https://dx.doi.org/10.4314/ijs.v26i3.3

BIOSURFACTANT PRODUCTION AND CONCOMITANT DEGRADATION OF SPENT ENGINE OIL BY *Alcaligens faecalis* ULAG3AF ISOLATED FROM LAGOS LAGOON

Ashamu, T. O.[1,*], Omotayo A. E.[1] and Adekunle, A. A.[2]

[1]Department of Microbiology, University of Lagos, Akoka-Yaba, Lagos, Nigeria.

[2]Department of Botany, University of Lagos, Akoka-Yaba, Lagos, Nigeria.

*Corresponding Author's Email: temitopeashamu@gmail.com

(Received: 5th June, 2024; Accepted: 15th October, 2024)

ABSTRACT

Alcaligens faecalis strain ULAG3AF was isolated from the Lagos Lagoon, an estuarine environment. The aim of the study was to investigate the biosurfactant production potentials of this organism and explore the antimicrobial potential of the biosurfactant. The bacterial isolate was screened for its biosurfactants production potentials using the drop collapse, oil displacement tests and emulsification index. The strain was cultivated in an enrichment medium, Bushnell Haas Medium, supplemented with 1%, 2%, 3% and 4% v/v spent engine oil and grown at different temperatures including 15 °C, 25 °C, 30 °C, 35 °C and 40 °C and salinity (10 mg/l, 20 mg/l, 30 mg/l and 40 mg/l of NaCl). The optimisation process involved analysing the effect of substrate concentration, temperature, and salinity on biosurfactant production by the bacterium using the one-variable-at-a-time (OVAT) approach. Gas Chromatography and Mass Spectrometry (GC-MS) and emulsification index were used to determine the rate of hydrocarbon degradation and the concentration of biosurfactant produced by the organism respectively. The bacterium demonstrated the ability to biodegrade different polyaromatic hydrocarbon components of spent engine oil and produced biosurfactants with an emulsifying index of 80.9% when cultivated in a medium containing 2% v/v of spent engine oil, 2% salinity (20 mg/l of NaCl) and a temperature of 40 °C after 7 days. The biosurfactant produced was a glycolipid with antimicrobial activities against *Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus.*

Keywords: Biosurfactant, Glycolipid, Biodegradation, Gas Chromatography and Mass Spectrometry, Polyaromatic hydrocarbon.

INTRODUCTION

Like chemical surfactants, biosurfactants are amphipathic molecules with both hydrophobic and hydrophilic moieties, which are produced by plants and microorganisms (Santos *et al.*, 2016). However, unlike chemical surfactants, they are less toxic and more environmentally friendly because of their degradability and stability over a wide range of environmental conditions, such as pH, temperature, and salinity (Franzetti *et al.*, 2018). As a result, there has been a growing interest in the study of biosurfactants over the past three decades. One of the important applications of biosurfactants is in the bioremediation of hydrocarbon-polluted environments (Peele *et al.*, 2016). Hydrocarbon pollutants are released into the environment as a result of the exploration, use, storage and disposal of petroleum hydrocarbon and its products, thereby affecting the ecology of such environments owing to their toxicity (Oyetibo *et al.*, 2017). While bioremediation offers a solution to the menace of hydrocarbon pollutants, the process is often limited by the hydrophobicity of the pollutants, which limits their bioavailability for degradation by microorganisms (Babaei & Habibi, 2019). Biosurfactants can increase the bioavailability of hydrocarbon pollutants by emulsifying them via pseudo-solubilisation and direct interfacial contact (Santos *et al.*, 2016). Typically, microorganisms that produce biosurfactants are hydrocarbonoclastic organisms that can metabolize hydrocarbons as their sole source of carbon (Joy *et al.*, 2017). The estuarine environment is a potential reservoir for hydrocarbonoclastic microorganisms, and consequently, biosurfactants producers because they harbour large deposits of hydrocarbon pollutants generated from anthropogenic activities in and around the estuarine (Di Gregorio *et al.*, 2016).

The Lagos Lagoon, which is the largest Lagoon in West Africa, is a brackish coastal lagoon that is exposed to diverse anthropogenic activities

including water transportation, industrialization and waste disposal (Alo *et al*., 2014). As a result, the lagoon is subjected to a significant level of hydrocarbon and metal pollutants (Oyeyiola *et al*., 2013) which is further enhanced by the unregulated environmental protection laws in the country (Edmund Emeka, 2015). For instance, Sogbanmu *et al*. (2016) reported that the Apapa Port, which is the busiest port along the Lagos Lagoon, is heavily contaminated due to the numerous petroleum tank farms located along the Port's terminals. Alo *et al*. (2014) also highlighted that the contamination of this large water body adversely affects the ecology of the Lagos Lagoon which is also a source of fisheries for the city. In a study by Sogbanmu *et al*. (2019), the authors reported that fish from four locations around the Lagos Lagoon were minimally contaminated with Polyaromatic Hydrocarbons (PAHs), which can pose a health risk to humans if such fishes are consumed.

Given the heavy contamination of the Lagos Lagoon, it is a rich source of hydrocarbon-degrading, biosurfactants-producing microorganisms (Di Gregorio *et al*., 2016). Several studies have reported the isolation of hydrocarbon-degrading bacteria from the Lagos Lagoon (Adebusoye *et al*., 2007; Buraimoh *et al*., 2020; Obayori *et al*., 2009, 2021; Obi *et al*., 2016). Biosurfactants-producing microorganisms isolated from estuarine environments like the Lagos Lagoon are desirable because the microorganisms from the estuarine environment can withstand extreme conditions owing to the fluctuating pH, salinity and temperature in that environment, thereby increasing the applications of the biosurfactants (Antoniou *et al*., 2015). Furthermore, hydrocarbon-contaminated environments also have extreme pH and salinity (Fathepure, 2014), thus, biosurfactants-producing organisms which can tolerate and produce biosurfactants under such conditions are important. This study, therefore, aims to investigate the biosurfactant production potentials of a bacterial isolate from the hydrocarbon-polluted Lagoon water; and explore the antimicrobial potential of the biosurfactant

MATERIALS AND METHODS

Sample Collection

Water samples were collected from the harbour region of three oil-contaminated sites on the Lagos Lagoon, Lagos State, Nigeria. The three sites included Apapa (3°22'40.233"E, 6°26'50.476"N), Kirikiri (3°18'22.674"E, 6°26'15.574"N) and Ijegun (3°14'40.547"E, 6°24'45.254"N). Approximately 1L of Lagoon water was collected in a sterile glass bottle and stored at 4 °C until use. The samples were collected at five different points at the sampling site and pooled together before being transported to the laboratory for further analysis.

Physicochemical Analysis of Samples

Physicochemical parameters including pH and conductivity were determined with pH (Pro2Go Portable Mettler Toledo, Germany) and conductivity meter (INE-EC100B conductivity meter) respectively, and total hydrocarbon (THC) was measured gravimetrically as described previously (BSI, 1990; Chopra and Kanwar, 1998). The Varian 720-ES Inductively coupled plasma optical emission spectrophotometer was used for the heavy metal analysis while the nitrate and phosphate Analysis were done using the Hach Model DR/2010 spectrophotometer.

Molecular Identification of Isolates

Bacterial DNA was extracted using the ABL-GA-GBB100 Presto Mini Genomic DNA Extraction Kit following the manufacturer's instructions. A NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was used to quantify DNA concentration at 260/280 nm. The quality of the PCR amplicon was viewed using 1% agarose gel electrophoresis, and the DNA was stored at –20 °C. The conserved region (16S rRNA gene) targeted for analysis was the V4 –V5 hypervariable region of the 16S rRNA gene, which was amplified using primers 515F-Y and 926R with Illumina adapters and barcodes. Pair-end sequencing was performed using a 600 – cycle MiSeq® Reagent Kit V3 (Illumina, U.S.A.).

Enrichment and Isolation of Hydrocarbon-Degrading, Biosurfactant-Producing Bacteria

The enrichment was carried out in 500 ml conical flasks containing 100ml Bushnell Hass Broth containing K_2HPO_4 (1 g/L); KH_2PO_4 (1 g/l); NH_4NO_3 (1 g/l); NaCl (30.0 g/l); $FeCl_3$ (0.5 g/l); $CaCl_2$ (0.02 g/l), $MgSO_4$ (0.02 g/l) as described by Adebusoye *et al.* (2007). The medium was amended with crude oil (1% v/v). This was then inoculated with 10 ml water sample and was sealed with a cotton plug before incubation at room temperature (28 ± 2 °C) at 150 rpm for 7 days. After 7 days, 10 ml aliquot was transferred into 100 ml of fresh Bushnell Haas broth amended with 1% (v/v) of crude oil and incubated under the same conditions. The transfer was repeated three times and 1 ml from the final transfer was plated out on Bushnell Haas agar and the pure isolates were selected for further studies.

Screening of Isolates for Biosurfactant Production and Biodegradation Studies

Axenic cultures of the isolate was further incubated in Bushnell Haas broth amended with 1% (v/v) of crude oil at 150 rpm and 28 °C for 7 days. The broth was then centrifuged at 10, 000 × g for 10 min at 4 °C and the cell-free supernatant (CFS) was filtered through Whatman filter paper No.1. The filtrate was then analysed for biosurfactant production using oil displacement test, drop collapsing assay and emulsification properties tests (Ram *et al.*, 2019). The experiment was set up in triplicate, and all broths in each set were sacrificed for total viable count (TVC) determination, biosurfactant quantification, and biodegradation studies every two days. The TVC was determined using the standard plate count technique to determine the microbial populations in the samples (Bloem, Hopkins and Benedetti, 2005), whereas the rate of degradation of hydrocarbons was determined by Gas Chromatography and Mass Spectrometry (GC-MS) using the Varian CP-3800 GC. The ability of the bacterial isolate to metabolize the Polyaromatic Hydrocarbons (PAH) in spent engine oil during the production of biosurfactants was determined. Using GC-MS, the quantities of selected PAH known to be present in motor engine oil were assessed in the samples over 14 days when the isolates were cultivated at 30 °C with 1% salinity and 1% (v/v) spent engine oil.

Drop Collapse Test

Two µl of olive oil were added to a 96-well microtitre plate, and the plate was equilibrated for 1 h at 37 °C and 5 µl of the CFS was added to the surface of the oil. The shape of the drop on the oil surface was observed after 1 minute. The culture supernatant that made the drop collapse was indicated as positive result and the drops that remained beaded were recorded as negative, and were compared with distilled water as control.

Oil Displacement

This was done using the methods proposed by Youssef *et al.* (2004). Forty microlitre of distilled water was placed in a Petri dish and 15 µl of spent engine oil was dropped on the surface of the distilled water to form an oil film. Ten microlitre of the cell-free supernatant was then gently dropped on the surface of the oil film. An area of clear halo was observed after 30 seconds and the diameter recorded.

Emulsification Index

As described by Kiran *et al.* (2009), 2 ml of the spent engine oil was added to an equal volume of cell-free supernatant and homogenized for two minutes in a vortex at very high speed. The emulsification stability was measured after 24 h, and the emulsification index was calculated by dividing the measured height of the emulsion layer by the total height of the liquid layer and multiplying by 100. The emulsification activity of the isolates was compared to that of Tween 80. The EI was also used to quantify the amount of biosurfactant produced.

Extraction of Biosurfactant

The biosurfactant in the cell-free filtrate was extracted using liquid-liquid extraction. The cell-free supernatant was acidified to pH 2.0 using 1 N HCl, and then mixed with an equal volume of chloroform and methanol (2:1) mixture. The organic phase containing the biosurfactant was then collected in a separation funnel. The aqueous phase was extracted three times to ensure a maximum yield, while the organic extract was dried using a rotary evaporator. Biosurfactant yield was determined using the equation proposed by Irorere *et al.* (2017).

Yield = Biosurfactant mass/ volume of cell-free supernatant

Eight strains which could utilise spent engine oil and produce biosurfactants were identified. The isolate with the highest biosurfactant yield and emulsification index was selected for further studies.

Optimisation of Biosurfactant Production Using One-Variable-at-a-Time Method

Optimisation involved analysing the effect of concentration, temperature, and salinity (% NaCl) on biosurfactant production in the isolates using the one-variable-at-a-time (OVAT) (Rane *et al*., 2017). Once one of the assessed parameters is optimised, it is set as the optimal level for the next round of OVAT studies to optimise other variables. The substrate/carbon source used was Spent Engine oil (SEO) added to the production medium (Bushnell Hass Medium) at different concentrations (1%, 2%, 3% and 4% volume per volume). The temperature range were 15 ^{o}C, 25 ^{o}C, 30 ^{o}C, 35 ^{o}C and 40 ^{o}C while the salinity was 10 mg/l, 20 mg/l, 30 mg/l and 40 mg/l of NaCl in the medium. These were the independent variables whereas the emulsification index was the dependent variable.

Characterisation of Biosurfactants

The characterisation of the biosurfactant produced involves identifying the chemical structure of the biosurfactant and its bioactive properties and was performed using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy to analyse the functional classes and types of bonds present in the biosurfactant produced. Spectroscopy was conducted using the Agilent Technologies Cary 630 FTIR Cary630 ZnSe spectrometer in the range of 4000 - 650 cm^{-1}. The analysis was conducted in accordance with the manufacturer's instructions.

Antimicrobial Activity of Biosurfactant

The antibacterial activity of the biosurfactant was determined using the agar well diffusion technique as described by Das *et al.* (2016). The bacterial strains used to test the antibacterial activity of the biosurfactant were *Staphylococcus aureus,* and *Pseudomonas aeruginosa* obtained from the Covenant University Microbial Collection Center. The test organisms were cultivated on Muller-Hinton agar plate into which wells had been made with sterile cork-borer. About 100 µl of the biosurfactant was added into the wells and incubated for 24 hours at 37^{o}C. Streptomycin was used for positive control whereas sterile distilled water was used for negative control. The antibacterial activity was evaluated based on the presence of a clear zone which indicates inhibition of growth and the diameter of the zone of inhibition was measured in millilitres.

RESULTS

Physicochemical and Microbial Analysis of Samples

Water samples collected from three sites on the Lagos Lagoon were heavily contaminated with hydrocarbons and heavy metals (Table 1). The Apapa site had the highest hydrocarbon pollution of the three sites. It also had the highest heavy metal contamination except for cadmium and chromium which are more abundant at the Ijegun site (0.16 and 1.10mg/l respectively). All three sites had heavy metal pollution at levels that were significantly higher than the national and (World Health Organisation) WHO standards. At the Apapa site, heavy metal contamination was Fe>Pb>Cr>Cd, at the Ijegun site heavy metal contamination was Cr>Pb>Fe>Cd while at the Kirikiri site, it was Cr=Pb>Fe>Cd. Surprisingly, however, the Apapa site had a lower concentration of hydrocarbon-utilizing bacteria compared to the Ijegun site, although it had the highest concentration of heterotrophic bacteria (Table 2).

Table 1: Physicochemical Analysis of Water Samples.

S/N	PARAMETER	WATER SAMPLE CODES			NESREA STANDARDS	WHO RECOMMENDATION
		AP1	JG1	KR1		
1	pH	6.80	7.20	7.13	-	-
2	Conductivity (µs/cm)	59,100	35,906	43,845	1000	NA
3	Salinity (mg/l)	35.73	20.96	32.73	-	-
4	THC (mg/l)	12.13	11.40	7.38		
5	Nitrate (mg/l)	5.94	6.09	4.87	10-20	10
6	Phosphate (mg/l)	2.98	3.15	3.12	-	1
7	Potassium (mg/l)	2.08	3.10	3.01	-	-
8	Sodium (mg/l)	1.55	1.05	1.29	-	-
9	Lead (mg/l)	1.06	0.96	1.02	0.01	0.05
10	Cadmium (mg/l)	0.05	0.16	0.07	0.003	0.002
11	Iron (mg/l)	1.62	0.89	0.97	0.300	0.300
12	Chromium (mg/l)	1.04	1.10	1.02	0.05	0.150

NB – Total Hydrocarbon Content (THC), Not Available (NA), National Environmental Standards and Regulations Enforcement Agency (NESREA), World Health Organisation (WHO).

Table 2: Total Viable Count

Sample	Bacteria		Fungi	
	THB (CFU/ml)	THD (CFU/ml)	THF (CFU/ml)	THD (CFU/ml)
AP1	8.01×10^5	1.10×10^4	1.15×10^5	2.10×10^3
KR1	3.14×10^5	0.90×10^4	1.30×10^5	3.09×10^3
JGI	3.91×10^5	1.41×10^4	1.70×10^5	2.60×10^3

Identification and Antimicrobial Sensitivity Profile Biosurfactant-Producing Isolates

Isolate K3A1 (Table 3) was identified by 16S rRNA sequencing. Sequence alignment and phylogenetic tree analysis showed that the 16S rRNA sequence obtained in this study was identified as *Alcaligenes faecalis* strain ULAG3AF (Figure 1). Antimicrobial sensitivity tests showed that *Alcaligens faecalis* strain ULAG3AF was sensitive to all the antibiotics tested, except Cefotuxime, Nitrofurantoin and Ampliclox (Figure 2).

Table 3: Capability of isolates to produce biosurfactants based on drop collapse, oil displacement and Emulsification Index.

Bacterial Isolates	Drop Collapse Assay	Oil Displacement Test	Emulsification Index (%)	Yield in 1000 ml of Broth (ml)
K3A1	**+**	**++**	**68**	**2.2**
K3B4	+	++	63	2.2
K3C3	+	+	24	1.8
K3D1	+	++	53	2.0
K3E1	-	+	29	0.3
K3F7	+	+	42	1.8
K3G1	+	+	32	0.9
K3H1	+	++	45	0.3

Drop Collapse Test: (-) indicates rounded drops of oil. (+) indicates spread of the oil.
Oil Displacement Test: (-) indicates no spread, (+) incomplete spread ranging between 0.5 and 1.0 cm, (++) indicates significant spread of diameter ranging from 1.0 cm to 2.0 cm, (+++) indicates complete spread with diameter >2cm. The isolate selected for further studies are in bold.

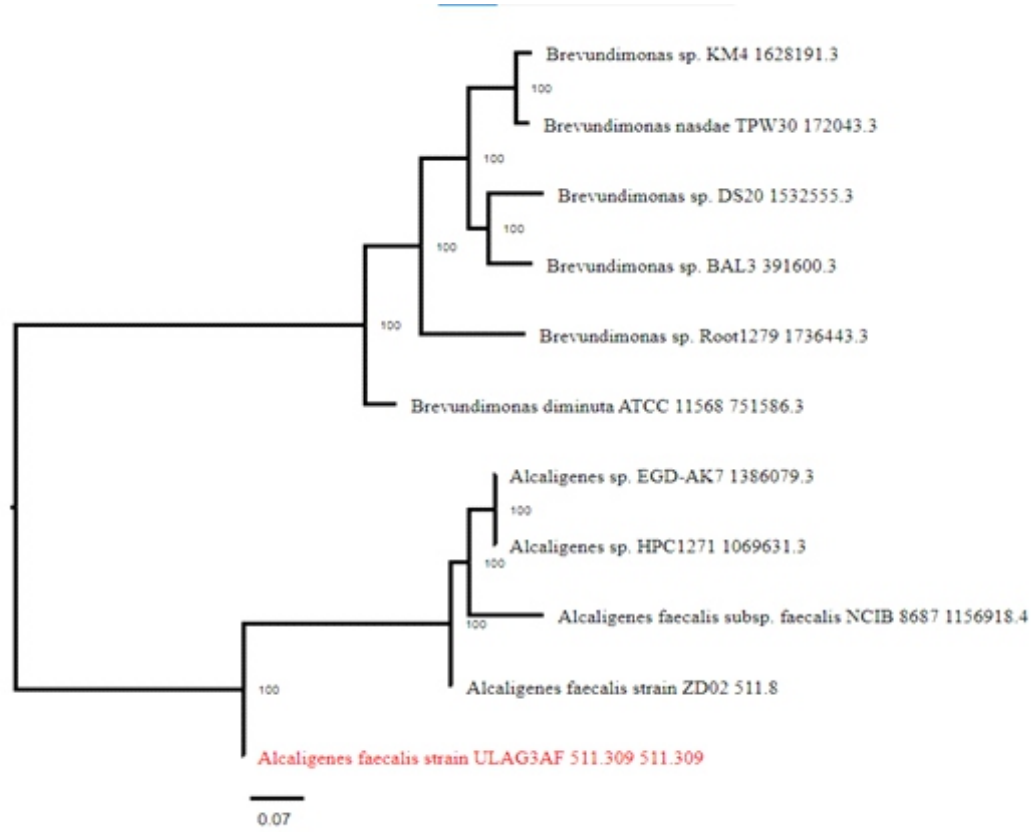

Figure 1: Phylogenetic Tree of *Alcaligens faecalis* strain ULAG3AF with the Unique Genome Identifier.

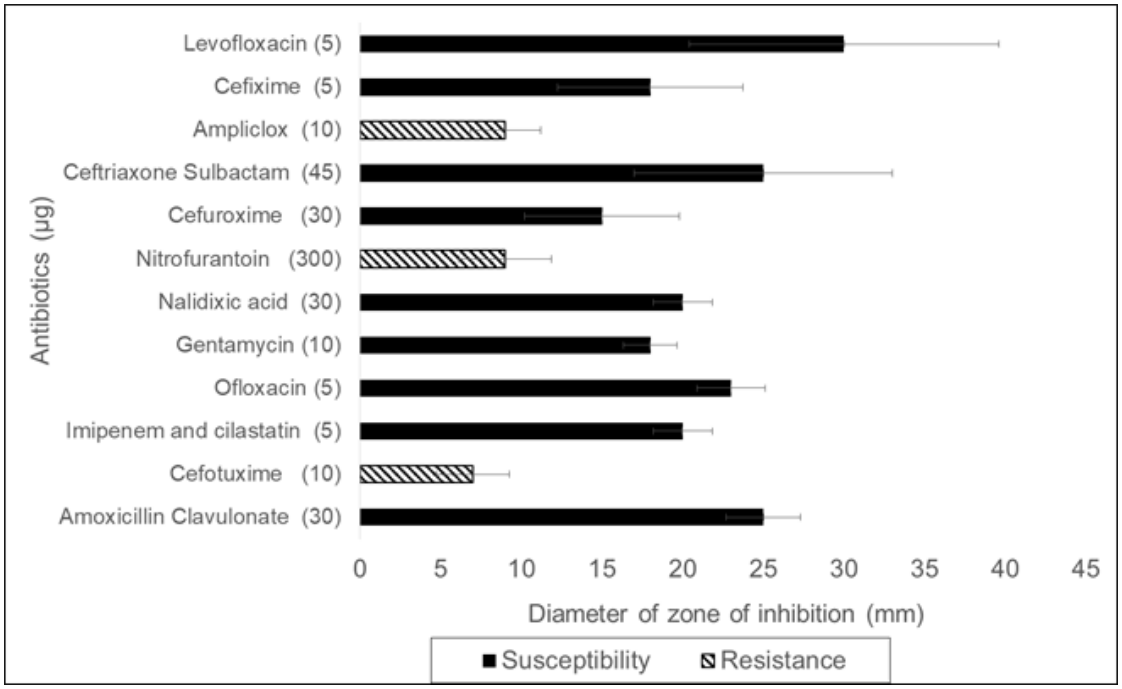

Figure 2: Antibiotics Resistance Profile of *Alcaligens faecalis* strain ULAG3AF
NB: Resistant = Diameter of zone of inhibition is <15 mm; Susceptible = Diameter of zone of inhibition is ≥15mm.

Biodegradation Ability of the Isolate

Alcaligens faecalis strain ULAG3AF utilised all the PAH during the production of biosurfactant with spent oil as a substrate, albeit at different rates (Table 4). *Alcaligens faecalis* strain ULAG3AF degraded between 50.34% (benzo (b) fluoranthene) 96.96% (benzo (a) anthracene) of the PAHs in 14 days. Significant utilisation of the PAHs was observed between days 1 and 7 when there was also significant emulsification activities, indicating biosurfactant production (Figure 3).

Table 4: The Degree of Degradation of Polyaromatic Hydrocarbons in Spent Engine Oil over 14 Days by *Alcaligens faecalis* strain ULAG3AF.

Polyaromatic Hydrocarbon	Degree of Degradation (%)
Benzo(a)anthracene	96.96
Napthalene	92.46
Dibenzo(a,h)anthracene	91.74
Fluorene	91.66
Indeno(1,2,3-c,d)pyrene	90.82
Acenaphtene	90.71
Pyrene	88.89
Acenaphtyylene	88.41
Benzo(a)pyrene	88.12
Phenanthrene	88.05
Fluoranthracene	87.98
Anthracene	87.84
Benzo(g,h,i)perylene	82.87
2-methyl napthalene	58.14
Chrysene	55.25
Benzo (b)fluoranthene	50.34

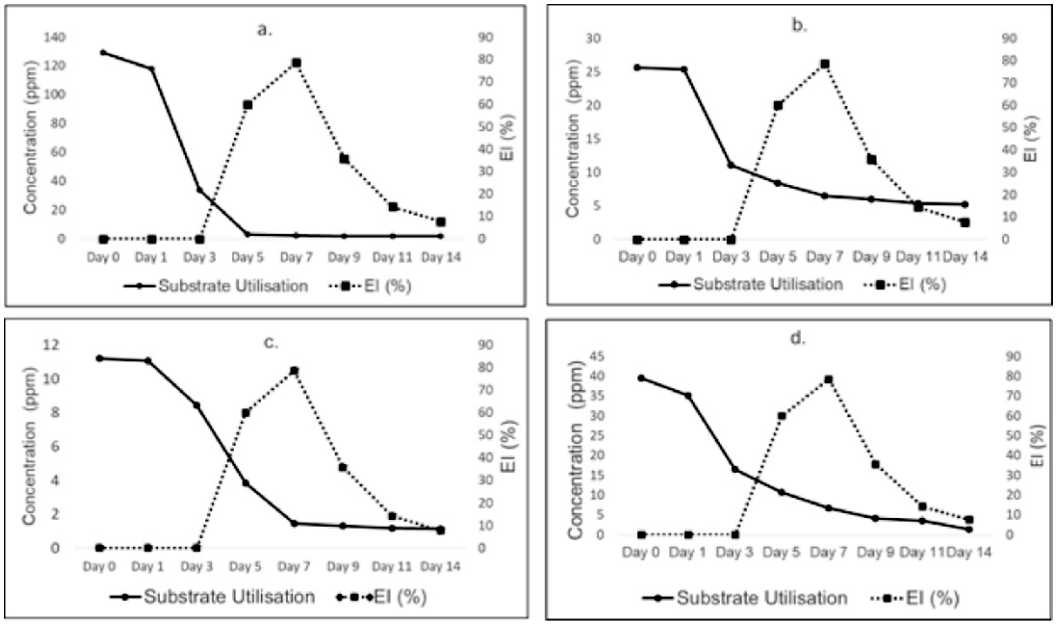

Figure 3: The Relationship between Emulsification Index (EI) and Utilisation of Naphtalene (a.), Dibenzo(a,h)anthracene (b.), Flourene (c.), and Benzo(a)anthracene (d.) by *Alcaligens faecalis* strain ULAG3AF during Biosurfactant Production.

Biosurfactant Production Process Optimisation

Effect of Substrate and Concentration on Biosurfactant Production

Figure 4 shows the emulsification index (biosurfactant production) over 14 days with different concentrations of spent engine oil. At all concentrations, biosurfactant production began after day 3 and peaked between days 7 and 9 (Figure 4). After day 9, biosurfactant production began to decline. Generally, biosurfactant production rate was directly proportional to the growth rate of the isolate. The highest Emulsification Index (EI) (78.9%) was observed on day 7 with 2% (v/v) spent oil. However, there was no significant difference between the EI values observed on day 7 with 2 and 3% (v/v) spent oil. With 3% and 4% v/v spent oil high EI was observed on days 9 and 11, respectively, suggesting delayed biosurfactant production with an increase in substrate concentration.

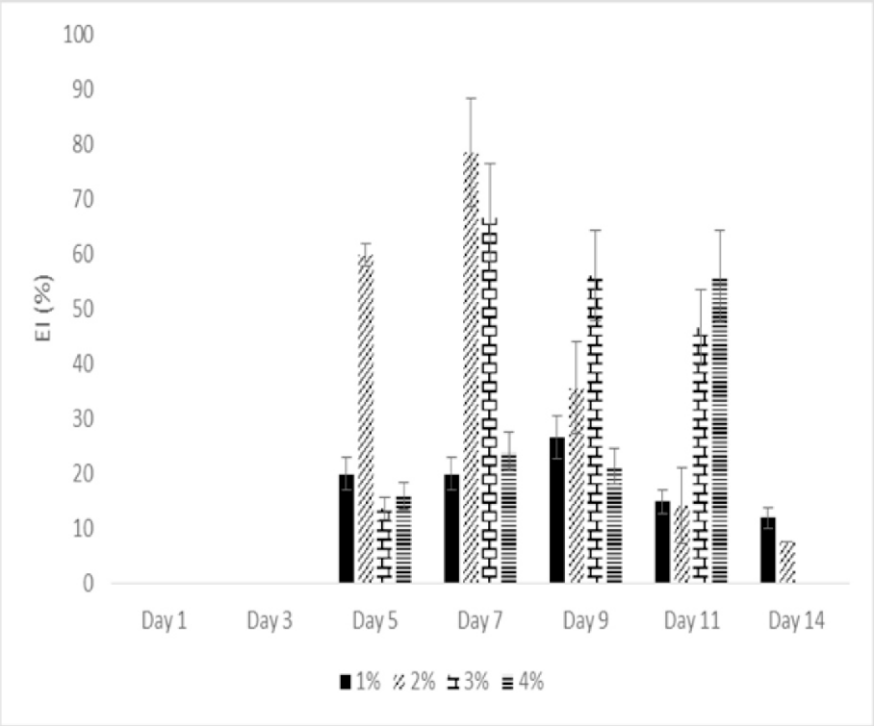

Figure 4: Biosurfactant Production (Measured in Emulsification Index) by *Alcaligens faecalis* strain ULAG3AF in Spent Oil at 1%, 2%, 3% and 4% v/v. (NB: EI- Emulsification Index).

Effect of Salinity on Biosurfactant Production

At 1 and 2% salinity (10 mg/l and 20 mg/l NaCl), bioproduction was not observed until day 5 when *Alcaligens faecalis* strain ULAG3AF was cultivated with 2% (v/v) SEO at 30 °C (Figure 5). The onset of bioproduction appeared to occur earlier as the level of salinity increased. The late onset observed at 1 and 2% salinity notwithstanding, the highest concentrations of biosurfactant production was observed at these levels of salinity once bioproduction commenced. The highest yield was observed on day 7 with 1% salinity producing an EI of 78.57% and 2% salinity an EI of 78.97%, suggesting that there was no difference in the ability of *Alcaligens faecalis* strain ULAG3AF to produce biosurfactants at both salinity levels. However at higher salinity levels (3-5%), bioproduction rates are considerably lower although they span for a longer period. In addition, beyond day 7, bioproduction rates reduced at both 1 and 2% salinity levels.

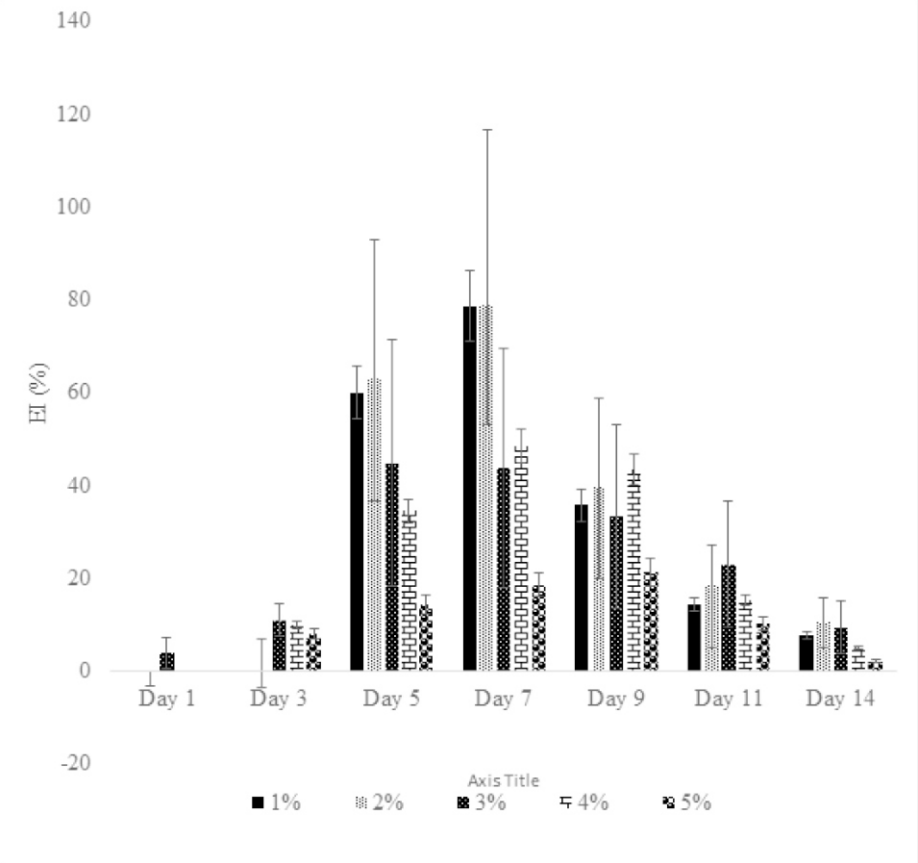

Figure 5: Biosurfactant Production (Measured in Emulsification Index) by *Alcaligens faecalis* strain ULAG3AF in Spent Engine Oil at 1% (a.), 2% (b.), 3% (c.), 4% (d.) and 5% (e) Salinity.

Effect of Temperature on Biosurfactant Production

When *Alcaligens faecalis* strain ULAG3AF was cultivated with 2% salinity and 2% (v/v) spent oil, significant EI was observed from 25°C, and reached its peak at 35°C (80.94%) with a slight decline at 40 °C (75.85%) (Figure 6). However, there was no significant difference between the yields obtained at the temperature ranging from 25 °C to 40 °C. Additionally, although biosurfactant production peaked on day 7 and declined significantly on day 9, there is no significant difference between the yields observed on days 5 and 7.

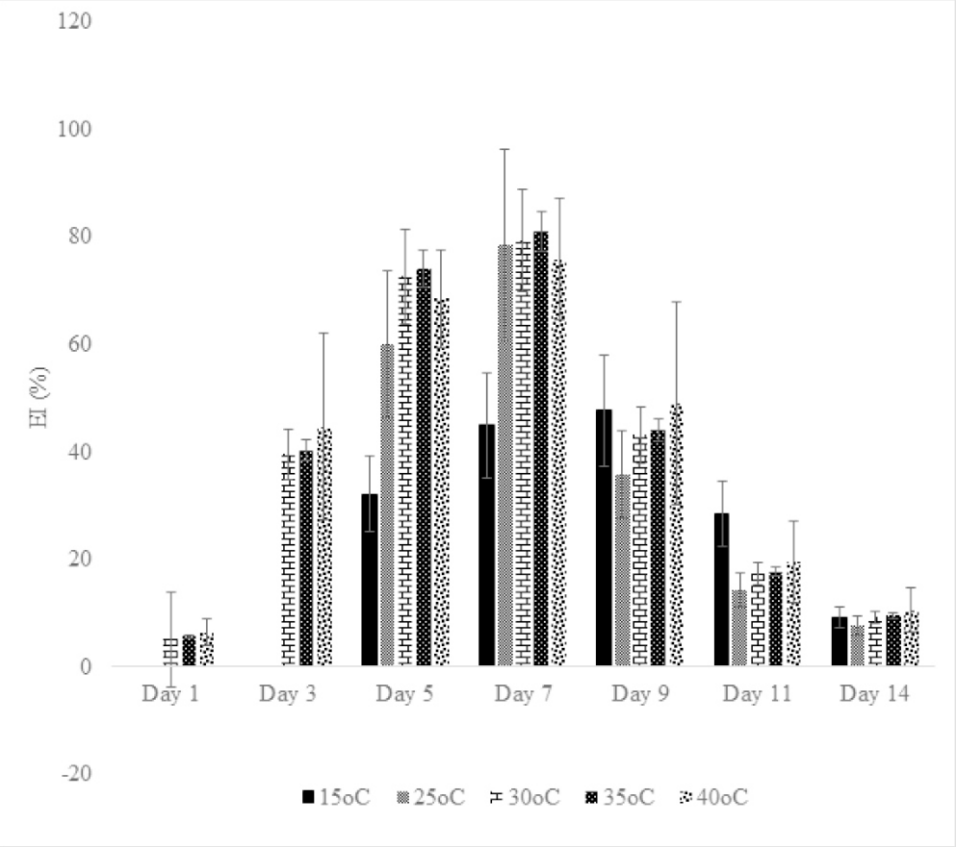

Figure 6: Biosurfactant Production (Measured in Emulsification Index) by *Alcaligens faecalis* strain ULAG3AF in 2% v/v Spent Oil at 15 °C, 25 °C, 30 °C, 35 °C and 40 °C.

Chemical Characterisation of Biosurfactants

The Attenuated Total Reflectance Fourier Transform Infrared (FTIR) spectrum of the biosurfactant produced by *Alcaligens faecalis* strain ULAG3AF is shown in Figure 6. The FTIR analysis of the biosurfactants produced by *Alcaligens faecalis* strain ULAG3AF showed a total of 22 absorbance peaks. Table 5 shows some peaks and the corresponding functional groups present in the biosurfactants based on evidence from literature (Das *et al*., 2016). The biosurfactant produced by *Alcaligens faecalis* strain ULAG3AF had OH, CO and CH bonds.

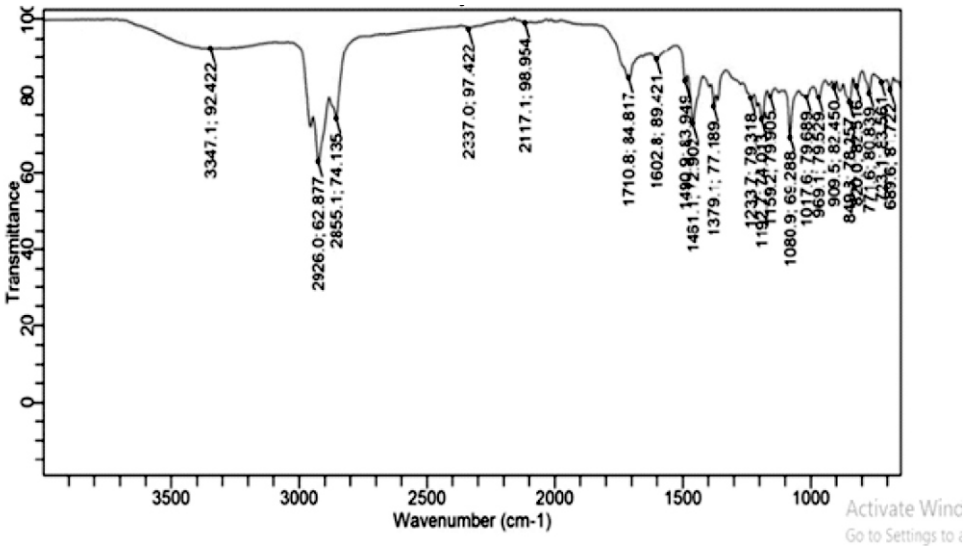

Figure 7: FTIR analysis of Biosurfactant Production by Alcaligens *faecalis* strain ULAG3AF.

Table 5: Peaks and corresponding functional groups present in the biosurfactants produced by *Alcaligens faecalis* strain ULAG3AF.

Organism	Wavelength	Bonds
Alcaligens faecalis strain ULAG3AF	3347.1	OH bond or N-H bond
	2926, 2855	C-H bond
	2337	O=C=O Bond
	1080	C=O Bond

Antimicrobial Activity

The biosurfactant produced by *Alcaligens faecalis* strain ULAG3AF showed antibacterial but not antifungal properties (Table 6). *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Gram-negative bacteria) showed the least susceptibility to the biosurfactant, while *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive bacteria) showed the higher susceptibility to the biosurfactant. However, the zones of inhibition with the biosurfactant were lower compared to the zones of inhibition observed with the chemical antibiotic, Streptomycin, which was used as the positive control.

Table 6: Antimicrobial Activity of Biosurfactant.

Organism Used	Diameter of Zone of Inhibition (mm)± SD		
	Biosurfactant	**Positive Control**	**Negative Control**
Pseudomonas aeruginosa	2.1 ± 1.05	24 ± 1.32	0
Bacillus subtilis	6.7 ± .41	19 ±3.16	0
Staphylococcus aureus	4.8 ± 0.76	36 ± 3.17	0
Klebsiella pneumoniae	2.1 ± 0.20	31 ± 0.62	0
Aspergillus niger	0	15.61± 0.16	0

NB: SD- Standard Deviation, Positive controls: Streptomycin (10 µg/ disc) for bacteria, Fluconazole (1.0 mg/disc) for the fungus.

DISCUSSION

The presence of heavy metal and hydrocarbon pollutants at the sampling points is unsurprising given that the Lagos Lagoon is exposed to diverse anthropogenic activities, including water transportation, industrialization, and waste disposal (Alo *et al*., 2014). In an earlier study, Sogbanmu *et al.* (2016) reported that the Apapa site of the Lagos Lagoon was the most contaminated site on the lagoon, which spans approximately 6000 km sq, owing to the presence of numerous petroleum tank farms located along the port's terminals.

In this study, the isolated hydrocarbon-utilizing and biosurfactant-producing bacterium was identified as *Alcaligenes faecalis* strain ULAG3AF. The isolate showed high emulsification indices and stronger oil displacement activity but weak drop collapse activity. Both oil displacement and drop collapse activities are typically used for the qualitative identification of biosurfactant producers, whereas the emulsification index (EI) allows the quantification of the biosurfactant production potentials of the producing organism (Irorere *et al*., 2017). The emulsification index shows a linear relationship between emulsification activity and the amount of biosurfactant produced; hence, it is ideal for quantifying the amount of biosurfactant produced (Mounira & Abdelhadi, 2015). In this study, multiple screening methods were used to identify biosurfactant producers, as this would help eliminate the drawbacks of individual screening methods, ensuring that true biosurfactant producers are identified (Walter *et al*., 2010).

Alcaligenes faecalis has previously been isolated from different hydrocarbon-contaminated sites, including sediment and water from the Lagos

Lagoon, soil from mechanical workshops, and wastewater amongst others (Igwo-Ezikp *et al*., 2009; Muftau *et al*., 2021; Salam *et al*., 2018). These studies demonstrate the hydrogen-utilizing abilities of the bacterium. However, despite its application in bioremediation, the isolate is a known opportunistic pathogen, and its presence in a body of water, such as the Lagos Lagoon, can pose an environmental threat (Tang *et al*., 2020). Infections caused by opportunistic pathogens, which are often transmitted by water, can cause significant public health concerns, particularly if they are resistant to many antibiotics (Stec *et al*., 2022). Furthermore, the use of biosurfactants from pathogenic bacteria is ethically unacceptable because of the possibility of the co-production of harmful toxins or pigments (Uzoigwe *et al*., 2015). Although the *Alcaligens faecalis* strain ULAG3AF isolated herein was sensitive to all the antibiotics tested, except Cefotuxime, Nitrofurantoin and Ampliclox, its potential pathogenicity may limit its applicability for bioremediation.

This isolate demonstrated the ability to utilize different polyaromatic hydrocarbons as substrates for the production of biosurfactants. The ability of the bacterium to utilise hydrocarbons may be attributed to the presence of hydrocarbon degradation enzymes in the organism (Adetitun *et al*., 2020). Undugoda, Kannangara, and Sirisena (2016) reported the presence of the *phnG* gene, which is involved in phenanthrene degradation, and the *nah*R and *nah*U genes both involved in the naphthalene degradation pathways in the genome of *Alcaligens faecalis*. The hydrocarbon-utilizing capability of the isolate makes it a candidate for the bioremediation of PAH-contaminated sites. As observed in this study, spent engine oil, which is a mixture of different PAHs, sustained both the growth and biosurfactant production potential of the isolate for 14 days, although the biosurfactant production rate began to decreased after day 7. The extended sustenance of the bacteria by the substrate (spent engine oil) was possibly due to the organisms assimilating the different PAHs present in the mixture at different rates. According to Brown and Jones (2024), the carbon use efficiency of substrates increases when there is substrate diversity, thereby, resulting in prolonged microbial carbon uptake and microbial carbon storage potential.

The results obtained herein show that at 1 to 5% salinity levels, *Alcaligens faecalis could* produce biosurfactants, although the optimum salinity levels were between 1% and 3%. The estuarine environment, from whence the bacterium was isolated, has variable levels of salinity and undergoes constant reflux; hence, the ability of the isolate to withstand a range of salinity levels is unsurprising (Hillmann *et al*., 2019). Additionally, this strain of *Alcaligens faecalis in* this study grew and produced biosurfactant optimally at temperature range from 25 °C to 40 °C, suggesting that the organism can withstand fluctuating environmental temperature. This result is similar to findings reported by Arabo *et al*. (2021) and Najafi-Marghmaleki *et al*. (2018), who reported optimal production of biosurfactant by *A. faecalis* at 35 °C and 28 °C, respectively. Bharali *et al*. (2011) also reported that the *A. faecalis* strain in their study grew and produced biosurfactants optimally at 42°C. This temperature is higher than the temperature reported in this study, suggesting that the organism is possibly mesophilic, considering that mesophiles can grow between 20 °C and 45 °C (Zuberer and Zibilske, 2021). However, Salehizadeh & Mohammadizad (2009) reported that at 50 °C, the strain of *Alcaligens faecalis* grew and produced biosurfactants optimally. This suggest that the bacterium may be a facultative thermophile because of its ability to withstand high temperatures (Zuberer and Zibilske, 2021). The ability of *A. faecalis* to produce biosurfactants at such high temperatures is an attractive feature for biotechnological and bioremediation studies (Antoniou *et al*., 2015).

The biosurfactant produced by *Alcaligens faecalis* strain ULAG3AF had OH, CO, and CH bonds, suggesting that the biosurfactants are glycolipids (Hamed *et al*., 2021). Bharali *et al*. (2011) also reported the production of glycolipids by *Alcaligens faecalis*. Furthermore, glycolipids such as rhamnolipids have been reported to be produced by the uptake of hydrophobic substances, including pure samples of pyrene, chrysene, naphthalene, and fluoranthene (Patowary *et al*., 2022) and hydrocarbon mixtures such as diesel and crude oil (Malavenda *et al*., 2015; Maneerat *et al*., 2019). According to Li *et al*. (2015), biosurfactants increase the hydrophobicity and

solubilisation of the cell surface, thereby increasing the bioavailability of the hydrophobic substrate to the microorganism, which then metabolizes or degrades it.

The biosurfactant produced by the strain of *Alcaligens faecalis* in this study exhibited antimicrobial activities. However, it appeared to be more effective against gram-positive isolates than gram-negative isolates. These results contradict those reported by Bharali *et al.* (2011), who reported that the biosurfactant produced by their strain of *Alcaligens faecalis had* a higher antimicrobial effect on gram-negative bacteria than gram-positive bacteria. The antimicrobial activity of biosurfactants is important for the survival of the biosurfactant producer to thrive in a competitive environment (Banat *et al*., 2014). Different authors have proposed the mode of action of biosurfactants as antimicrobial agents. For instance, Gomaa (2012) reported that the addition of lipopeptide biosurfactant to *Staphylococcus aureus* cells resulted in the reduction of the protein and lipid content of the cells, possibly due to the biosurfactant preventing protein synthesis by binding to the 30S ribosome and preventing the aminoacyl-tRNA from binding to the 30S ribosome. Additionally, Cortés-Sánchez *et al.* (2013) biosurfactants can alter the structure of proteins in the cell wall, thereby increasing their permeability and resulting in cell lysis.

The findings from this study also showed that the inhibition zones produced by the biosurfactant was lower than those produced by chemical antimicrobials (positive controls). These findings suggest that biosurfactant may be required at higher concentration to be more effective. However, this study did not investigate the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the biosurfactant. While MIC is the minimum concentration of a substance that can elicit a bacteriostatic effect on a bacterium, MBC is the minimum concentration that is lethal to a particular bacterium (Bindayna and Al-Salman, 2023). Determining the MIC and MBC of the biosurfactant would provide a quantitative assessment of the effectiveness of the biosurfactant as an antimicrobial agent (de Freitas *et al*., 2019). Nonetheless, this study highlights the antimicrobial potential of the biosurfactant produced by *Alcaligenes faecalis* strain ULAG3AF.

CONCLUSION

This study explored the hydrocarbon-degrading and biosurfactant-producing capabilities of *Alcaligens faecalis* strain ULAG3AF, a mesophile which could produce biosurfactant optimally at 40°C. Using spent engine oil, this study demonstrated the ability of the bacterium to utilise different polyaromatic hydrocarbon components of spent engine oil while producing a biosurfactant with an emulsifying index of 80.9%, when cultivated in a medium with 2% v/v salinity (20 mg/l NaCl) and substrate at 40 °C in 7 days. The bacterium also demonstrated the ability to degrade a wide range of PAHs including pyrene, naphthalene and chrysene. Although the bacterium is a known opportunistic pathogen, a quality that could discourage its use for environmental bioremediation owing to the public health threat it poses, it showed susceptibility to a wide range of antibiotics. The biosurfactant it produces was also identified as a glycolipid with antimicrobial activities against selected bacteria and fungi.

ACKNOWLEDGEMENT

Not Applicable

FUNDING

This research was not funded by any organisation

CONFLICT OF INTERESTS

The authors declare they have no conflict of interest

AUTHORS' CONTRIBUTIONS

A.T.O designed and conducted the research, analysed and interpreted the data and wrote the manuscript. A.E.O. designed and supervised the research and edited the manuscript. A.A.A. proofread and edited the manuscript.

REFERENCES

Adebusoye, S. A., Amund, O. O., Illori, M. O., Domeih, D. O., and Okpuzor, J. 2007. Growth and biosurfactant synthesis by Nigerian hydrocarbon-degrading estuarine bacteria. *Revista de Biología Tropical*, *56*: 1603–1611. doi:10.15517/rbt.v56i4.5746

Adetitun, D. O., Fagbemi, F. O., and Oguntoye, M. B. 2020. Hydrocarbon Degradative Potential of Alcaligenes sp. strain 3k Under Modified Nitrate Concentrations. *Nigerian Journal of Pure and Applied Sciences*, *2*: 3764–3770. doi:10.48198/NJPAS/20.B03

Alo, B., Olayinka, K., Oyeyiola, A., Oluseyi, T., Alani, R., & Abayomi, A. 2014. Studies and Transactions on Pollution Assessment of the Lagos Lagoon System, Nigeria. In The Land/Ocean Interactions in the Coastal Zone of West and Central Africa. Estuaries of the World., S. Diop, J. P. Barusseau, & C. Descamps (Editors), Springer, London, pp. 65–76. doi:10.1007/978-3-319-06388-1_6

Antoniou, E., Fodelianakis, S., Korkakaki, E., & Kalogerakis, N. 2015. Biosurfactant production from marine hydrocarbon-degrading consortia and pure bacterial strains using crude oil as carbon source. *Frontiers in Microbiology*, 06:1-14. doi:10.3389/fmicb.2015.00274

Arabo, A. A., Bamanga, R. A., Fadilu, M., Abubakar, M., Shehu, F. A., Yakasai, H. M., & Abdullahi, N. 2021. Isolation and Characterization of Biosurfactant-producing Alcaligenes sp. YLA11 and its Diesel Degradation Potentials. *Bioremediation Science and Technology Research*, *9*: 7-12. doi:10.54987/bstr.v9i2.619

Babaei, F., and Habibi, A. 2019. Fast biodegradation of diesel hydrocarbons at high concentration by the sophorolipid-producing yeast *Candida catenulata* KP324968. *Journal of Molecular Microbiology and Biotechnology*, *28*:240–253. doi:10.1159/000496797

Banat, I. M., De Rienzo, M. A. D. D., and Quinn, G. A. 2014. Microbial biofilms: biosurfactants as antibiofilm agents. *Applied Microbiology and Biotechnology*, *98*:9915–9929. doi:10.1007/s00253-014-6169-6

Bharali, P., Das, S., Konwar, B. K., & Thakur, A. J. 2011. Crude biosurfactant from thermophilic Alcaligenes faecalis: Feasibility in petro-spill bioremediation. *International Biodeterioration and Biodegradation*, *65*:682–690. doi:10.1016/j.ibiod.2011.04.001

Bindayna, K. M., and Al-Salman, J. 2023. Basic concepts in microbiology and immunology. Elseivier, New York, 366 pp. doi:10.1016/b978-0-323-95092-3.00001-9

Brown, R. W., and Jones, D. L. 2024. Plasticity of microbial substrate carbon use efficiency in response to changes in plant carbon input and soil organic matter status. *Soil Biology and Biochemistry*, *188*:109230. doi:10.1016/j.soilbio.2023.109230

Buraimoh, O. M., Ogunyemi, A. K., Oyegbite, I. E., Amund, O. O., and Ilori, M. O. 2020. Potentials of hydrocarbon utilization by wood-digesting bacteria isolated from the Lagos Lagoon, Nigeria. *Journal of Tropical Life Science*, *10*: 1-10. doi:10.11594/jtls.10.01.01

Cortés-Sánchez, A. de J., Hernández-Sánchez, H., & Jaramillo-Flores, M. E. 2013. Biological activity of glycolipids produced by microorganisms: New trends and possible therapeutic alternatives. *Microbiological Research*, *168*: 22–32. doi:10.1016/j.micres.2012.07.002

Das, M., Patowary, K., Vidya, R., & Malipeddi, H. 2016. Microemulsion synthesis of silver nanoparticles using biosurfactant extracted from Pseudomonas aeruginosa MKVIT3 strain and comparison of their antimicrobial and cytotoxic activities. *IET Nanobiotechnology*, *10*: 411–418. doi:10.1049/iet-nbt.2015.0119

de Freitas Ferreira, J., Vieira, E. A., and Nitschke, M. 2019. The antibacterial activity of rhamnolipid biosurfactant is pH dependent. *Food Research International*, *116*:737–744. doi:10.1016/j.foodres.2018.09.005

Di Gregorio, S., Siracusa, G., Becarelli, S., Mariotti, L., Gentini, A., and Lorenzi, R. 2016. Isolation and characterization of a hydrocarbonoclastic bacterial enrichment from total petroleum hydrocarbon contaminated sediments: potential candidates for bioaugmentation in bio-based processes. *Environmental Science and Pollution Research*, 23: 10587-10594 doi:10.1007/s11356-015-5944-y

Edmund Emeka, E. 2015. Comparative Analysis of Effluent Discharge from Emene Industrial Area of Enugu, Nigeria, With National and International Standards. *Civil and Environmental Research*, *7*: 84-93.

Fathepure, B. Z. 2014. Recent studies in microbial degradation of petroleum hydrocarbons in hypersaline environments. *Frontiers in Microbiology*, *5*:1-7. doi:10.3389/fmicb.2014.00173

Gomaa, E. Z. 2012. Antimicrobial activity of a biosurfactant produced by Bacillus licheniformis strain M104 grown on whey. *African Journal of Microbiology Research*, *6*:1–7. doi:10.5897/AJMR11.463

Hamed, M. M., Abdrabo, M. A. A., & Youssif, A. M. 2021. Biosurfactant production by marine actinomycetes isolates streptomyces althioticus rg3 and streptomyces californicus rg8 as promising sources of antimicrobial and antifouling effects. *Microbiology and Biotechnology Letters*, *49*:356–366. doi:10.48022/mbl.2106.06007

Hillmann, E., DeMarco, K., & La Peyre, M. 2019. Salinity and water clarity dictate seasonal variability in coastal submerged aquatic vegetation in subtropical estuarine environments. *Aquatic Biology*, *28*:175–186. doi:10.3354/ab00719

Igwo-Ezikp, M. N., Gbenle, O. G., Ilori, M. O., Okpuzor, J., & Osuntoki, A. A. 2009. Evaluation of *Alcaligenes faecalis* Degradation of Chrysene and Diesel Oil with Concomitant Production of Biosurfactant. *Research Journal of Environmental Toxicology*, *3*:159–169. doi:10.3923/rjet.2009.159.169

Irorere, V. U., Tripathi, L., Marchant, R., McClean, S., and Banat, I. M. 2017. Microbial rhamnolipid production: a critical re-evaluation of published data and suggested future publication criteria. *Applied Microbiology and Biotechnology*, 101: 3941–3951. doi:10.1007/s00253-017-8262-0

Joy, S., Rahman, P. K. S. M., & Sharma, S. 2017. Biosurfactant production and concomitant hydrocarbon degradation potentials of bacteria isolated from extreme and hydrocarbon contaminated environments. *Chemical Engineering Journal*, 317:232–241. doi:10.1016/j.cej.2017.02.054

Kiran, G. S., Hema, T. A., Gandhimathi, R., Selvin, J., Thomas, T. A., Rajeetha Ravji, T., & Natarajaseenivasan, K. 2009. Optimization and production of a biosurfactant from the sponge-associated marine fungus Aspergillus ustus MSF3. *Colloids and Surfaces B: Biointerfaces*, 73:250–256. doi:10.1016/j.colsurfb.2009.05.025

Li, S., Pi, Y., Bao, M., Zhang, C., Zhao, D., Li, Y., Sun, P., & Lu, J. 2015. Effect of rhamnolipid biosurfactant on solubilization of polycyclic aromatic hydrocarbons. *Marine Pollution Bulletin*, 101:219–225. doi:10.1016/j.marpolbul.2015.09.059

Malavenda, R., Rizzo, C., Michaud, L., Gerçe, B., Bruni, V., Syldatk, C., Hausmann, R., & Lo Giudice, A. 2015. Biosurfactant production by Arctic and Antarctic bacteria growing on hydrocarbons. *Polar Biology*, 38:1565–1574. doi:10.1007/s00300-015-1717-9

Maneerat, S., Phetrong, K., Jahan, R., Bodratti, A. M., Tsianou, M., Alexandridis, P., Nurfarahin, A. H., Mohamed, M. S., Phang, L. Y., Biniarz, P., Łukaszewicz, M., Janek, T., Santos, D. K. F., Rufino, R. D., Luna, J. M., Santos, V. A., Sarubbo, L. A., Fechtner, J., Cameron, S., … Zinjarde, S. 2019. Identification and characterisation of short chain rhamnolipid production in a previously uninvestigated, non-pathogenic marine pseudomonad. *Bioresource Technology*, 102: 1–6. doi:10.2139/ssrn.3323582

Mounira, A., & Abdelhadi, G. 2015. Assessment of four different methods for selecting biosurfactant producing extremely halophilic bacteria. *African Journal of Biotechnology*, 14:1764-1772. doi:10.5897/AJB2015.14611

Muftau, U. K., Juwon, A. D., & Olanipekun, Ekundayo, F. 2021. Appraisal on Microbiologal Qualities of Hospital Wastewaters from off a Local Government Area of Kwara State, Nigeria. *Journal of Advances in Microbiology*, 1: 25–38. doi:10.9734/jamb/2021/v21i930381

Najafi-Marghmaleki, A., Kord, S., Hashemi, A., & Motamedi, H. 2018. Experimental investigation of efficiency of MEOR process in a carbonate oil reservoir using *Alcaligenes faecalis*: Impact of interfacial tension reduction and wettability alteration mechanisms. *Fuel*, 232: 27–35. doi:10.1016/j.fuel.2018.05.117

Obayori, O. S., Fashola, M. O., Ashade, A. O., Opere, B. O., Adeoye, S. P., & Adeyeye, M. O. 2021. Isolation and characterization of biosurfactant producing bacteria from Mile 2 and Ologe Lagoons, Nigeria. *Malaysian Journal of Microbiology*, 18: 37–46. doi:10.21161/mjm.211149

Obayori, O. S., Ilori, M. O., Adebusoye, A. A., Oyetibo, G. O., Omotayo, A. ., & Amund, O. O. 2009. Degradation of hydrocarbons and biosurfactant production by Pseudomonas sp. strain LP1. *World Journal of Microbial Biotechnology*, 25: 1615–1623. doi:10.1007/s11274-009-0053-z

Obi, C. C., Adebusoye, S. A., Ugoji, E. O., Ilori, M. O., Amund, O. O., & Hickey, W. J. 2016. Microbial Communities in Sediments of Lagos Lagoon, Nigeria: Elucidation of Community Structure and Potential Impacts of Contamination by Municipal and Industrial Wastes. *Frontiers in Microbiology*, 7: 1213. doi:10.3389/fmicb.2016.01213

Oyetibo, G. O., Chien, M. F., Ikeda-Ohtsubo, W., Suzuki, H., Obayori, O. S., Adebusoye, S. A., Ilori, M. O., Amund, O. O., and Endo, G. 2017. Biodegradation of crude oil and phenanthrene by heavy metal resistant Bacillus subtilis isolated from a multi-polluted industrial wastewater creek. *International Biodeterioration and Biodegradation*, 120:143–151. doi:10.1016/j.ibiod.2017.02.021

Oyeyiola, A. O., Davidson, C. M., Olayinka, K. O., Oluseyi, T. O., & Alo, B. I. 2013. Multivariate analysis of potentially toxic metals in sediments of a tropical coastal Lagoon. *Environmental Monitoring and Assessment*, 185:2167–2177. doi:10.1007/s10661-012-2697-7

Patowary, R., Patowary, K., Kalita, M. C., Deka, S., Borah, J. M., Joshi, S. J., Zhang, M., Peng, W., Sharma, G., Rinklebe, J., & Sarma, H. 2022. Biodegradation of hazardous naphthalene and cleaner production of rhamnolipids — Green approaches of pollution mitigation. *Environmental Research*, 209: 112875. doi:10.1016/j.envres.2022.112875

Peele, K. A., Teja, V. R., & Vidya, C. 2016. Emulsifying activity of a biosurfactant produced by a marine bacterium. *3 Biotech*, 6:1–6. doi:10.1007/s13205-016-0494-7

Salam, L. B., Ilori, M. O., Amund, O. O., LiiMien, Y., & Nojiri, H. 2018. Characterization of bacterial community structure in a hydrocarbon-contaminated tropical African soil. *Environmental Technology*, 39:939–951. doi:10.1080/09593330.2017.1317838

Salehizadeh, H., & Mohammadizad, S. 2009. Microbial enhanced oil recovery using biosurfactant produced by *Alcaligenes faecalis*. *Iranian Journal of Biotechnology*, 7: 216-223.

Sogbanmu, T. O., Nagy, E., Phillips, D. H., Arlt, V. M., Otitoloju, A. A., & Bury, N. R. 2016. Lagos Lagoon sediment organic extracts and polycyclic aromatic hydrocarbons induce embryotoxic, teratogenic and genotoxic effects in Danio rerio (zebrafish) embryos. *Environmental Science and Pollution Research*, 23:14489–14501. doi:10.1007/s11356-016-6490-y

Sogbanmu, T. O., Osibona, A. O., & Otitoloju, A. A. 2019. Specific polycyclic aromatic hydrocarbons identified as ecological risk factors in the Lagos Lagoon, Nigeria. *Environmental Pollution*, 255: 113295. doi:10.1016/j.envpol.2019.113295

Stec, J., Kosikowska, U., Mendrycka, M., Stępień-Pyśniak, D., Niedźwiedzka-Rystwej, P., Bębnowska, D., Hrynkiewicz, R., Ziętara-Wysocka, J., & Grywalska, E. 2022. Opportunistic Pathogens of Recreational Waters with Emphasis on Antimicrobial Resistance—A Possible Subject of Human Health Concern. *International Journal of Environmental Research and Public Health,* 19: 7308 doi:10.3390/ijerph19127308

Tang, W., Mao, Y., Li, Q. Y., Meng, D., Chen, L., Wand, H., Zhu, R., and Zhang, W. X. 2020. Prevalence of Opportunistic Pathogens and Diversity of Microbial Communities in the Water System of a Pulmonary Hospital. *Biomedical and Environmental Sciences*, 33: 248–259. doi:10.3967/bes2020.034

Undugoda, L. J. S., Kannangara, S., & Sirisena, D. 2016. Genetic Basis of Naphthalene and Phenanthrene Degradation by Phyllosphere Bacterial Strains Alcaligenes faecalis and Alcaligenes sp. 11SO. *Journal of Bioremediation & Biodegradation*, 07: 333. doi:10.4172/2155-6199.1000333

Uzoigwe, C., Ennis, C. J., & Rahman, P. K. S. M. 2015. Production of biosurfactants using eco-friendly microorganisms. In Environmental Sustainability: Role of Green Technologies, P. Thangavel, G. Sridevi, New Delhi, Springer, pp 185-204. doi:10.1007/978-81-322-2056-5_11

Walter, V., Syldatk, C., & Hausmann, R. 2010. Screening concepts for the isolation of biosurfactant producing microorganisms. *Advances in Experimental Medicine and Biology*, 672:1–13. doi:10.1007/978-1-4419-5979-9_1

Youssef, N. H., Duncan, K. E., Nagle, D. P., Savage, K. N., Knapp, R. M., & McInerney, M. J. 2004. Comparison of methods to detect biosurfactant production by diverse microorganisms. *Journal of Microbiological Methods*, 56: 339-347 doi:10.1016/j.mimet.2003.11.001

Zuberer, D. A., & Zibilske, L. M. 2021. Composting: the microbiological processing of organic wastes. In Principles and Applications of Soil Microbiology, Elsevier, New York, pp. 655–679. doi:10.1016/B978-0-12-820202-9.00024-1