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# Biosurfactant-producing bacteria recovered from hydrocarbon contaminated soil in Awka, Anambra State, Nigeria

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## Abstract

The increasing concern over environmental sustainability and the adverse ecological impact of synthetic surfactants has fueled interest in microbial biosurfactants as safer and more sustainable alternatives. Biosurfactants are valued for their biodegradability, low toxicity, and stability under extreme environmental conditions.

The aim of this study was to isolate, characterize, and determine the effects of environmental parameters on biosurfactant-producing bacteria from hydrocarbon-contaminated soils in Anambra State, Nigeria.

Samples from hydrocarbon contaminated soil from different locations in Awka, Anambra state were examined for biosurfactant-producing bacteria. Distinct colonies recovered and purified, were partially characterized and then subjected to preliminary screening tests which include drop collapse, oil spreading and emulsification index for biosurfactant production. The isolate with the highest biosurfactant production was further identified and characterized using 16S rRNA gene sequencing. The effects of carbon and nitrogen sources on biosurfactant production were examined. The crude biosurfactant produced by the isolate was extracted and its stability assessed under different temperature, pH, and salinity conditions.

Four bacterial isolates were recovered and one of them was observed to produce an emulsification index ($E_{24}$) above 50% for all the substrates tested. The isolate was identified as Ochrobactrum anthropi MO419. Sucrose (2%w/v) and $NH_4NO_3$ (0.1%w/v) gave the highest biosurfactant production. The biosurfactant was stable at 100$^{o}$C, pH 3-11, and 1-10% NaCl concentration.

This study reveals the ability of O. anthropi to produce stable biosurfactant under optimized conditions, making it a promising candidate for applications in environmental bioremediation, enhanced oil recovery, and pharmaceutical formulations.

**Keywords:** *Ochrobactrum Anthropi*; Biosurfactants; Emulsification Index; Carbon/Nitrogen Sources

## 1. Introduction

Increasing environmental concerns over the adverse impacts of synthetic surfactants have intensified interest in eco-friendly alternatives such as biosurfactants [1, 2]. Biosurfactants are structurally diverse, amphiphilic compounds produced by bacteria, yeast, and filamentous fungi. They are valued for their high surface and interfacial activity, stability across broad temperature and pH ranges, biodegradability, low toxicity, and anti-adhesive properties. Their synthesis is influenced by environmental and nutritional factors, including temperature, pH, aeration, agitation, salinity,

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and the availability of carbon and nitrogen sources [3, 4]. Key microbial producers include bacterial genera such as Acinetobacter, Arthrobacter, Agrobacterium, Antarctobacter, Bacillus, Clostridium, Lactobacillus, Halomonas, Serratia, and Rhodococcus; filamentous fungi such as Aspergillus and Penicillium; and yeast genera such as Candida, Yarrowia, Torulopsis, Pseudozyma, and Saccharomyces [4].

Biosurfactant-producing microorganisms inhabit diverse ecological niches which include; aquatic, terrestrial, extreme, and industrial environments, from marine sediments and polar seas to petroleum-contaminated soils and hypersaline salterns [5]. Marine sediment isolates such as Pseudomonas aeruginosa N33, Bacillus paralicheniformis Nian$_2$, and Stenotrophomonas nematodicola T10 exhibit remarkable thermal stability (up to 120 °C) and enhanced oil recovery potential [4, 6]. Coastal sediments and marine invertebrate guts host strains with exceptional resilience to extreme temperature, pH, and salinity, while polar and deep-sea microbes produce cold-active biosurfactants with applications in low-energy industrial bioprocesses [7]. Terrestrial sources such as petroleum-contaminated soils, rhizospheres, and phyllospheres yield biosurfactants that facilitate pollutant degradation, nutrient cycling, and plant growth, whereas extremophiles from hot springs, acidic mines, and salt flats generate products stable under severe industrial conditions [8, 9]

Industrial and agro-waste streams, including molasses, wastewater, and food waste, not only serve as reservoirs for biosurfactant-producing microbes but also provide cost-effective substrates for large-scale production; for instance, kitchen waste oil has been used to produce rhamnolipid biosurfactants exhibiting stability across 20–100 °C, pH 6–12, and salinity of 2–20 % (w/v) [10].

Compared with synthetic surfactants, biosurfactants generally exhibit lower critical micelle concentrations, greater functional stability under extreme pH, salinity, and temperature conditions, and higher substrate specificity [3]. Despite the widespread use of chemical surfactants, their drawbacks; environmental persistence, toxicity, bioaccumulation, algal bloom formation, reduced bioavailability, microbial resistance, and operational inefficiencies are increasingly recognized [11]. These challenges have accelerated the shift towards “green” surfactants, which are natural, biodegradable, and sustainable alternatives [12].

Biosurfactants have growing applications in pharmaceuticals, healthcare, enhanced oil recovery, oil spill remediation, and drug delivery systems, particularly in improving the solubility and bioavailability of poorly water-soluble drugs [13]. They also contribute to advancements in bioenergy and enzyme production technologies [14].

Given their versatility, the isolation of biosurfactant-producing bacteria from soil is attracting considerable interest for biotechnological applications. However, developing sustainable and efficient production methods remains essential. This study aims at isolating biosurfactants-producing bacteria from hydrocarbon-contaminated soils within Awka metropolis.

## 2. Materials and Method

### 2.1. Sample area/ sample collection

Soil samples were collected from hydrocarbon-contaminated sites such as mechanic workshops, sewage channels, abattoirs, and refuse dumpsites within Awka metropolis (Latitude 6.25 oN and longitude 7.14 oE). These locations were selected based on the fact that prolonged exposure of hydrocarbon-rich substances, such as used engine oil, grease, proteinaceous waste, and mixed organic materials, are known to support the proliferation of biosurfactant-producing and hydrocarbon-degrading microorganisms [15, 16]. At each site, surface soil (10–15 cm depth) was aseptically collected using sterile spatula, transferred into sterile containers, and transported to the laboratory. The samples were stored at 4°C and processed within 24h for bacterial isolation.

### 2.2. Isolation of bacteria

The bacteria were isolated following a modified method of [17] Soil sample (1 g) was suspended in 50 mL of sterile distilled water, and shaken at 120 rpm for 30 min on a rotary shaker. The soil solution was diluted ten-fold, and 0.1 mL of the $10^{-2}$ dilution spread inoculated on starch casein agar plates. The plates were incubated at 28 °C for 7 days. Morphologically distinct colonies were picked, purified through repeated streaking, to obtain pure cultures and stored on starch casein agar slants at 4 °C.

### 2.3. Characterization of bacterial isolates

The isolates were characterized following the method described by [18], which include colonial morphology and Gram staining.

### 2.4. Preliminary Screening of the Isolates for Biosurfactant Activity/Production

#### *2.4.1. Preparation of broth culture/ crude biosurfactant*

The pure isolate suspended in 1 ml of sterile distilled water was transferred into a 250 ml Erlenmeyer flask containing 50 ml of Kim's medium (g/l): NaNO3, 1.0 g; KH2PO4, 0.1 g; MgSO4. 7H2O, 0.1 g; CaCl2, 0.1 g; yeast extract, 0.2g; and 3% olive oil as the sole source of carbon at pH 6 [19]. The broth culture was incubated on a rotary shaker at 28 OC for 7 days and the cell-free supernatant obtained by centrifugation at 3500 rev/ min for 30 min.

#### *2.4.2. Drop collapse test*

The drop collapse test was done using a modified method described by [20]. A 20 µl of olive oil was added into each well of a 96-well plate, and then equilibrated at 25°C for 1 h. A 50 µl of the cell-free supernatant was added to the well, and the shape of the drops examined after 1 min. Collapse of the olive oil by the cell-free supernatant indicates a positive result. Tween-80 at a concentration of 1 mg/mL served as a control.

#### *2.4.3. Oil displacement method*

This assay was performed by a modified method described by [21]. Distilled water (40 ml) was poured into a Petri dish, and 10 µL of olive oil was gently added to form a thin film on the surface. Then, 10 µL of the cell-free supernatant was carefully applied to the center of the oil film and allowed to stand for 60 sec. The result was read by measuring the diameter (in millimeters) of the clear zone that formed on the oil film. A clear zone indicates biosurfactant activity. Distilled water served as a negative control.

### 2.5. Emulsification Index (E24%) assay

The emulsification assay was carried out following the procedure described by [22]. Two milliliters of the cell-free supernatant of the isolate mixed with an equal volume of kerosene, was vortexed using a vortex mixer for 2 min and left undisturbed at 28 oC. After 24 h, the height of the emulsion layer was measured and the emulsification index calculated thus:

$$E24 = \frac{\text{Height of emulsified layer after 24 h}}{\text{Total height of liquid}} \times 100$$

Similarly, the emulsification index for palm oil, diesel and spent engine oil were determined.

### 2.6. Molecular identification and phylogenetic analysis of the bacterial isolates with high biosurfactant producing activity

Molecular identification and phylogenetic analysis of the bacterial isolates with high producing activity was determined using 16S rDNA sequencing, at Molecular Research Foundation for Staff and Students (MRFSS), Nnamdi Azikiwe University, Awka. The 16S rRNA gene sequence was submitted to NCBI GenBank for accession number. The isolate with the highest biosurfactant activity (B3) was used for further studies.

### 2.7. Preparation of crude biosurfactant

The cell-free supernatant was collected and acidified to pH 2.0 using 6 N HCl, followed by storage at 4 °C overnight to precipitate the biosurfactant. The precipitate was then recovered by centrifugation, washed twice with distilled water to remove residual medium components, and oven-dried at 50 °C. The dried product obtained was used as the crude biosurfactant for subsequent analyses.

### 2.8. Medium optimization for biosurfactant production in Kim's medium

Optimization studies for enhancing the biosurfactant activity was done using the approach of changing one variable at a time and keeping the other factors fixed at a specific set of conditions. To enhance the biosurfactant productivity, four factors were chosen: the different sources of carbon and nitrogen, C/N ratio and incubation period [23, 24].

Four different carbon sources namely glucose, glycerol, sucrose and starch and three different nitrogen sources; ammonium nitrate (NH4NO3), ammonium sulphate (NH4)2SO4), sodium nitrate (NaNO3), were used in the fermentation medium [25]. Kim's medium served as the basal medium, and emulsification index (E24%) of the biosurfactant was estimated using kerosene. Carbon concentrations were varied at 1%, 2%, 3%, and 4% (w/v), and nitrogen at 0.1%, 0.5%, 0.75%, and 1% (w/v). An uninoculated flask served as control [26].

### 2.9. Determination of environmental parameters affecting biosurfactant activity of isolate B3

The effects of temperature, pH, and sodium chloride (NaCl) concentration on biosurfactant activity of isolate B3 were evaluated using the cell-free supernatant obtained from the culture broth of the isolate. All experiments were performed in triplicate as described by [27].

#### *2.9.1. pH stability test*

Five milliliters of the cell-free supernatant was used to assess pH stability. The pH was adjusted from 3.0 to 11.0 with 1 M NaOH or 3 N HCl. The samples were allowed to equilibrate at room temperature, and the emulsification index (E24%) at various pH was measured to determine biosurfactant activity.

#### *2.9.2. Thermal stability test*

Thermal stability was evaluated by heating 1 ml aliquots of the cell-free supernatant in test tubes at various temperatures (30 - 100 °C) for 1 h in a water bath. The samples were cooled to room temperature, and the emulsification index (E24%) of each sample was determined.

#### *2.9.3. Salt tolerance test*

The effect of various concentrations of NaCl (1-10% w/v) on the biosurfactant activity was determined. Following incubation for 24 h, the emulsification index (E24%) of the biosurfactant with kerosene was measured, to assess the activity under varying NaCl levels.

#### *2.9.4. Statistical Analysis*

All data collected was expressed as Mean ± SEM. The results obtained were analyzed for statistical significance by one-way ANOVA using Statistical Package for Social Sciences (SPSS) software version 22. Results with $p< 0.05$ were considered statistically significant.

## 3. Results

### 3.1. Isolation and Characterization of Bacterial Isolates

Four bacterial isolates with biosurfactant activity were recovered from the hydrocarbon-contaminated soils. The isolates were code-named B3, D4, F1 and F2. Three of the isolates were Gram positive and one of them Gram negative.

### 3.2. Preliminary screening of the isolates for biosurfactant activity/production

All the isolates were positive for drop collapse assay with B3 having high biosurfactant activity (Table 1). As shown in Table 1, B3 had the highest oil displacement, while the least was D4. The emulsification index of the isolates is as recorded in Table 1, with B3 having the highest biosurfactant activity. Isolate B3 was chosen for further studies.

**Table 1** Preliminary screening test of the isolates for biosurfactant production

| Isolates | Drop Collapse Oil | Oil Displacement (cm) | Emulsification Palm oil | Index ($E_{24}$) Kerosene | % Spent Engine Oil | Diesel |
|---|---|---|---|---|---|---|
| B3 | +++ | $5.97\pm0.15^{b}$ | $99.97\pm0.06^{c}$ | $54.73\pm0.11^{d}$ | $60.23\pm0.85^{c}$ | $14.97\pm0.21^{b}$ |
| D4 | ++ | $4.00\pm0.00^{a}$ | $99.83\pm0.11^{c}$ | $27.76\pm0.25^{c}$ | $62.77\pm0.25^{d}$ | $0.00\pm0.00^{a}$ |
| F1 | + | $4.40\pm1.15^{ab}$ | $85.60\pm0.56^{a}$ | $17.57\pm0.15^{b}$ | $50.37\pm0.57^{a}$ | $0.00\pm0.00^{a}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| F2 | ++ | 4.20±0.72 [a] | 94.57±0.45[b] | 9.80±1.08[a] | 54.33±0.31[b] | 0.00±0.00[a] |

Mean values along same column with different affixes (a, b, c, d, e, ab) are significantly different (P<0.05); Key: + =Weak positive; ++ = Moderate positive; +++ = Strong positive

### 3.3. Molecular identification of the bacterial isolate with the high biosurfactant activity

The bacterial isolate exhibiting high biosurfactant activity was subjected to 16S rRNA gene sequencing, and the resulting sequences were compared with those in the NCBI GenBank database using BLAST analysis. Phylogenetic characterization, as illustrated in Fig. 1, revealed that isolate B3 corresponded to Ochrobactrum anthropi strain MO419.

### 3.4. Gel Image of Sample

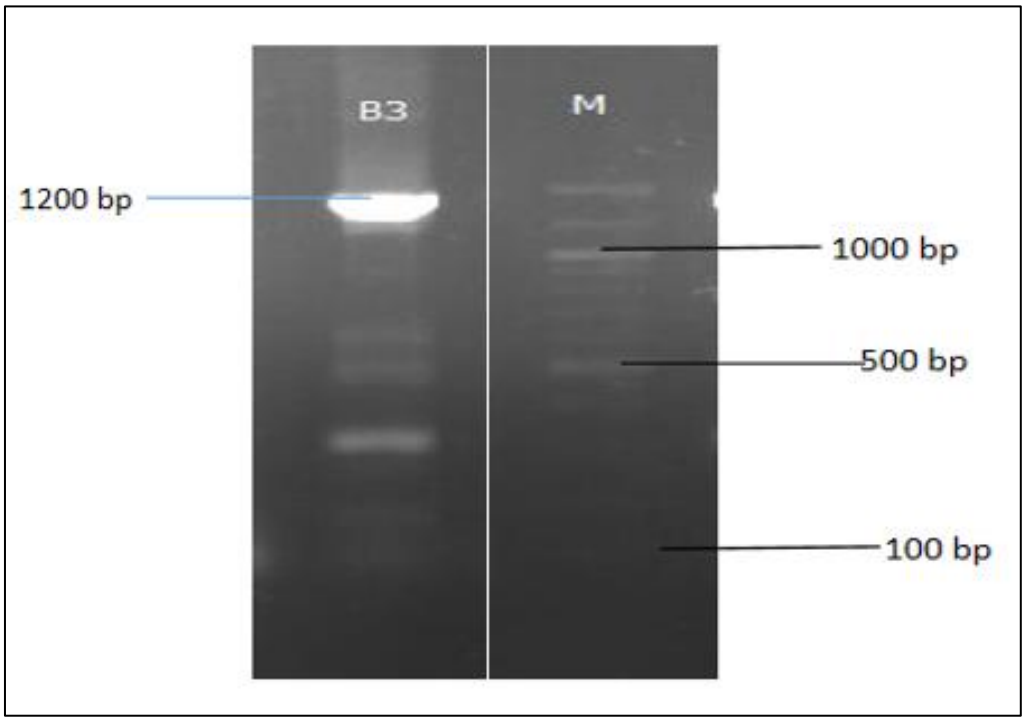


**Figure 1** Agarose Gel Electrophoresis of the Amplicon. Lane M: DNA marker; Lanes B3 Showing Visible Amplification of the 16S rRNA gene for Achromatium anthropic strain MO419

### 3.5. Medium optimization for biosurfactant production in Kim's medium

In Achromatium anthropic strain MO419, enhanced biosurfactant production, as indicated by the emulsification index, showed that Sucrose and NH4NO3, gave the highest E24 of 57.13%, while the least E24 (5.71%) was obtained with Glucose and NH4NO3. No emulsion was recorded with Starch/NH4NO3 (Table 2).

The optimum concentration of biosurfactant produced by Achromatium anthropic strain MO419 is presented in Table 3. The highest emulsification index (E24) of 55.83% was obtained using 2% Sucrose and 0.1% NH4NO3 as the carbon and nitrogen sources, respectively.

**Table 2** Effects of carbon and nitrogen sources on biosurfactant produced by Achromatium anthropic MO419

| Isolates | Carbon Source | Nitrogen Source | Emulsification Index ($E_{24}$) % |
|---|---|---|---|
| *Achromatium anthropic* Strain MO419 | Glycerol | $NaNO_3$ | 14.03±0.12 |
| | Starch | $NaNO_3$ | 16.93±0.15 |
| | Sucrose | $NaNO_3$ | 30.60±0.10 |
| | Glucose | $NaNO_3$ | 10.00±0.00 |
| | Glycerol | $(NH_4)_2SO_4$ | 34.33±0.15 |

| | | | |
|---|---|---|---|
| | Starch | $(NH_4)_2SO_4$ | 21.13±0.06 |
| | Sucrose | $(NH_4)_2SO_4$ | 13.30±0.17 |
| | Glucose | $(NH_4)_2SO_4$ | 23.30±0.00 |
| | Glycerol | $NH_4NO_3$ | 8.63±0.06 |
| | Starch | $NH_4NO_3$ | 0.00±0.00 |
| | Sucrose | $NH_4NO_3$ | 57.13±0.06 |
| | Glucose | $NH_4NO_3$ | 5.71±0.01 |

**Table 3** Effects of varying concentrations of sucrose and NH4NO3 on biosurfactant produced by Achromatium anthropic MO419

| **Isolate** | **Sucrose Concentration (%w/v)** | **$NH_4NO_3$ Concentration (%w/v)** | **Emulsification Index ($E_{24}$) %** |
|---|---|---|---|
| *Ochrobactrum anthropi* MO419 | 1 | 0.1 | 40.00±0.00 |
| | | 0.5 | 33.50±0.02 |
| | | 0.75 | 15.07±0.06 |
| | | 1 | 6.45±0.01 |
| | 2 | 0.1 | 55.83±0.21 |
| | | 0.5 | 45.33±0.15 |
| | | 0.75 | 40.00±0.00 |
| | | 1 | 35.86±0.12 |
| | 3 | 0.1 | 48.58±0.03 |
| | | 0.5 | 45.28±0.03 |
| | | 0.75 | 40.00±0.00 |
| | | 1 | 35.91±0.02 |
| | 4 | 0.1 | 44.08±0.10 |
| | | 0.5 | 35.03±0.15 |
| | | 0.75 | 25.00±0.00 |
| | | 1 | 17.09±0.11 |

### 3.6. Effect of Temperature, pH and Sodium Chloride on the Stability of the Crude Biosurfactant

*3.6.1. Temperature*

As shown in Fig. 2, it can be observed that as the temperature increases the E24 increases. At a temperature of 100oC, Achromatium anthropic strain MO419 gave the highest emulsification index of 68.37%.

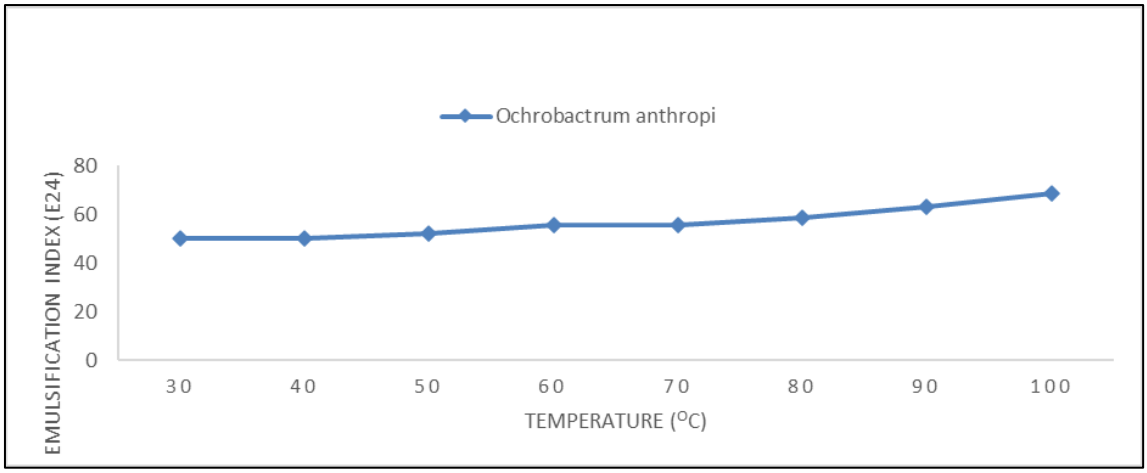


**Figure 2** Effect of temperature on biosurfactant production by O. anthropi MO419

Table 4, shows the effect of pH on the emulsification index capacity (E24 %) of the biosurfactant produced by the isolate. Achromatium anthropic strain MO419 produced stable emulsion at an acidic pH 4 and an emulsification index value of 60.69%.

**Table 4** Effects of varying pH on biosurfactant produced by Ochrobactrum anthropi MO419

| **Isolates** | **pH** | **Emulsification Index (E24) %** |
|---|---|---|
| Ochrobactrum anthropi strain MO419 | 3 | 52.57±0.45d |
| | 4 | 60.69±0.18f |
| | 5 | 58.86±0.06g |
| | 6 | 50.00±0.00g |
| | 7 | 53.40±0.61c |
| | 8 | 58.17±0.38e |
| | 9 | 53.00±0.00d |
| | 10 | 53.20±0.35b |
| | 11 | 57.93±2.00a |

Mean values along same column with different affixes (a, b, c, d, e, f,) are significantly different (P<0.05)

### 3.7. Sodium Chloride (NaCl)

The effects of varying concentration of NaCl on biosurfactant production by Ochrobactrum anthropi strain MO419 are shown in Table 5. The highest emulsification index of 61.33% was obtained with 8% sodium chloride.

**Table 5** The effects of varying concentrations of NaCl on biosurfactant produced by Ochrobactrum anthropi sMO419

| **Isolates** | **NaCl (%)** | **Emulsification Index ($E_{24}$) %** |
|---|---|---|
| *Ochrobactrum anthropi* strain MO419 | 1 | $50.20\pm0.36^{b}$ |
| | 2 | $50.00\pm0.00^{b}$ |
| | 3 | $57.20\pm0.35^{c}$ |
| | 4 | $57.97\pm2.05^{bc}$ |
| | 5 | $50.00\pm2.65^{a}$ |

| | | |
|---|---|---|
| | 6 | 50.00±0.00$^{ab}$ |
| | 7 | 59.93±1.30$^{d}$ |
| | 8 | 61.33±1.53$^{d}$ |
| | 9 | 55.50±0.62$^{d}$ |
| | 10 | 55.50±0.46$^{e}$ |

Mean values along same column with different affixes (a, b, c, d, e, f,) are significantly different (P<0.05)

## 4. Discussion

Samples contaminated with diesel oil, motor oil, and other hydrocarbons were collected from mechanic workshops, sewage channels, abattoirs, and refuse dumpsites within Awka metropolis. These sites were examined because petroleum oil-contaminated environments have been known for harboring native bacterial populations capable of producing biosurfactants and degrading crude oil hydrocarbons [28]. Similar environments have also been studied for biosurfactant-producing microorganisms. Aliyu et al [29] isolated biosurfactant-producing bacteria from mechanic workshop soil using sterile mineral salt medium (MSM) supplemented with 10% actual gasoline oil (AGO) [29]. Olukunle et al [15], isolated biosurfactant-producing bacteria from soils of auto mechanic workshops in Akure, Ondo State, using Bushnell Haas medium. Izomor et al [16], identified biosurfactant-producing microorganisms from abattoir soil collected in Awka metropolis using MSM with engine oil as the supplement.

Satpute et al. [24], recommended employing multiple screening methods during primary screening to effectively identify potential biosurfactant producers. In this study, drop collapse tests, oil displacement tests, and emulsification index assay (E24) with various hydrocarbons (palm oil, kerosene, spent engine oil, and diesel) were employed to screen the isolates for biosurfactant production (Table 1). Jain et al [30], affirmed that the qualitative drop collapse test has a high correlation with surface tension due to the biosurfactant's ability to destabilize the liquid droplets on the oily surface. The drop collapse test performed showed that the isolates have strong, moderate and weak activities. This result is similar to that obtained by [31], in which they reported that several microorganisms isolated presented high, moderate, and weak capacity to collapse the oily surface. However, [16] noted that the isolated microorganisms in their study displayed only high and weak capacity to collapse the oily surface. The oil displacement test measures the diameter of the oil film displaced by the biosurfactant, with larger values indicating higher activity [32]. The values obtained in this study are higher than those obtained from [16], [15], and [33]. These variations can likely be attributed to differences in medium composition, as each study employed a distinct growth medium. [16] used mineral salt medium (MSM) supplemented with 1% engine oil as the sole carbon source, while [15] utilized Bushnell Haas medium (BHM) supplemented with 2% hydrocarbon, and [33] used a broth medium. Also, the sample sources in these studies and types of organisms isolated may have influenced the variations in biosurfactant activity observed.

The emulsification index assay (E24) is a straightforward quantitative method to prospect biosurfactant-producing microorganisms [34]. [35], reported that an emulsification index (E24) higher than 50% is a criterion to select biosurfactant-producing isolates. Although in this study, all isolates showed emulsification indices above 50% when Palm oil and Spent engine oil were used. Isolate B3 gave an emulsification index higher than 50% for all the substrates tested except diesel, suggesting it to be a good candidate for biosurfactant production. Under kerosene-based assay conditions, isolate B3 exhibited E24 value as high as 54.73%, which is markedly higher than the value of 13.5% reported by [16] in Streptomyces sp. However, [15] reported E24 values of 60% in Clostridium sp.

Ochrobactrum anthropi strain MO419 was identified as the microorganism with the most promising ability for biosurfactant synthesis (Fig. 1). The ability of these bacteria in hydrocarbon degradation and biosurfactant production has been reported by many researchers. [36], documented the biosurfactant properties of Ochrobactrum anthropi isolated from a fuel contaminated soil, while [37] isolated O. anthropi from used engine oil-contaminated soil. [38], reported Ochrosin, a multifunctional biosurfactant, isolated from the halophilic Ochrobactrum sp. strain BS-206 (MTCC 5720).

The concentrations of biosurfactants produced by microorganisms varies based on the nutrient sources provided [20, 19, 4]. Ochrobactrum anthropi M0419 produced a biosurfactant concentration of 1.24 g/L when cultured with sucrose (2%w/v) and NH4NO3 (0.1%w/v) as the carbon and nitrogen sources, respectively. Contrary to our findings, [39] reported a higher yield of 2.5 g/L of crude surfactant by Ochrobactrum anthropi after 72 h under optimized conditions when cultured with glucose and hexadecane as carbon source and NH4NO3 as the nitrogen source. The variation in the biosurfactant produced in this study, may have been as a result of the carbon source used.

The production of biosurfactant is strongly influenced by its stability under extreme environmental and operational conditions, such as pH, temperature, and salinity [37]. The emulsification index (E24) values obtained from varying temperatures (Fig. 2), indicate that the biosurfactants produced by Ochrobactrum anthropi MO419 retained its emulsification activity across a wide temperature range (30–100 °C). Ibrahim [37], however, reported an emulsification index of 92% at 50oC, and 72.75 at 120 oC for biosurfactants produced by O. anthropi HM-1. This shows that Ochrobactrum anthropi is thermotolerant.

The emulsification activity (E24) % of biosurfactant produced by Ochrobactrum anthropi MO419 was evaluated at a pH range of 3 to 11 (Table 4). The biosurfactant exhibited its highest E24 value of 60.69% at pH 4, indicating optimal activity under acidic conditions. As can be observed the biosurfactant produced by O. anthropi MO419 was also stable under alkaline conditions with its highest activity at pH 8.0. This shows that biosurfactant from O. anthropi MO419 has a broad range (pH 3-11) of activity, which is in line with the reports of [40]. They noted that rhamnolipids and other biosurfactants produced in their study, maintain structural integrity under both acids and alkaline conditions. Ibrahim [37], also reported that biosurfactants produced by O. anthropi- HM1 displayed high stability across a wide pH range (2–12), with maximum emulsification activity at pH 6 (E24 values of 95–97%). The biosurfactant produced by Ochrobactrum anthropi MO419 was assessed across a gradient of NaCl concentrations (1-10%w/v) (Table 5). The results show that the biosurfactants produced by O. anthropi were stable at different NaCl concentrations examined, with its maximum activity at 8.0 % concentration. Thus, this observation is contrary to the report of Ibrahim [37], who noted a maximum emulsification index of 97% for O. anthropi- HM1 at 4% NaCl concentration.

## 5. Conclusion

Soil samples from hydrocarbon-contaminated environments were examined for biosurfactant-producing bacteria. Four of the isolates recovered were screened for their biosurfactant activity and one of them was observed to produce an emulsification index above 50% for all the substrates used. Based on the molecular characterization the organism was identified as Ochrobactrum anthropic MO419. The biosurfactant produced was quite stable at 100 oC, various pH levels and NaCl concentrations. The adaptation of this organism to various environmental conditions makes it a good candidate for microbial enhanced oil recovery.

## Compliance with ethical standards

*Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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