Journal of Advances in Biology & Biotechnology
7(4): 1-15, 2016; Article no.JABB.27467
ISSN: 2394-1081

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Experimental Investigation of Sophorolipid Biosurfactants Produced by *Candida* and *Pleurotus* Species Using Waste Oils and Rice Bran and Their Oilfield Benefits

E. F. Ahuekwe[1,2*], B. E. Okoli[3], O. H. Stanley[2] and B. Kinigoma[4]

[1]World Bank African Centre for Oilfield Chemicals and Research, Institute of Petroleum Studies, University of Port Harcourt, P. M. B. 5323, Choba, Port Harcourt, Nigeria.
[2]Department of Microbiology, Faculty of Science, University of Port Harcourt, Nigeria.
[3]Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria.
[4]Department of Petroleum Engineering, Faculty of Engineering, University of Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study. Authors BEO, OHS and BK offered technical expertise and proof-read the manuscript. Authors EFA and OHS wrote the protocol and conducted the experiments. Author EFA wrote the first draft of the manuscript. All authors managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2016/27467
Editor(s):
(1) Joana Chiang, Department of Medical Laboratory Science and Biotechnology, China Medical University, Taiwan.
Reviewers:
(1) Ahmed Z. Abdel Azeiz, Misr university for Scienceand Technology, Egypt.
(2) Banu Kaskatepe, Ankara University, Turkey.
Complete Peer review History: http://sciencedomain.org/review-history/15400

Original Research Article

Received 2nd June 2016
Accepted 4th July 2016
Published 14th July 2016

ABSTRACT

Conventional chemical surfactants applications usually linked with environmental unfriendliness and toxicity are associated with high production costs resulting from fluctuations in oil prices and thermal energy requirements. Sophorolipid biosurfactants can potentially be implemented with a remarkably low operating cost. Besides economic interest, sophorolipids and their derivatives have shown promise as emulsifiers, antimicrobials, surfactants and a source of specialty chemicals

**Corresponding author: E-mail: aezefrank@yahoo.com;*

possessing both hydrophilic and hydrophobic structural moieties that impart surface tension reduction capacities, thus, facilitating microbial hydrocarbon and heavy metal emulsification and uptake. In this study, sophorolipids produced by *Candida* and *Pleurotus* species respectively isolated and harvested from oil contaminated soils from Ukwa West LGA of Abia State were investigated. Mineral salt media supplemented with different hydrophilic (rice bran, spent mushroom substrate and cassava peels) and hydrophobic (food industry waste oil) renewable wastes as sources of carbon were tested on nine (9) of the potential isolates to select the best medium and organism that maximized sophorolipids production. This was supported by the emulsification index after 24 hours (E_{24}), FT-IR and GC-MS analysis. All isolates were subjected to biosurfactants production screening, to find the best sophorolipid producer among the available strains. Results showed that isolate CP1SP6c, a hydrocarbon utilizing fungi (HUF) exhibiting β-haemolysis and 92.4% microbial adhesion to hydrocarbon (MATH), gave the maximum E_{24} of 69.2%, within 6 days of incubation on media fortified with rice bran and food industry waste oil in a rotary shaker. Further studies on CP1SP6c were carried out to assess the interaction of the produced sophorolipids with porous media in core flooding experiments as a tertiary recovery technique. The results showed high promises of using this bio-product in hydrocarbon recovery, where 12.3% of crude oil was recovered after injecting the culture supernatant. An additional recovery of 15.7% of residual oil was observed after concentrating the sophorolipid solution.

Keywords: Sophorolipid biosurfactants; emulsification index; renewable agro-wastes; EOR; biodegradation; cheap; oilfield chemicals.

1. INTRODUCTION

Biosurfactants are valuable microbial amphiphilic compounds with effective surface active and biological properties applicable to several industries and process materials. Owing to their superior properties of diversity, higher biodegradability, ecological acceptability and production from wide range of raw materials over petroleum-based surfactants, interests in their use in various industrial applications has increased [1,2]. Surfactants possess both hydrophilic and hydrophobic structural moieties, which impart many unusual properties, including an ability to lower the surface tension, thus, facilitating hydrocarbon emulsification and uptake [3,4]. In bioremediation, studies show that the introduction of biosurfactants increases the bioavailability of long-chain hydrocarbons to microbes and renders the hydrocarbons more accessible to microbial enzyme systems for degradation and utilization [5]. The environmental uses of biosurfactants are mostly related to their bioremediation of petroleum hydrocarbons in soils and groundwater, dispersion of oil spills and degradation of harmful compounds [6] of industrial and agricultural origin. In the oil industry, they are employed in microbial-enhanced oil recovery, cleaning of contaminated vessels and to facilitate transportation of heavy crude oil by pipeline [7,8]. Other potential applications of biosurfactants relate to the pharmaceutical, cosmetic and food processing industries [9].

2. SOPHOROLIPID-PRODUCING FUNGI

Biosurfactant production by yeasts is largely reported by the genera *Candida* sp*., Pseudozyma* sp*. and Yarrowia* sp. This great advantage of using yeasts in bioprocessing is due to their generally regarded as safe (GRAS) status. Organisms with GRAS status are generally neither non-toxic nor pathogenic; a fact that widens their range of possible use in biodegradation and biosurfactant production [10,11].

Among biosurfactants, the sophorolipids, generally produced by yeasts, (primarily *Starmerella bombicola*) are among the most promising types because they present a considerably high production yield of 100 g/L and are recovered efficiently from culture broths [4]. Most recently, Velioğlu and Öztürk-Ürek, [12] reported biosurfactant production by *Pleurotus ostreatus*, a white-rot fungus known for lignin degradation, using two separate production media and conditions involving a submerged fermentation (SmF) and solid-state fermentation (SSF).

Sophorolipids exist in the lactone and acidic conformations. The lactone form results from the esterification of the carboxylic acid group to the disaccharide ring (Fig. 1a) while the acidic form has two head groups of carboxylic acid and acetylated sophorose head (Fig. 1b) [13,14].

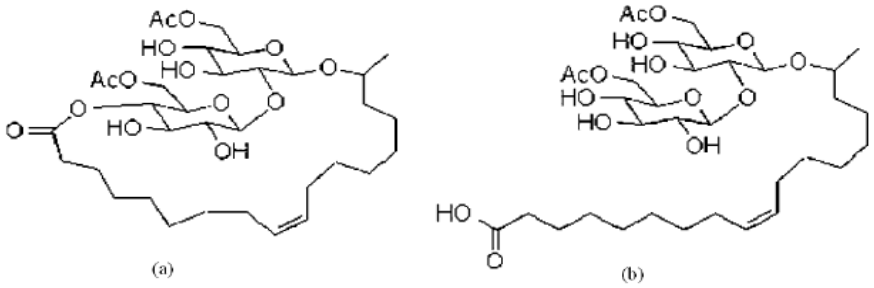

Fig. 1. Sophorolipids structures (Ac = Acetyl): (a) Lactone form and (b) Acidic form [15]

High costs of biosurfactants production have been a great challenge in meeting its global demand. As such, in order to obtain large amounts of biosurfactants at reduced costs, much attention has focused on the search for efficient microbial strains as well as cheap and readily accessible substrates [16]. This study reports the best substrates for the production of sophorolipid biosurfactants and the ability of these fungal isolates to produce large amounts of sophorolipids on water-immiscible waste oils and water-soluble substrates at a laboratory scale. Further investigations were done to assess the interaction of the sophorolipids with porous media in core flooding experiments as an index to tertiary recovery technique.

3. MATERIALS AND METHODS

3.1 Sample Collection

In this study, oil contaminated soils from Ukwa West Local Government Area (LGA) of Abia State, Nigeria were investigated. Different hydrophilic (rice bran, spent mushroom substrate and cassava peels) and hydrophobic (food industry waste oil) renewable wastes served as sources of carbon in the bioreactors.

3.2 Fungal Isolates and Biosurfactant Screening

Two fungal species used in this study include *Candida* sp and *Pleurotus* sp isolated and harvested respectively from a hydrocarbon impacted farmland in Ukwa West LGA of Abia State following the protocols of Daverey et al., [17], and identified on the basis of their morphological and physiological characteristics as outlined by Aneja et al. [18]. The isolates were assessed for hydrocarbon utilizing capacity following the protocols of Chikere and Ekwuabu, [19], before onward screening for biosurfactant production.

3.3 Screening for a Potential Biosurfactant Producing Strain

3.3.1 Haemolytic activity

Blood agar containing 5% (v/v) human blood [20], were streaked with each strain using sterile inoculation loops and plates visually examined for haemolytic zones around the colonies after 48 hours incubation at 28°C. Colonies presenting clear zones were presumed to produce biosurfactants [21,22].

3.3.2 Cetyl trimethyl ammonium bromide (CTAB)/ methylene blue agar test

This was conducted by introducing 0.2 g/l CTAB and methylene blue in a modified Bushnell-Haas agar (Merck, Germany) and incubating at 28°C for 48 h. Positive results indicated a dark blue halo around the inoculation spot [23].

3.3.3 Oil displacement test

This was assessed by placing 100 µl of cell-free supernatant onto a 100µl diesel oil overlay on 50ml distilled water in a large petri dish [24]. The diameter of the displaced oil overlay was measured and compared against 100µl of distilled water as the negative control.

3.3.4 Microbial adhesion to the hydrocarbon (MATH)

Isolates harvested after centrifugation at 10,000 g at 4°C for 10 min, were washed twice with a buffered salt solution containing KH_2PO_4, Urea and $MgSO_4$ and maintained at pH 7.0. The cells were re-suspended in the buffer to fit a ca. 1.0 (A_0) optical density and measured at 600nm on UV visible spectrophotometer (Agilent 8453 UV-VIS, United States).

Diesel (500 µl) was added to a microbial suspension (5 ml) and shaken vigorously for 2 min. The optical density of the aqueous phase

was measured (A_1) after 10 mins while the degree of hydrophobicity was calculated as [1– (A_0 - A_1) / A_0. 100%].

3.3.5 Emulsification activity assay

A mixture of kerosene and the cell free supernatant in a 1:1 ratio were shaken vigorously for 2 min. After 24 h of stabilization at 28°C, the emulsification index (%) was obtained by measuring the column height of emulsified oil against its total height multiplied by 100 times [25,26].

All assays were conducted in triplicates. A sample serving as control was prepared by using 1 ml of non-inoculated culture medium in place of the cell-free supernatant.

3.4 Experimental Set Up

3.4.1 Culture condition

Sophorolipid biosurfactant production was performed in a 250 ml Erlenmeyer flasks containing 50 ml of modified Bushnell-Haas medium of Haba et al. [27], Nalini et al. [28] and Ismail et al. [29] made up of 1.2 g KH_2PO_4, 2.0 g K_2HPO_4, 0.02 g CaCl, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 14.0 g $NaNO_3$, yeast extract 5.0 g and 20.0 g glucose per liter at pH 7.2. A solution of trace element (2 ml) was added per litre of the medium. The composition of the trace element solution was 0.02 g $FeSO_4.7H_2O$, 0.01 g $MnSO_4.H_2O$ and 0.006 g $(NH_4)_6Mo_7O_{24}.4H_2O$ dissolved in 10ml distilled water.

Each flask was inoculated with 2% (v/v) of a 48h old seed culture and fermentation flasks incubated in a rotary shaker (160 rpm) at 28°C. Samples were collected 24-hourly for the 7-day period of incubation for pH, fungal biomass and sophorolipid biosurfactant production assessments. The emulsification index was used as the criterion of sophorolipids production, following the methods of Luna et al. [3]. At different treatment conditions, sodium dodecyl sulphate (SDS) and distilled water served as the positive and negative controls respectively.

3.4.2 Effect of carbon source

To economize biosurfactant production, glucose was replaced with 0.1–1.0% (w/v) of the hydrophilic carbon sources (rice bran, spent mushroom substrate and cassava peels), and each medium supplemented with 4% (v/v) of the hydrophobic carbon source (food industry waste oil) [30].

3.5 Extraction of the Sophorolipids

The yeast cells were removed by centrifugation at 8,000 rpm for 20 minutes using a centrifuge (Thermo Scientific, Canada) and the separated culture supernatant acidified with (0.1 M) HCl to obtain the pH of 2.0. The biosurfactants extraction was done using a chloroform-methanol (2:4 v/v) mixture introduced into the supernatant, and vigorously shaken, and allowed to stand until phase separation. Rotary evaporation and water removal with anhydrous sodium sulfate concentrated the extracts [31]. The biosurfactants concentrate appeared as dirty-white or brown crystals. Sophorolipid yield was estimated gravimetrically and expressed in g/L [29].

3.6 Sophorolipids Stability Test

Stability tests were performed as outlined by Obayori et al. [32], Mabrouk et al. [26] and Adamu et al. [31]. The thermal stability of the broth was assessed by maintaining the culture supernatant at constant temperatures in the range of 5-120°C for 1 h and cooling to room temperature of 28°C, before measuring the emulsification index. For pH stability, the cell free supernatant was adjusted to various acid and alkaline pH regimes between 2 and 12, by adjusting with 1 N HCl or 1 N NaOH [33]. The emulsifying indices were measured after 15 min. Different concentrations of NaCl (2–10% w/v) were added to the cell free culture supernatant and E_{24} (%) measured after 1 h incubation at 30°C [34]. These experiments were performed in triplicates.

3.7 Fourier Transform Infrared Spectroscopy

According to the methods described by Donio et al. [35] and Velioglu and Ozturk-Urek, [12], one milligram of dried purified biosurfactants was ground with 100 mg of KBr, pressed to obtain translucent pellets and analyzed using a KBr pellet as the background reference. The FT-IR spectra were recorded on a Happ Genzel FT-IR system (Shimadzu, Japan) in the spectral region of 4000–500 cm^{-1}.

3.8 Gas Chromatograph-Mass Spectrometry (GC-MS)

The hydroxyl fatty acids of the sophorolipid biosurfactants were extracted in a 2:1

chloroform-methanol mixture and washed with n-hexane before concentrating by evaporation at room temperature. The concentrated sample was analysed with an Agilent (United States) 7890A gas chromatograph interfaced with an Agilent 5975C mass selective detector apparatus previously calibrated under specific temperature programmed inlet, oven and detector conditions using helium as the carrier gas at 1 ml/min.

3.9 Application of Sophorolipids in Recovery of Hydrocarbon from a Porous Media

The interaction of sophorolipids with porous media in core flooding experiments as a tertiary recovery index was assessed using the sand packed column methods of Abu-Ruwaida et al. [36] and Adamu et al. [31]. Glass columns packed with 100 g of properly rinsed acid-washed beach sand were saturated with 50 ml Bonny light crude oil and stood for 24 h. The potential of the cell-free supernatant and the concentrated solution for recovery of hydrocarbon was estimated by running 50 ml of the cell free supernatant through the column in an upward displacement technique. Afterwards, the displaced crude oil was recovered in a measuring cylinder [37]. The control assays were performed simultaneously at the same conditions, using SDS and distilled water as positive and negative controls. All experiments were conducted in triplicate.

4. RESULTS AND DISCUSSION

4.1 Screening for Biosurfactant Production

The isolated nine morphologically distinct hydrocarbon utilizing fungal colonies were evaluated for biosurfactants production capacity by the hemolytic activity, CTAB/Methylene blue test, oil displacement test and cell hydrophobicity. Two of the isolates that exhibited high cell hydrophobicity (92.04% and 77.41% respectively) and emulsification index (29.2% and 23.7%) were initially identified as CP1SP6 (*Candida* sp.) and CP1SP8 (*Pleurotus* sp.). Further studies were conducted with CP1SP6 (*Candida* sp.) only, since it showed the highest emulsification activity. The cell hydrophobicity was related to the extracellular biosurfactants secreted on the cell surface aiding adhesion of fungal cells to hydrocarbons [38].

Table 1. The physicochemical properties of the soil samples

Parameters	Sample point 1 (0-15cm)	Sample point 2 (15-30cm)
pH	4.96	4.86
Temperature	26.0	26.0
Density	1.5832	1.5113
Specific gravity	1.6359	1.5618
Total organic carbon (g/kg)	15.7	12.2
Total inorganic carbon	1.07	0.64
Phenol (mg/kg)	0.024	0.022
Extractable nitrite (mg/kg)	<0.02	<0.02
Extractable nitrate (mg/kg)	0.67	0.32
Extractable sulphate (mg/kg)	85.0	<2.00
Extractable phosphate (mg/kg)	<0.02	<0.02
Exchangeable cations (mg/kg)		
Sodium (Na)	<101	<101
Potassium (K)	0.09	0.17
Calcium (Ca)	9.96	12.7
Magnesium (Mg)	0.54	1.00
Cation exchange capacity (mEq/100g)	0.055	0.072
Heavy metals (mg/kg)		
Chromium (Cr)	27.2	25.4
Lead (Pb)	7.80	5.90
Cadmium (Cd)	<2.00	<2.00

Table 2. The physicochemical properties of the food industry waste oil

Parameters	Food industry waste oil
Density	0.8941
Specific gravity	0.9199
Exchangeable cations; (mg/L)	
Sodium (Na)	7.11
Potassium (K)	0.71
Calcium (Ca)	16.3
Magnesium (Mg)	12.8
Cation exchange capacity (mEq/100 g)	0.19
Heavy metals (ppm);	
Manganese (Mn)	1.9
Cadmium (Cd)	<0.006
Iron (Fe)	19.7
Zinc (Zn)	1.2
Copper (Cu)	0.8

The hydrocarbon utilizing isolates exhibited β-hemolysis, agreeing with the results of Okore et al. [22], who reported that biosurfactants producing microorganisms can displace oil on water-oil interface and also show β-hemolysis on blood agar. Mulligan et al. [39] had recommended blood agar lysis as a preliminary method for biosurfactants production screening. However, the CTAB/Methylene blue test and oil displacement test were included to confirm biosurfactants production. The CTAB/methylene blue test revealed a dark blue halo around the inoculation spot, suggesting the formation of an insoluble ion pair between the produced anionic glycolipid and the cationic CTAB-methylene blue complex [23]. The oil displacement test measures the surface activity of biosurfactant samples against oil; thus, a larger diameter suggests a higher surface activity of the testing solution [40]. These qualitative tests are indicative of the wetting and surface activities of sophorolipids [21]. Sodium dodecyl sulfate and distilled water were employed as positive and negative controls in the oil displacement and emulsification index analyses.

4.2 Sophorolipid Biosurfactants Production

The result from Table 1 showed that the emulsification index E_{24} (%) of the biosurfactant produced by *Candida* sp using glucose as the sole source of carbon in the basal medium was 29.22% after 7 days period. The time for optimum production of biosurfactant by this organism was 6 days. Similarly, the E_{24} (%) of the biosurfactant produced by *Pleurotus* sp using glucose was 23.7%, after 7 days. Fig. 2 shows the time-course study of sophorolipids production on basal medium using *Candida* sp. Emulsification activity increased with increasing biomass throughout the process. Beginning at a pH value of 5.00, the observed final pH was 3.38. The pH values constantly decreased and together with that, a constant increase in the biomass and emulsification activity was also observed showing that the biosurfactants production occurred mostly during the exponential growth phase. This suggests that the sophorolipids are produced as primary metabolites accompanying cellular biomass increase [41].

Table 3. The physicochemical properties of the cellulosic fermentation substrates

Parameter	Ricebran	Cassava peel	Spent mushroom substrate
Total organic carbon (g/kg)	369	752	558
Total inorganic carbon	2.17	2.35	3.48
Nitrate (mg/Kg)	0.95	4.82	8.00
Sulphate (mg/Kg)	85	278	585
Phosphate (mg/Kg)	2,270	169	2,778
Exchangeable cations (mg/Kg);			
Potassium (K)	49.5	62.8	82.2
Calcium (Ca)	22.1	70.1	171
Magnesium (Mg)	19.7	15.2	67.3
Cation exchange capacity (mEq/100g)	0.402	0.638	1.627
Heavy metals (mg/Kg);			
Manganese (Mn)	213.8	190.8	162.1
Silicon (Si)	170,800	62,730	11,920
Iron (Fe)	572.6	5628	628
Zinc (Zn)	41.9	45.0	35.7
Copper (Cu)	4.2	13.9	12.1

Table 4. Screening methods for biosurfactants producing isolates

Isolates	Haemolytic activity	CTAB/Methylene blue test	Oil displacement test (cm)	Microbial adhesion to hydrocarbon (MATH) (%)	Emulsification index E_{24} (%)
CP1SP1	β	+	1.60	53.60	11.5
CP1SP2	γ	-	0.91	66.91	10.3
CP1SP3	α	-	1.16	43.62	14.6
CP1SP4	β	-	2.63	34.25	20.1
CP1SP5	β	+	0.32	51.32	16.3
CP1SP6	β	+	4.49	92.04	29.2
CP1SP7	α	-	0.61	44.12	11.4
CP1SP8	β	+	4.11	77.41	23.7
CP1SP9	γ	-	0.36	33.51	0.0
SDS (+ve control)			5.03		50.8
Distilled water (-ve control)			0		0

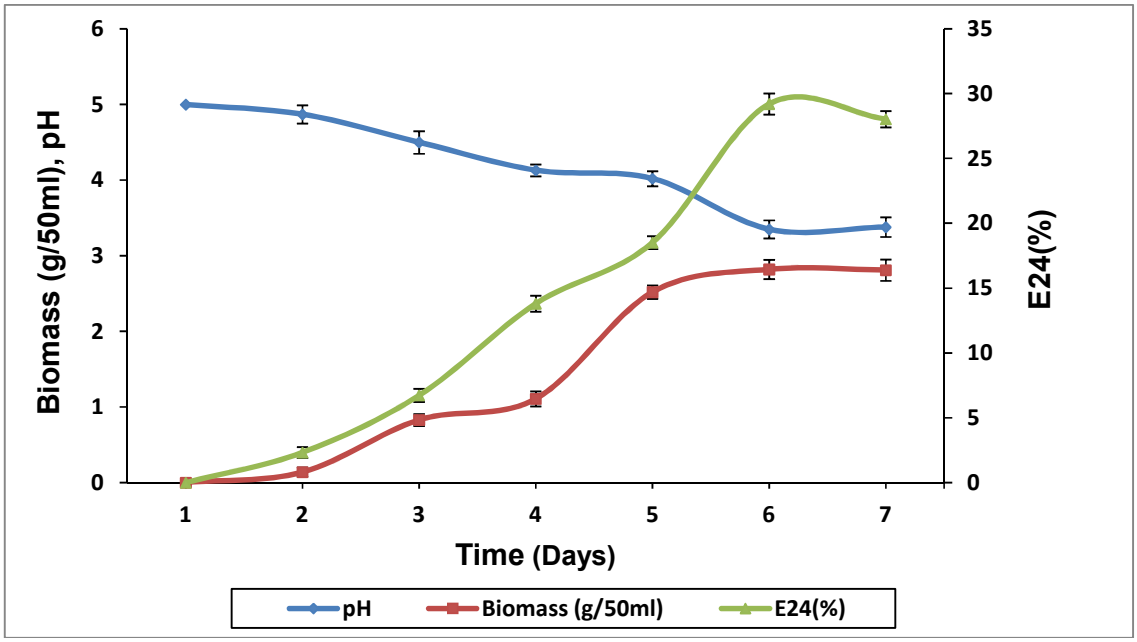

Fig. 2. The time-course study of sophorolipids production on basal medium using *Candida* sp

According to Bednarski et al. [42] and Accorsini et al. [43], constant acidity found in the culture medium is a parameter which correlates to the efficacy of cell biomass increase and glycolipid synthesis by yeasts such as *Candida antarctica* and *Candida apicola*. They further opined that a greater production of glycolipids is attained to the control and maintenance of pH value within the acidic region during the assays. However, when pH is not adjusted in the media, a negative effect is exerted on the efficacy of the synthesis.

4.3 Carbon Sources

The sophorolipid biosurfactants can be produced from a single hydrophilic carbon (carbohydrates) source, however, the production will considerably increase if a second hydrophobic source of carbon (lipids, hydrocarbon, vegetable oil and animal fat) is added [44]. The different carbon sources examined in this study comprised of three hydrophilic carbon sources (rice bran, spent mushroom substrate and cassava peelings) alongside a hydrophobic carbon source

(food industry waste oil) in all culture media. Fig. 3 shows the results of the emulsification index E_{24} (%) of the produced sophorolipids using agro-waste carbon sources (RB: Rice bran, CP: Cassava peeling, SMS: Spent mushroom substrates) at 0.1%, 0.5% and 1.0% (w/v). All the cultures were supplemented with 4% (v/v) food industry waste oil, as a hydrophobic carbon source. The media supplemented with 0.5% (w/v) rice bran exhibited the highest E_{24} of 69.22% after 144h of culture. The media with 0.1% rice bran (44.8%) gave the second highest E_{24}. The spent mushroom substrate (0.1% w/v) media showed the least emulsification activity of 11.1%.

Basically, the main source of hydrophilic carbon used in the production of the sophorolipids is glucose, used for cellular growth and the production of sophorolipids, although, other sugars can be used as a hydrophilic substrate [45,46]. In a bid to find a use for our wastes, we observed that rice bran can be a viable hydrophilic source alongside the food industry waste oil, due mainly to the waste oil's composition of fatty acids.

4.4 Stability Studies of Sophorolipids on Temperature Regimes, Salinity and PH Ranges Related to Emulsification Indices

The effect of temperature on the stability of the sophorolipids revealed that the biosurfactant was stable during incubation for 1 h at temperatures between 70 and 120°C as shown in Fig. 4. The sophorolipid retained its emulsification index at these temperatures. Higher emulsification was observed at temperatures between 30 and 50°C (39.2% and 38.7%). This result corresponds with that of Haba et al. [27] and Luna et al. [47] in stability patterns relation to temperature, but contradicts the reports by Adamu et al. [31] and Luna et al. [3] which showed higher emulsification at these tested temperature ranges.

Studies on the stability of the sophorolipids in the cell-free culture supernatant showed a stable but closely related low emulsification activity (7–9%) at lower concentrations (2-6%) of NaCl. However, the emulsification index increased with increasing concentrations of the salt and was highest (55.6%) at 10% NaCl concentration. This is presented in Fig. 5. Our findings differ with the results of Muthusamy et al. [7] who reported a higher activity at 5% concentrations and lower activity at 10% concentrations with *Candida tropicalis*. However, the high salt tolerance of our biosurfactants suggests that it may be a good candidate for marine environments and some emulsion related industries [3].

The assessed effect of pH on the stability of the biosurfactants produced at pH range of 2 to 12 is shown in Fig. 6. Sophorolipids produced by *Candida* sp. was stable over the tested pH

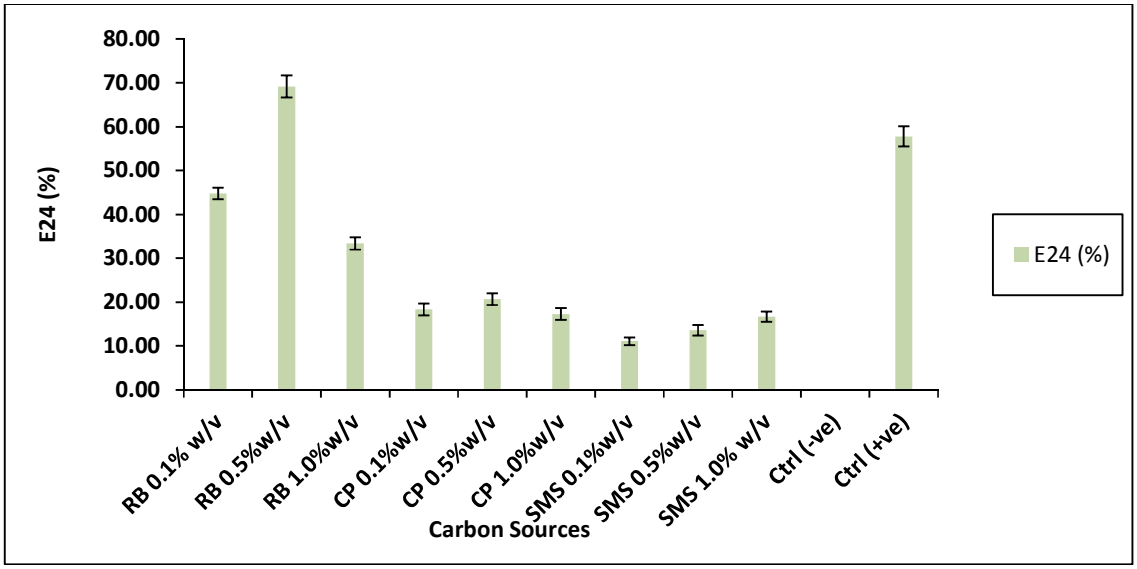

Fig. 3. The emulsification index E_{24} (%) of the produced sophorolipids using agro-waste carbon sources (RB: Rice bran, CP: Cassava peeling, SMS: Spent mushroom substrates) at 0.1%, 0.5% and 1.0% (w/v) and CTRL (Control)

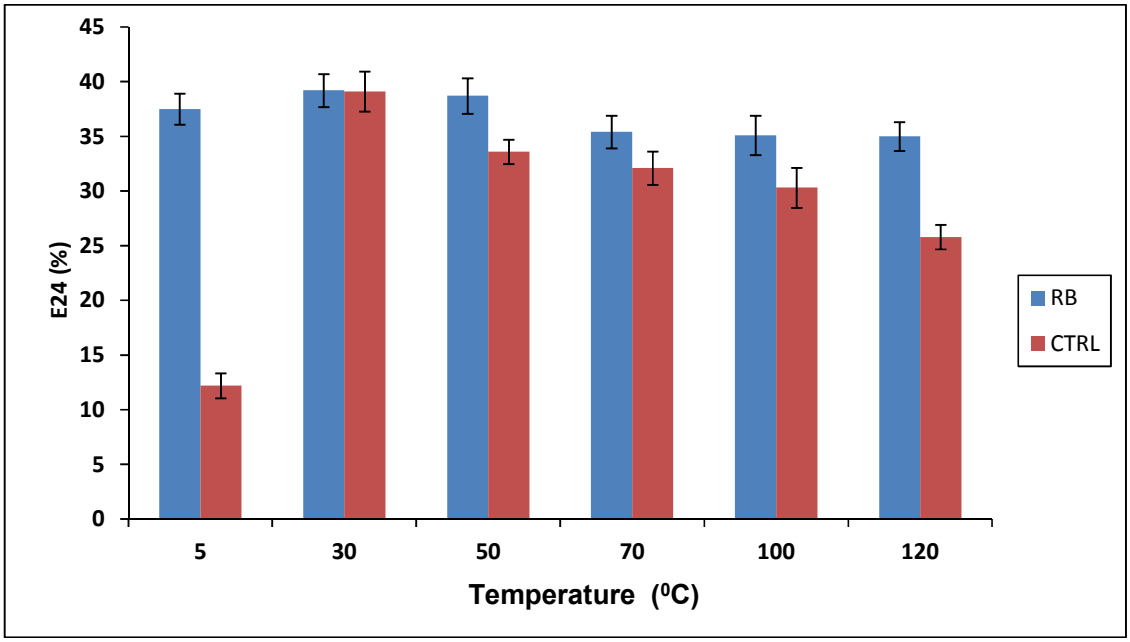

Fig. 4. Effect of different temperature regimes on Emulsification Index E_{24} (%) of sophorolipids produced using rice bran (RB) and the control (CTRL)

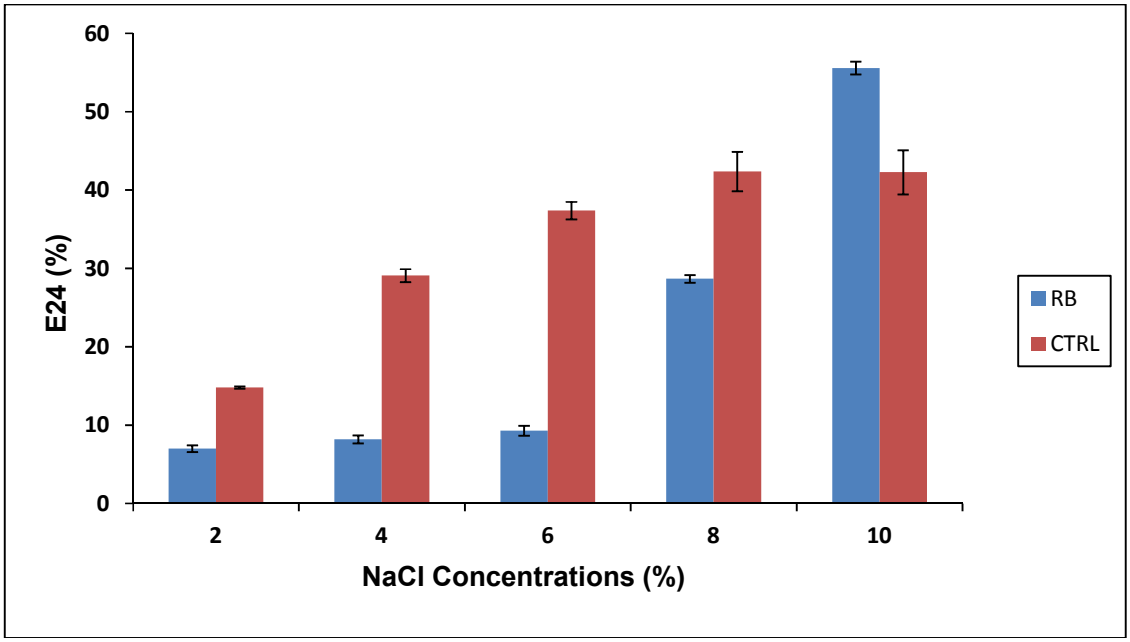

Fig. 5. Effect of salinity on Emulsification Index E_{24} (%) of sophorolipids produced using rice bran (RB) and the control (CTRL)

range, with highest activity at pH12.0 (E_{24}= 66.7%). There were no much variations in E_{24} values (54.5 – 57.7) with regards to pH levels 2 to 8. This result agrees with the findings of Luna et al. [3] and Adamu et al. [31] who reported high emulsification indices at this pH range. More so, Sarubbo et al. [48] reported the limited effectiveness of the emulsification activity of *Candida lipolytica* to the acid to neutral pH range, with a corresponding increase in activity at pH 12.0.

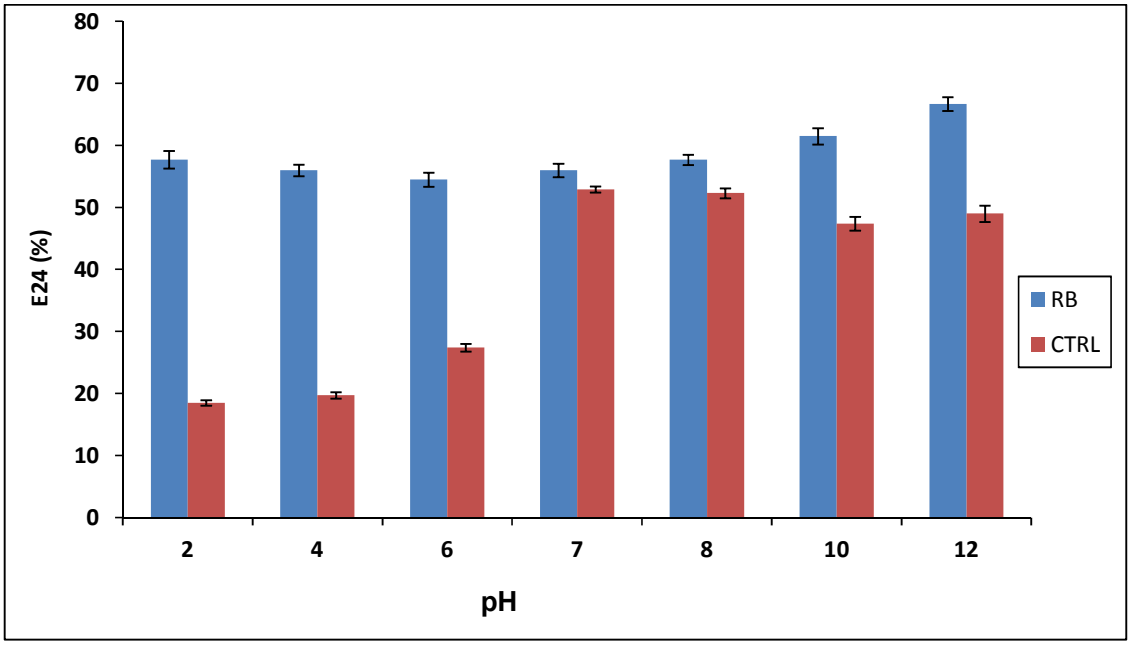

Fig. 6. Effect of different pH on Emulsification Index E_{24} (%) of sophorolipids produced using rice bran (RB) and the control (CTRL)

These stability studies indicate that the constituents of the biosurfactants are not degraded within the tested physicochemical conditions, with the emulsification activity more pronounced at 30-50°C, 10% NaCl and pH 12 with E_{24} of 39.2%, 55.6% and 66.7% respectively. Reports on the production and stability of biosurfactants at extreme conditions abound [32,49]. Considering the optimum conditions for the sophorolipids activity, the potential applicability of these surfactants in microbial enhanced oil recovery (MEOR), could be suggested and taken under advisement, since these extremes of temperature, salinity and pH occur in oil reservoirs [31].

Molecular composition of the sophorolipid was evaluated by FT-IR and GC-MS to respectively assess the functional groups and fatty acids present. Fig. 7 represents the bands of the concentrated biosurfactant from our test organism, *Candida* sp. Chandran and Das [38] had reported that biosurfactants isolated from yeast species had almost similar absorption bands, and our result agrees with theirs and the findings of Thavasi et al. [50]. The broadband observed in the biosurfactants isolated from *Candida* sp. at 3417.86 – 3439.08 cm^{-1} matches to the O-H group. The assymetrical stretch (CH_2) of methylene occured at 2922.16 cm^{-1}. The band 1637.56 cm^{-1} is from stretching of unsaturated C=C bonds. The C=O absorption band at 1735.93 cm^{-1} may include influences from that of the esters or acids and lactones. This characteristic band is usually observed within the spectra of biosurfactants of three yeast species [51]. The stretch C-O band of C (=0) –O-C in lactones which appears at 1109.07 cm^{-1} is observed in the spectra of biosurfactants isolated from *Candida* sp. The sugar C-O stretch of C-O-H groups is at 1033 cm^{-1} as was characteristically seen in the spectra of the sophorolipid produced. From this data, it is evident that the lactone form of sophorolipid is dominant in our test isolate, *Candida* sp. Researchers have reported that sophorolipids are the only surfactants produced in enormous amounts by yeast species with similar FT-IR spectra of sophorolipids from *Candida bombicola* and *Candida rugosa* obtained previously by Shah and Prabhune, [52] and Chandran and Das, [38] respectively.

The GC-MS spectra as presented in Fig. 8 revealed the abundance of the constituent fatty acids and their retention times. n-Hexadecanoic acid with a percentage composition of 25.8 showed a retention time of 24.105. Also, 9-hydroxyoctadecanoic acid with a percentage composition of 25.6 showed a retention time of 26.194 min as well as 16-hydroxyoctadecanoic acid with 16.501% composition at a retention

time of 26.104 min. Other constituents include the methyl stearate (7.21%, 26.444 min.), oleic acid (3.14%, 26.630 min) and methyl esters of 9-octadecanoic acid with 1.84% and 1.08% compositions showing retention times of 32.181 and 49.184 minutes respectively. This result is close to the reports of Cavalero and Cooper, [53] who recorded the major peak of standard sophorolipid identified as 17-hydroxyoctadecanoic acid at 36.9 minutes as well as the hydroxyl-acid methyl esters liberated by the methanolysis of the sophorolipids produced by *Candida bombicola* confirmed through their fragmentation pattern [54].

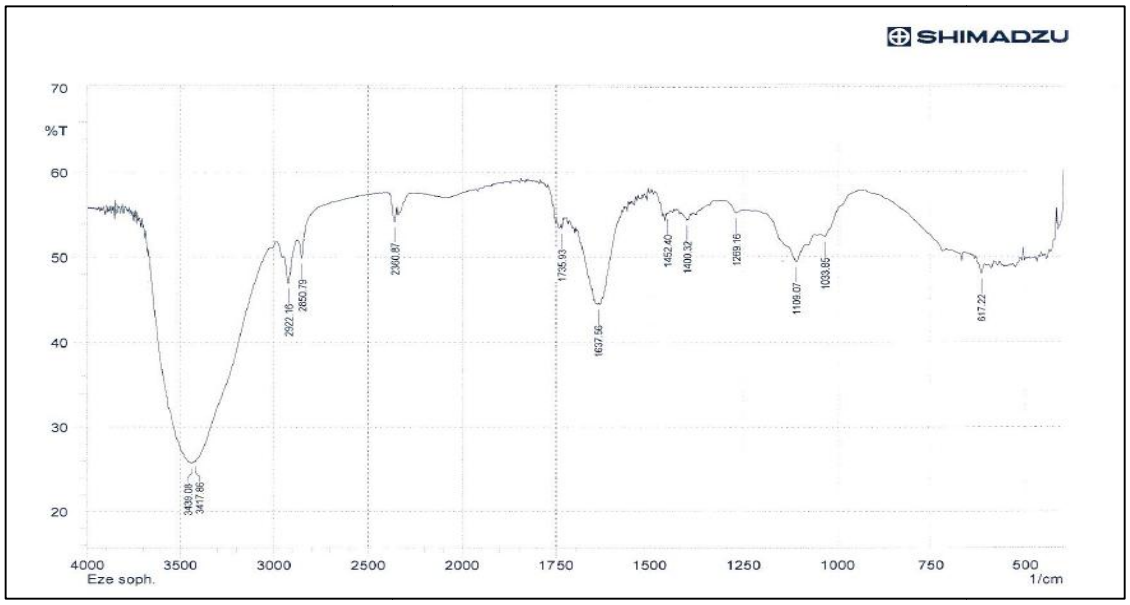

Fig. 7. FT-IR Scan of the produced sophorolipid biosurfactant showing the peaks of the hydroxyl, fatty acid and sugar functional groups of the sophorolipids

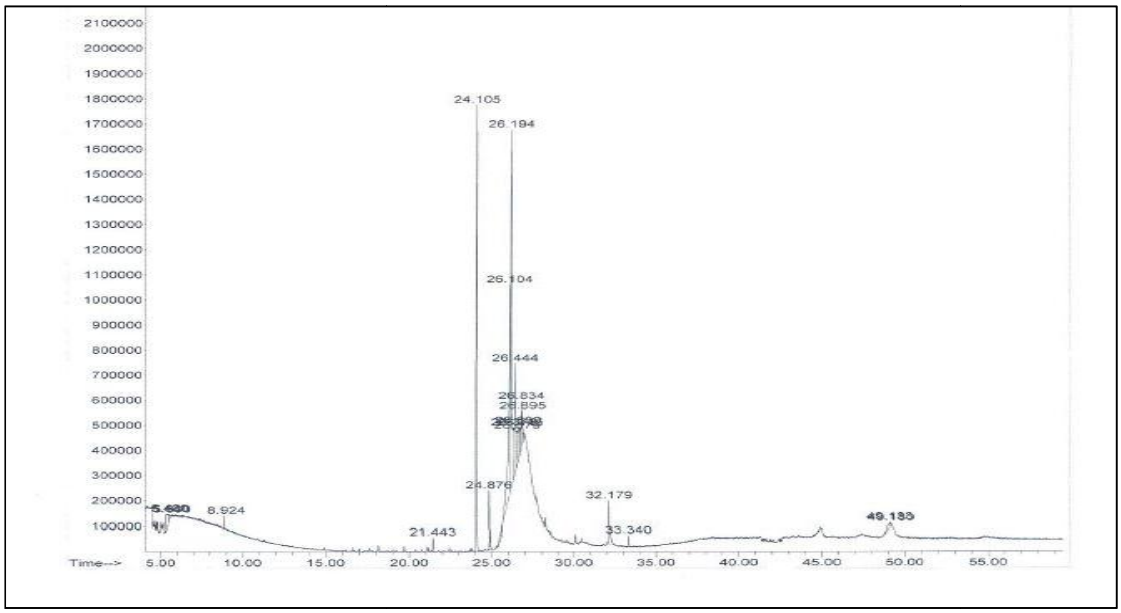

Fig. 8. The GC-MS profile of the produced sophorolipid biosurfactant showing the constituent fatty acids

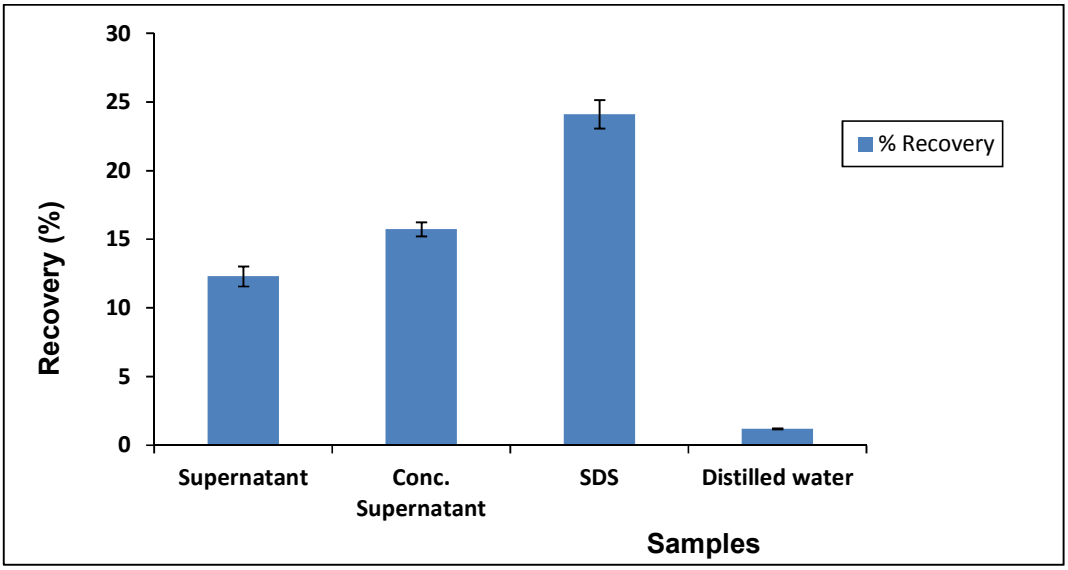

Fig. 9. A chart for the displacement efficiency of the sophorolipids

The chart for the hydrocarbon displacement efficiency is represented in Fig. 9. The result indicates 12.31% of residual oil was recovered after injecting the culture supernatant through a sand packed column. However, an additional recovery of 15.74% of residual oil was observed after concentrating the cell-free-supernatant containing the sophorolipid biosurfactants. Sodium dodecyl sulphate used as the positive control recovered 24.11% of the residual oil, while distilled water used as a negative control recovered 1.21%.

This result shows high promises of using this bio-product in hydrocarbon recovery, although the experimental value recorded does not appropriately portray field values since real life conditions were not fully mimicked in the study. Volkering et al. [55] and Salihu et al. [56] suggest that this surface-active compound enhanced the recovery of the crude oil through a mechanism that involves the decrease in interfacial tension between an aqueous and a non-aqueous phase, resulting in formation of emulsions that lead to improved mass transfer of the non-aqueous hydrocarbon to the aqueous phase.

5. CONCLUSION

The yeast *Candida* species used in this research was isolated from a hydrocarbon contaminated site. The organism presented as a potent producer of sophorolipid biosurfactants from agro-industrial wastes (rice bran and food industry waste oil) having high emulsification activity. The stability of the sophorolipids over a wide range of pH, salinity and temperature enables their application in extreme environments such as temperate marine compartments and oil reservoirs as well as industry systems where these physicochemical conditions and extremes are integral elements.

ACKNOWLEDGEMENTS

The authors gratefully thank the World Bank African Centre of Excellence in Oilfield Chemical and Research (CEFOR), University of Port Harcourt, Nigeria for the scholarship. We also thank Fugro Nigeria Limited (FNL) for providing the necessary laboratory facilities for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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