Eze and Okpokwasili, 2010). Similar to the present findings, other authors have reported high counts of hydrocarbon degraders in spent engine oil-polluted soil (Luepromchai *et al*., 2007; Jones *et al*., 2020). *Staphylococcus* species were the main hydrocarbon-utilizing bacterial isolates found in the engine oil-polluted soil samples followed by the *Bacillus* species. This agreed with the findings of other researchers (Jones *et al*., 2020) that had found similar organisms petroleum-polluted soil samples. The properties examined to evaluate the biosurfactant-producing potentials of the bacterial isolates showed that the *Bacillus* species were suspected to have the highest biosurfactant-producing potentials. The suspected biosurfactant-producing bacteria identified in the present

study belong to genera previously identified in a myriad of studies as efficient petroleum hydrocarbon biodegraders and biosurfactant producers (Jemil *et al*., 2016; Parthipan *et al*., 2017; Chaprão *et al*., 2018; Khamis *et al*., 2020).

Conclusion

The present study revealed that bacterial species, particularly the *Bacillus* species, isolated from the spent engine oil-polluted sites in some automobile workshop situated at Okada, Edo State, Nigeria exhibited biosurfactant-producing potentials. However, further purification and characterization of biosurfactants produced by the bacterial species harboured in these oil-polluted locations is warranted to enable the future use of these biosurfactants in a variety of environmental and industrial applications such as bioremediation operations and enhanced oil recovery.

References

Banerjee, B., Kaur, G., & Priya, A. (2022). Naturally occurring bioactive biosurfactants. In *Green Sustainable Process for Chemical and Environmental Engineering and Science* (pp. 337-350). Academic Press.

Barbosa, F. G., Ribeaux, D. R., Rocha, T., Costa, R. A., Guzmán, R. R., Marcelino, P. R., ... & Silva, S. S. D. (2022). Biosurfactants: sustainable and versatile molecules. *Journal of the Brazilian Chemical Society*, *33*, 870-893.

Bhadra, S., Chettri, D., & Kumar Verma, A. (2022). Biosurfactants: secondary metabolites involved in the process of bioremediation and biofilm removal. *Applied Biochemistry and Biotechnology*, *7*, 1 – 27.

Chaprão, M. J., da Silva, R. D. C. F. S., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2018). Production of a biosurfactant from *Bacillus methylotrophicus* UCP1616 for use in the bioremediation of oil-contaminated environments. *Ecotoxicology*, *27*(10), 1310 – 1322.

Eze, V. C., & Okpokwasili, G. C. (2010). Microbial and other related changes in Niger Delta River sediment receiving

industrial effluents. *Continental Journal of Microbiology*, *4*, 15 – 24.

Gayathiri, E., Prakash, P., Karmegam, N., Varjani, S., Awasthi, M. K., & Ravindran, B. (2022). Biosurfactants: potential and eco-friendly material for sustainable agriculture and environmental safety—a review. *Agronomy*, *12*(3), 662 – 666.

Georgiou, G., Lin, S. C., & Sharma, M. M. (1992). Surface–active compounds from microorganisms. *Bio/technology*, *10*(1), 60 – 65.

Ibrahim, M. L., Ijah, U. J. J., Manga, S. B., Bilbis, L. S., & Umar, S. (2013). Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. *International Biodeterioration & Biodegradation*, *81*, 28 – 34.

Ingsel, T., de Souza, F. M., & Gupta, R. K. (2022). Biosurfactants for industrial applications. In *Green Sustainable Process for Chemical and Environmental Engineering and Science*. Academic Press, pp. 467 – 493.

Jones, A. M., James, I. I., Akpan, P. S., Eka, I. I., Oruk, A. E., & Ibuot, A. A. (2020). Characterization of hydrocarbon utilizing bacteria in waste engine oil-impacted sites. *BioRxiv 2(3)*, 3 – 8.

Jemil, N., Ben Ayed, H., Hmidet, N., & Nasri, M. (2016). Characterization and properties of biosurfactants produced by a newly isolated strain Bacillus methylotrophicus DCS1 and their applications in enhancing solubility of hydrocarbon. *World Journal of Microbiology and Biotechnology*, *32*(11), 1 – 13.

Khamis, N., Abdul Rahim, A. I., Ibrahim, N A., Abdul Kadir, K., & Zaman, P. (2020). Characterization of biosurfactant production by indigenous bacteria from Sungai Dungun estuary, Terengganu by surface activity and emulsification test. *Earth and Environmental Science*, *5*, 596 – 602.

Krieg NR and Holt JC (1984). Bergey's Manual of Systematic Bacteriology, 1st ed., vol. 1. Williams and Wilkins, Baltimore.

Lane DJ (1991). 16S/23S rRNA sequencing. In Nucleic Acid Techniques in Bacterial Systematics eds. Stackebrandt, E. and Goodfellow, M. pp. 115–175. New York: John Wiley and Sons.

Lang, S. (2002). Biological amphiphiles (microbial biosurfactants). *Current Opinion in Colloid & Interface Science*, *7*(1-2), 12 – 20.

Luepromchai, E., Lertthamrongsak, W., Pinphanichakarn, P., Thaniyavarn, S., Pattaragulwanit, K., & Juntongjin, K. (2007). Biodegradation of PAHs in petroleum-contaminated soil using tamarind leaves as microbial inoculums. *Biodegradation*, *29*(2), 516 – 522.

Parthipan, P., Preetham, E., Machuca, L. L., Rahman, P. K., Murugan, K., & Rajasekar, A. (2017). Biosurfactant and degradative enzymes mediated crude oil degradation by bacterium Bacillus subtilis A1. *Frontiers in Microbiology*, *8*, 193 – 199.

Pinto, M. I. S., Campos Guerra, J. M., Meira, H. M., Sarubbo, L. A., & de Luna, J. M. (2022). A Biosurfactant from Candida bombicola: Its synthesis, characterization, and its application as a food emulsions. *Foods*, *11*(4), 561 – 568.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977) DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Science USA*, 74, 5463 – 5467.

Sarubbo, L. A., Maria da Gloria, C. S., Durval, I. J. B., Bezerra, K. G. O., Ribeiro, B. G., Silva, I. A., ... & Banat, I. M. (2022). Biosurfactants: Production, properties, applications, trends, and general perspectives. *Biochemical Engineering Journal*, 108377.

Thavasi, R. (2011). Microbial biosurfactants: from an environmental application point of view. *Journal of Bioremediation and Biodegradation*, *2*(5), 121 – 126.

Udeani, T. K. C., Obroh, A. A., Okwuosa, C. N., Achukwu, P. U., & Azubike, N. (2009). Isolation of bacteria from mechanic workshops' soil environment contaminated with used engine oil. *African Journal of Biotechnology*, 8(22), 6301 – 6303.