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Production and Characterization of Biosurfactants by *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15 for Crude Oil Recovery and Cleaning of Oil Contaminated Fabrics

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ABSTRACT:
The production and characterization of biosurfactant by *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15 were examined in this study. Seven agro-waste substrates (corn cob, cassava effluent, maize bran, rice straw, banana peels, corn husk and sugar cane bagasse) were used for yeast growth. The substrates supported yeast growth with banana peels having the highest optical density (OD) of 0.704 for *S. cerevisiae* H02; 0.417 for C. boleticola H09 and 0.458 for *R. bogoriensis* H15. This was followed by corn husk, maize bran and cassava effluent. These four substrates, which supported growth of yeasts were used in biosurfactants production by the yeasts. The result revealed that *R. bogoriensis* H15 had the highest yield of 2.62 g/l on corn husk, 2.02 g/l on maize bran, 1.16 g/l on cassava effluent and 0.5 g/l on banana peels. Chemical characterization of the biosurfactants was done using GC-MS and the results obtained indicated that they were glycolipids composed of palmitic hexadecanoic, octadecanoic acids, methyl esters and hydroxylated fatty acids linked to a decanoic acid. Fourier transform infrared (FTIR) analysis showed that the surfactants consisted of carboxyl, hydroxyl and sugar derivative groups. Specifically, C. boleticola H09 and R. bogoriensis H15 produced biosurfactants designated as lactonic sophorolipid Bios-H09 and acid sophorolipid Bios-H15 respectively. The potentials of the biosurfactants to serve as detergent additive as well as detergent was evaluated on a white cotton cloth soiled with 3 ml motor oil using shake flask method. So-Klin detergent had 66.15 % wash followed by mixture of So-Klin and Bios-H09 (58.8 %), So-Klin and Bios-H15 (28 %), Bios-H09 (24.7 %), Bios-H15 (15.8 %) and distilled water (4 %). The potential of the biosurfactant to enhance crude oil recovery was also examined using sand pack column method. Bios-H09 enhanced oil recovery of 47.75 %, Bios-H15 recovered 32.25 % and distilled water (control) recovered 15.25 % of crude oil. The results suggest that *C. boleticola* H09 and *R. bogoriensis* H15 are good candidates for the production of biosurfactants, particularly sophorolipids that can be used for enhanced oil recovery.

Keywords: Biosurfactant, *Candida boleticola* H09, *Rhodotorula bogoriensis* , agro-waste, detergent additive, crude oil recovery

INTRODUCTION:

Biosurfactants are amphilic compounds that are secreted extracellularly and contains both hydrophilic moieties, which reduce the surface and interfacial tension of the surface and interface respectively [1]. They are complex molecules, comprising different structures that include glycolipids, lipopeptides, polysaccharide, protein complexes, fatty acids and phospholipids [2-3]. Because of these properties, biosurfactants have potential applications in food, pharmaceutical and cosmetic industries [4-6]. Structurally, biosurfactants are polymers totally or partially extracellular, amphipathic molecules containing polar and non-polar moieties which allow them to form micelles that accumulate at interphase between liquids of different polarities such as water and oil [4, 7-8].

Unlike the chemically produced biosurfactants that are classified according to the type of the polar groups present and their dissociation pattern in water, biosurfactants are classified by their microbial origin, mode of action, chemical composition, molecular weight and physicochemical properties. According to their

molecular weight, they are grouped as low-molecular mass biosurfactant which comprise glycolipids, phospholipids, and lipopeptides and high molecular weight mass biosurfactant/bioemulsifiers which comprise particulate biosurfactant, amphipathic polyschaarides, lipopolysaccharides, proteins, lipoproteins and either complex mixtures of these biopolymers [6,9-10].

Biosurfactant with low-molecular mass tend to be efficient in both reducing and interfacial tensions, whereas those belonging to the high – molecular mass category are more effective and efficient at stabilizing oil-in-water emulsions [11-12]. They act by accumulating at the interface between two immiscible liquids or between a liquid and a solid thereby lowering surface (liquid-air) and interfacial (liquid-liquid) tension, by reducing the repulsive forces between two non-similar phases thus allowing these two phases to mix and interact more easily [13. Majority of these biosurfactants are ionic, neutral or anionic and the hydrophobic moieties are usually based on long-chain fatty acids or fatty acid derivatives, whereas the hydrophilic moieties can be a phosphate, amino acid, carbohydrate or cyclic peptide [14].

The success of biosurfactant production depends on the development of cheaper processed and use of low cost raw materials, which account for 10-30% of the overall cost [15-16]. Research on the selection of suitable substrates has mainly centred on tropical agro-industrial crops and residues. Additional substrates have been suggested for biosurfactant production, especially eater-miscible wastes such as banana peels, corn husk, cassava effluent, molasses, whey milk or distillery wastes [16-18].

To reduce the costs of biosurfactants production, it is necessary to select microorganisms capable of high-yield biosurfactants production as well as use of cheaper substrates such as agricultural and industrial wastes [4, 11, 18]. Various microorganisms such as bacteria and fungi are known to produce specific kind of biosurfactant [19]. Yeasts are known to produce biosurfactants in higher concentrations than bacteria but minimal studies have reported biosurfactants produced by yeasts [20]. Therefore, the aim of this study was to screen yeasts for production of biosurfactants for use in crude oil recovery and cleaning of oil contaminated fabric.

MATERIALS AND METHODS

Collection of Agrowastes substrates

The agrowaste substrates used in this study were corn cob, cassava effluent, maize bran, rice straw, banana waste, corn husk and sugarcane bagasse. The corn cob was obtained from corn stock at Gidan Mangoro village, Minna Nigeria. The cassava effluent and maize bran were obtained from Tunga Market Minna, Nigeria. The rice straw was obtained from a rice farm located at the frontage of Hill-Top Model School along David Mark Road Minna, The banana waste was obtained by peeling of ripe green banana obtained from Tunga Market area in Minna. Sugarcane bagasse was obtained by feeding on sugarcane obtained from Tunga Market, Minna and the resultant bagasse was used in this study. The corn cob, maize bran, rice straw, banana waste, corn husk and sugarcane bagasse were sun dried for three days and then grounded with mortar and pestle into fine powder, 100 g of each of the sample was boiled in water using kerosene stove and aluminium pot for two hours. The liquid substrate was then reconstituted to 200 ml in a 250 ml conical flask and sterilized by autoclaving at 121°C for 15 minutes after which it was allowed and stored in the refrigerator for further use.

The kerosene and diesel used for this study were obtained from Oando Petrol Filling Station Bosso Minna Nigeria
The soil sample used for this study was a sandy soil obtained in Tunga Market Area Minna. The nature of the soil was determined at Geology Department Federal University of Technology Minna using the Unite States Standard Sieve Mech Size sand analysis method

Yeast strains

Two yeast isolates *Candida boleticola* and *Rhodotorula bogoriensis* were used for this study. The yeasts were obtained from the Department of

Microbiology, Federal University of Technology, Minna, Nigeria. The isolates were screened from spent lubricating oil polluted soil and pristine soil in Bosso Niger State Nigeria. The yeast isolates were subcultured on yeast extract-malt agar and incubated at 28°C for 48 hours. The viable yeasts were screened for their ability to produce biosurfactant.

Screening of substrates for biosurfactant production

Four agro-waste substrates cassava effluent, maize bran, banana peel and corn husk) that best supported yeast growth were tested for their potential use as carbon sources for biosurfactant production. This method was carried out in 250 ml conical flasks containing 100 ml of Bushnell-Hass medium, supplemented with 4 ml of agro waste substrate and sterilised by autoclaving at 121°C for 15 minutes. This was allowed to cool and then inoculated with 1 ml of 48 hours old nutrient broth yeast culture and incubated on a rotary shaker at room temperature for 14 days. It was then centrifuged at 6000 rpm for 30 minutes and then /culture supernatant was extracted using hexane and the quantity of biosurfactant produced was measured by determining the dry weight. This procedure was carried out for each of the three yeasts using four different substrates.

Biosurfactant extraction

Extraction of biosurfactant was done using acid precipitation method according to Ibrahim *et al.* [21]. In this method, 14 days old broth culture was centrifuged at 6,000 rpm for 30 minutes where the deposit was the yeast cells which were discarded and the supernatant which contained the biosurfactant was extracted by adding 50mls of the supernatant with 50 ml of Chloroform and methanol (2:1 v/v) in a separating funnel. The mixture was then shaken vigorously and allowed to stay for one minute after which the mixture differentiated into lower and upper layer. The biosurfactants were retained within the lower layer which was the solvent layer while the upper layer was the liquid medium used. The solvent layer was collected in a conical flask through the tap of the funnel. The solvent was then removed by rotary evaporation and the resultant residue obtained was the crude biosurfactant

Determination of dry weight of biosurfactants

The weight of the biosurfactant was determined using the method of Chandran and Das [20]. The cell free culture broth was centrifuged at 6000rpm for 30 minutes and extracted with chloroform and methanol (2:1, v/v). The solvent was removed by rotary evaporation and the resultant residue obtained was the crude biosurfactant. Clean empty Petri plate was weighed and the residue was placed into the Petri plate and weighed. This was placed in the hot air oven for drying at 1000C for 30 minutes, after which the dry weight of the biosurfactant was calculated using the formula: DWB = WPBAD – WEP, where: DWB = dry weight of biosurfactant, WEP = weight of empty petri dish and WPBAD = weight of petri dish containing biosurfactant after drying

Characterization of Biosurfactants using Gas chromatography – mass spectrophotometry

The GC-MS analysis of crude biosurfactant (1 mg) was carried out to elucidate the chemical structure of the biosurfactants. This was carried out with GC-MS (Model QP2010 PLUS, Shimadzu, Japan) equipped with a RTX-5MS (20mx0.2mm) capillary column and mass selective detector (AOC-20i) set to scan from m/z 40 to m/z 800 at a scan rate of 1.2 scans per second using methods of National Research Institute for Chemical Technology, NARICT [22].

Characterization of Biosurfactants using Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) analysis was carried out to determine the functional groups in the biosurfactant according to the method used by NARICT [22]. One milligram of lyophilized purified biosurfactant was grounded with 100 mg of potassium bromide (KBr) and pressed with a silver coated hand presser at 7, 500 kg of pressure for 30 seconds to obtain translucent pellets. The pellet obtained was inserted into Fourier transform infrared spectrophotometer (FTIR-8400S, Shimadzu, Japan) where the infrared spectra were recorded within the range of 500-4500 cm-1 wave number. All measurements consisted of 500 scans, and KBr pellet was used as background reference.

Detergency Test of the Biosurfactants

The detergency property of the biosurfactant to remove oil with respect to commercially available detergent was studied with a view to establish the potential of biosurfactants as a detergent as well as a detergent additive. This test was carried out using 12 pieces of white cotton fabrics measuring 25 cm x 25 cm, motor oil, so-klin detergent and biosurfactants. Three millilitres of motor oil was poured on a white cotton fabric and oven dried at 400C for 24 hours. To test the oil removal capability, each piece of the white cotton cloth impregnated with oil was soaked in 250 ml conical flasks containing 100ml each of tap water (control), so-klin detergent, biosurfactants, and a mixture of so-klin detergent and biosurfactants. The flasks were kept on a shaker at room temperature, 100 rpm for 60 minutes after which the fabrics were removed from the conical flaks. The experiment was carried out in duplicate. The post water wash was used to measure the amount of oil removed from the cotton cloth by extracting it with hexane. The extraction process was done by adding 50 ml of hexane to the post water wash in a separation funnel, shaken vigorously and allowed to stay for 1 minute. The oil washed is trapped in the hexane which is drained out through the tap of the separation funnel. The hexane was recovered using a rotary evaporator and the residual motor oil was measured gravimetrically [1, 21].

Crude Oil Recovery Experiment using Sand Pack Column

The enhance oil recovery potential of biosurfactants produced by *Candida boleticola* and *Rhodotorula bogoriensis* was investigated in duplicate using the sand packed column assay. Distilled water and a mixture of sand and crude oil served as the controls. Glass column measuring 57cm in height x 6cm in diameter was vertically fixed on a retort stand and filled with a mixture containing 200g of soils and 20ml of crude oil. The surface was then inundated with 200ml of the biosurfactant solution and percolation of biosurfactant solution under the action of gravity was observed after 10 hours. After percolation of biosurfactant solutions through the columns, the soil samples were then transferred into 250 ml conical flask and washed with 20ml of hexane for removal of the residual oil. The solvent was recovered using rotary evaporator and the amount of oil removed was determined gravimetrically [23-24].

Statistical Analysis

Data was analyzed statistically using error mean square and correlation analysis. Computer statistical package SPSS 9.0 was used.

RESULTS

Biosurfactant Production by *Candida boleticola* and *Rhodotorula bogoriensis* H15 using different agro wastes

Four agro wastes (Corn husk, maize bran, banana peel, cassava effluent) were examined for their potential as substrates for biosurfactant production by *Candida boleticola* and *Rhodotorula bogoriensis.* The result (Table 1) showed that corn husk had the highest biosurfactant (2.62 g/l) produced by *Rhodotorula bogoriensis* H15 followed by maize bran (2.02 g/l) while banana peel gave the least biosurfactant (0.50 g/l). However, Banana peel biosurfactant (0.50 g/l) had the highest biosurfactant by *Candida boleticola* H09.

Characterization and Identification of Biosurfactants

The GC-MS analysis revealed the presence of unknown compounds especially the fatty acyl components in the biosurfactants, each peak representing a compound. From the results of the GC-MS analysis for biosurfactant produced by *Candida boleticola* H09 coded as Bios-H09 (Figure 1; Table 2), peak numbers 1,2,3,4 and 7 represent fatty acids such as palmitic acid (C16), stearic acid (C18) and Oleic acid (C18), (all saturated), the fatty acid moiety of peak no. 5,6,8 and 10 consists of unsaturated fatty acids of n-C18 carbon length. Peaks no. 5 and 10 consists of unsaturated acetyl and aldehyde moiety.

Fourier Transform Infra-Red (FTIR) analysis of biosurfactants

The spectra for biosurfactants produced from the FTIR analysis revealed peaks of different shapes, each represents specific functional groups that are present on the molecular chain in the biosurfactants studied. From the infra-red (IR)

analysis for Bios-H09 (Figure 3), the broad band at region 3219.30cm-1 and 3390.97cm-1 shows the hydroxyl group (O-H) stretching from carboxylic acids (COOH), the band at 2459.32cm-1 and the attached 2420.74cm-1 represent an sp2 CH group comprising –CH3-CH2- of alkane, the unsaturated C=C bonds is evident in the weak nature of the band at 1427.37 cm-1 and this is true for the fact that fatty acids of sophorolipids contain one or more unsaturated bonds, carbonyl (C=O) band stretching from the esters group was found at 1653.05 cm-1, Lactones and esters have two absorption bands arising from C=O and C-O stretching. The C=O absorption band at 1653.05 cm-1 may include contributions from that of lactones, esters, or acids [25]. The stretch of C-O band of C- (=O) –O-C in lactones appear at 1105.25cm-1 (Table 3). From the FT-IR data it is evident that lactonic form of sophorolipids was produced by *Candida boleticola* H09.

From the infra-red (IR) analysis for Bios-H15 (Figure 4), the broad band that corresponds to hydroxyl group (O-H) that stretches from carboxylic acid (COOH) was at 3435.34cm-1, the band at 2387.95cm-1 and the attached 2306.94 and 2154.56cm-1represent an sp2 CH group comprising –CH3-CH2-CH3- of alkane, the unsaturated C=C band appeared at 1384.94cm-1, the C=O band stretching was observed at 1643.41cm-1, the C-O band occurred at 1084.03cm-1. The carbonyl C=O and the C-O may belongs to carboxyl group (COOH) due to the absence of a peak showing for lactone ring.

Detergency Potential of Biosurfactants Produced by *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15 in Cleaning of Oil Contaminated Fabrics

The ability of biosurfactants produced by the two yeasts to serve as detergent as well as detergent additive was tested. The results revealed that the use of a commercial detergent (So-klin) in cleaning oil contaminated fabrics was more effective as it removed 66.15% of the oil contained in the fabric (Figure 5). When used as a detergent additive, the biosurfactants produced by *Candida boleticola* H09 was able to remove 58.8% while biosurfactant produced by *Rhodotorula bogoriensis* H15 was able to remove 28.5% of oil contained in the fabric. When compared to the performance of the commercial detergent, the poor performance obtained with the use of biosurfactants in addition to the commercial detergent signifies the poor potential of the biosurfactants for use as a detergent additive as it reduced the detergency activity of So-Klin detergent.

However, the detergency potential achieved by the use of So-Klin on oil contaminated fabric was not statistically significantly different (p>0.05) from level attained by the use of *Candida boleticola* H09 biosurfactant and So-Klin in combination. Furthermore, the detergency potential achieved by the use of biosurfactant produced by *Candida boleticola* H09 as detergent additive was significantly different and performed better when compared to biosurfactant produced by *Rhodotorula bogoriensis* H15 as detergent additive (28.5%; p<0.05).

Enhanced Oil Recovery from Soil by Biosurfactants Produced by *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15

The effectiveness of biosurfactants produced by the two yeasts to enhance crude oil recovery from soil was investigated using column packed with sandy soil of varying particle size. The results revealed that biosurfactants produced by *Candida boleticola* H09 was quite effective as it was able to enhance the recovery of 47.75% of crude oil from soil (Figure 6).

DISCUSSION

The results in Table 1 revealed that corn husk was a better substrate for biosurfactant production as R. bogoriensis H15 produced 2.62g/l of biosurfactant on corn husk than other substrates with 2.02g/l, 0.50g/l and 1.16g/l on maize bran, banana peels and cassava effluent. On the other hand, significant differences (P<0.05) existed in quantity of biosurfactant produced by R. bogoriensis H15 as compared to and C. boleticola H09 and this shows that *R. bogoriensis* H15 was a better biosurfactant producer than *Candida boleticola* H09. Banana peels gave amounts of biosurfactant (0.50g/l) when the test organisms

were grown on it respectively. However, banana peel was used in biosurfactant production by Halobacteriaceae archaeon in a study conducted by Chanika *et al.* (25) where the bacterium produced 5.30g/l of biosurfactant in a minimal salt medium containing 35 % (w/v) banana peel and 1 g/l commercial monosodium glutamate at 30 °C and 200 rpm after 54 hours of cultivation.

The presence of various fatty acids as revealed by the GC-MS analysis has confirmed the hydrophobic part of the biosurfactant. Bios-H09 is suspected to be a sophorolipid because of the presence of unsaturated fatty acids, fatty aldehyde and an acetylated compound since one or both of the 6'-hydroxyl groups on the glucose units are acetylated. The carboxylic end of this fatty acid is either free (Acidic form) or internally esterified (Lactonic form). The hydroxyl fatty acid itself counts in general 16 or 18 carbon atoms and can have one or more unsaturated bonds [27-28]. Several researchers have reported biosurfactants produced by *Candida* species as sophorolipids [28, 30]. In a similar study conducted by Zhang et al. [31] where sophorolipid was produced when *Rhodotorula bogoriensis* was grown on rapeseed oil and meadow foam oil, rapeseed oil gave 1.26g/l while meadow foam oil gave 0.77g/l of sophorolipid. Based on these established facts, it can be deduced that Bios-H09 is a sophorolipid consisting of unsaturated fatty acids that have been esterified to form a lactone (Lactonic form) attached to disaccharide sophorose sugar on which one or both of the 6'-hydroxyl groups have been acetylated.

From the chromatogram for biosurfactant Bios-H15 (Figure 2), the peaks number 7, 8, 10 and 13 represents fatty acids such as palmitic acid and stearic acid. Peak no. 14 represents hexadecanoic acid which was esterified to 2,3-dihydroxypropyl ester, 3, 4, 5 and 6 consists of fatty acids of C8, C9, C10 and C12 carbon length respectively, 11 and 16 indicates the unsaturated fragments of the fatty acid.

The presence of various fatty acids of varying chain lengths as revealed by GC-MS analysis has confirmed the presence of the hydrophobic part of the biosurfactant. The presence of unsaturated fragments further indicates the unsaturated nature of the fatty acids present in the biosurfactant. Comparing the compounds present in the biosurfactant Bios-H15 produced by *Rhodotorula bogoriensis* H15 and Bios-H09 from *Candida boleticola* H09 as revealed from the chromatographic analysis, it was observed that Bios-H15 differs from Bios-H09 in the absence of acetyl and an aldehyde group, implying that the hydroxyl groups of the sophorose sugar in Bios-H15 were not acetylated making it less suitable in biomedical applications [32].

The fatty acids in Bios-H15 were not esterified to form lactonic ring as compared to Bios-H09 which is a lactonic form of sophorolipids, thus Bios-H15 is an acid form of sophorolipids. The information from the respective wave numbers confirmed the glycolipid nature of the biosurfactants (Table 3). Even with slight differences in wave numbers for a particular functional group, this result is in agreement with the report of Chandran and Das [33] that characterized sophorolipids produced by Trichosporon asahii. Similar results were reported by Chandran and Das [20] and Basak and Das [38].

However, the differences in wave numbers as represented by various peaks may be due to difference in organisms, which may have resulted in variation in the composition of fatty acid chains of sophorolipids or different positions of the functional groups present in the compounds. From the GC-MS and FTIR analysis and the kind of organism that produced this biosurfactant (Bios-H09), it is confirmed that the biosurfactant is a sophorolipids and belong to glycolipid group, made up of aliphatic acid, acetyl and ester; therefore this study designates the biosurfactant as sophorolipid Bios-H09.

Table 1 Dry weight of biosurfactants produced by yeast

Substrate	*C. boleticola* H09 (g/l)	*R. bogoriensis* H15 (g/l)
Corn husk	0.30±0.01[a]	2.62±0.89[c]
Maize bran	0.40±0.020[a]	2.02±0.09[c]
Banana peel	0.50±0.011[a]	0.50±0.014[a]
Banana peel	0.30±0.017[a]	1.16±0.07[b]

Values are Mean ± SEM for n=2. Values of the same superscript alphabet are not significantly different at p>0.05

Table 2 Compounds present in the biosurfactant Bios-H09

Peak	Retention time (min)	%Height	Compound name	Molecular formular
1	9.375	0.31	Nonanoic acid	$C_9H_{18}O_2$ (158)
2	11.842	2.62	Undecanoic acid	$C_{11}H_{22}O_2$ (186)
3	14.242	4.65	Palmitic acid	$C_{16}H_{32}O_2$ (256)
4	18.117	18.05	Palmitic acid	$C_{16}H_{32}O_2$ (256)
5	19.542	1.35	Z,Z-10,12-Hexadecadien-1-ol-acetate	$C_{18}H_{32}O_2$ (280)
6	21.017	19.19	Oleic acid,9-Hexadecanoic acid	$C_{18}H_{34}O_2$ (282)
7	21.217	18.18	Stearic acid	$C_{18}H_{36}O_2$ (284)
8	21.817	8.80	1,E-11,Z-13-Octadecatriene	$C_{18}H_{32}$ (248)
9	23.125	6.63	Octadecane,1-(ethenyloxy)	$C_{20}H_{40}O$ (296)
10	23.808	7.43	(E)-13-Docosenoic acid	$C_{22}H_{42}O_2$ (338)
11	24.033	4.14	Oxalic acid	$C_{24}H_{46}O_4$ (398
12	24.383	3.79	2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$ (330)
13	26.058	4.87	7-Tetradecenal,(Z)-	$C_{14}H_{26}O$ (210)

Table 3 Compounds present in the biosurfactant Bios-H15

Peak	Retention time (min)	%Height	Compound name	Molecular formular
1	5.575	2.71	1,6-octadien-3-ol, 3,7-dimethyl	$C_{10}H_{18}O$
2	6.758	0.40	3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)	$C_{10}H_{18}O$
3	6.867	0.97	Octanoic acid	$C_8H_{16}O_2$
4	8.150	0.69	Nonanoic acid	$C_9H_{18}O_2$
5	9.442	0.50	Decanoic acid	$C_{10}H_{20}O_2$
6	11.908	2.97	n-Hexadecanoic acid, Palmitic acid	$C_{16}H_{32}O_2$
7	14.350	6.30	n-Hexadecanoic acid, Palmitic acid	$C_{16}H_{32}O_2$
8	18.500	17.43	n-Hexadecanoic acid, Palmitic acid	$C_{16}H_{32}O_2$
9	21.050	17.03	9,17-Octadecadienal,(Z)-	$C_{18}H_{32}O$
10	21.500	17.34	Octadecanoic acid, Stearic acid	$C_{18}H_{36}O_2$
11	22.117	9.23	1,E-11,Z-13-Octadecatriene	$C_{18}H_{32}$
12	23.31	10.49	1-Octanol, 2-butyl	$C_{12}H_{26}O$

13	23.425	4.20	n-Hexadecanoic acid, Palmitic acid	$C_{16}H_{32}O_2$
14	24.433	3.15	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$
15	25.475	2.83	Nonadeca-2(9),10(17)-diene,19,19-dimethyl-	$C_{21}H_{32}$
16	26.100	3.75	E-9-Tetradecenal	$C_{14}H_{26}O$

Table 4 Infra-red interpretation for the biosurfactant Bios-H09 produces by *Candida boleticola* H09

Peak	Wave no (cm^{-1})	Functional groups
1	453.29	C-H, bending
2	532.37	C-H
3	607.60	C-H
4	763.84	C-H
5	904.64	C-H
6	1105.25	C-O
7	1427.37	C=C
8	1653.05	C=O,ester
9	2420.74	$-C-H_2$ alkane
10	2459.32	$-C-H_2$ alkane
11	3219.30	O-H, COOH
12	3390.97	O-H, COOH

Table 5. Infra-red interpretation for the Bios-H15 produced by *Rhodotorula bogoriensis* H15

Peak	Wave no (cm^{-1})	Functional groups
1	405.06	C-H
2	869.92	C-H
3	937.44	C-H
4	1084.03	C-O
5	1384.94	C=C
6	1643.41	C=O,COOH
7	2154.56	CH_2
8	2306.94	CH_2
9	2387.95	CH_2
10	3435.34	O-H,COOH

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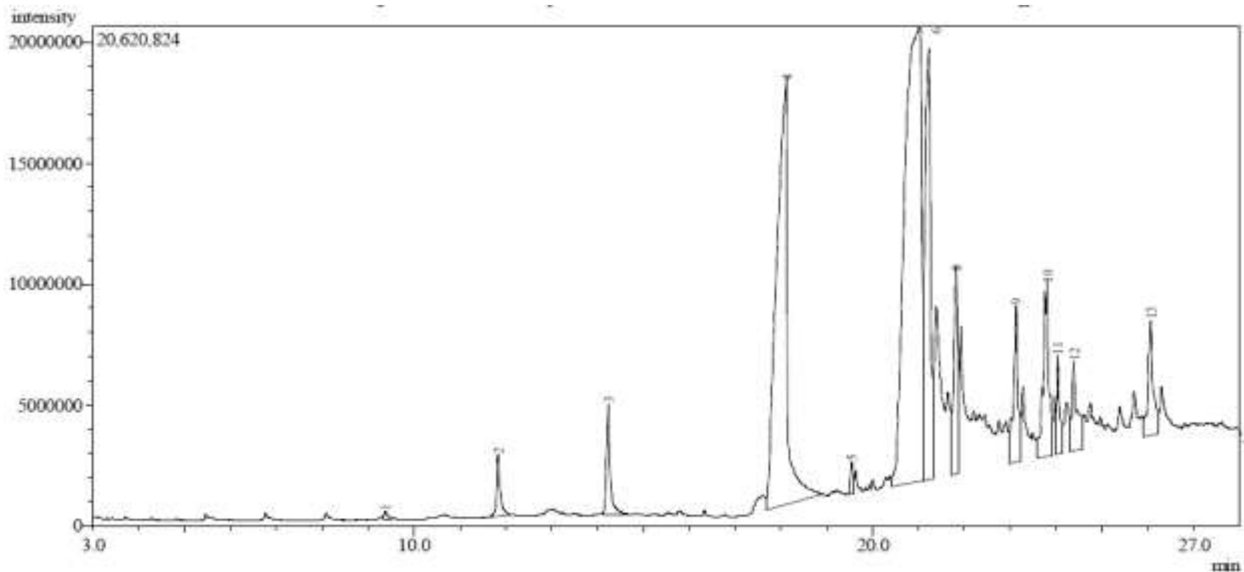

Figure 1. Chromatogram of biosurfactant Bios-H09

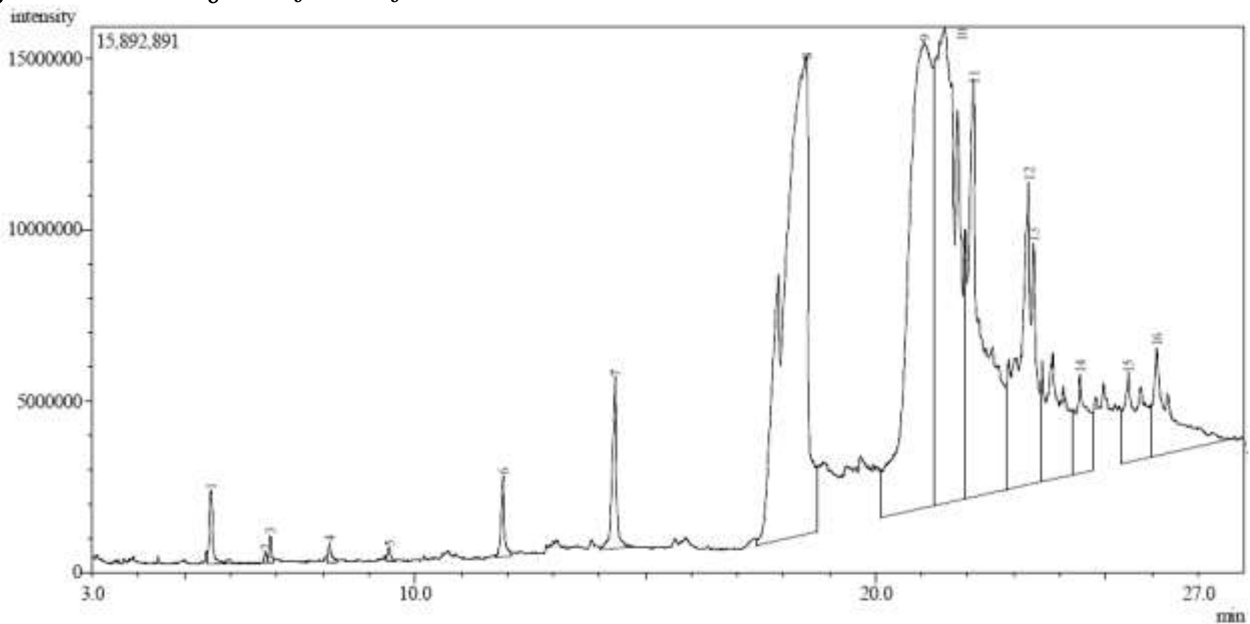

Figure 2. Chromatogram of biosurfactant Bios-H15

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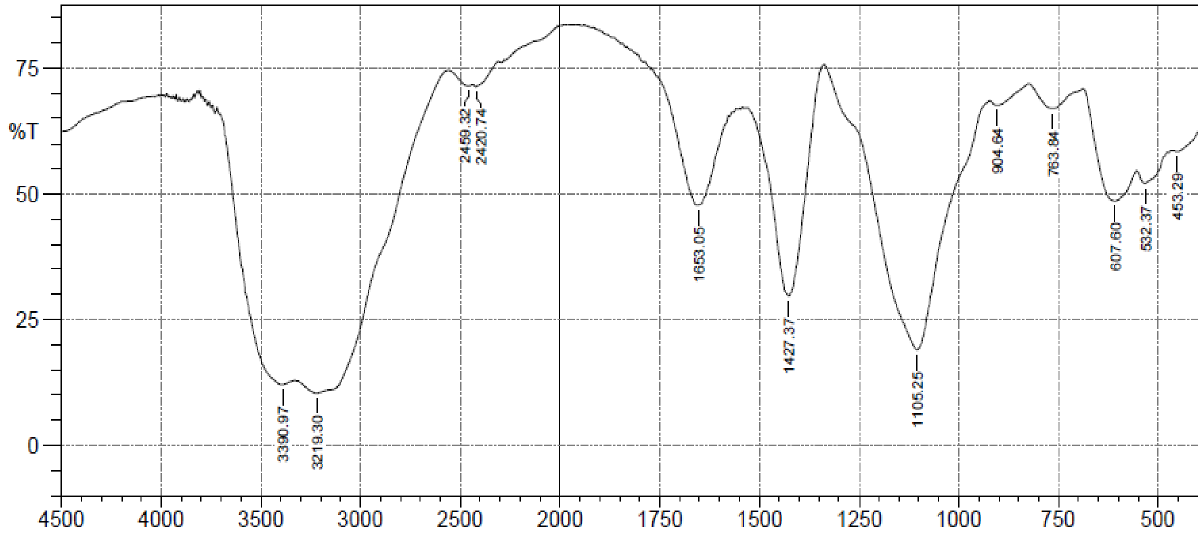

Figure 3. Infra-red spectra for the biosurfactant Bios-H09 from *Candida boleticola* H09
The various numbers attached to peaks are the wavelength (cm) corresponding to each functional group

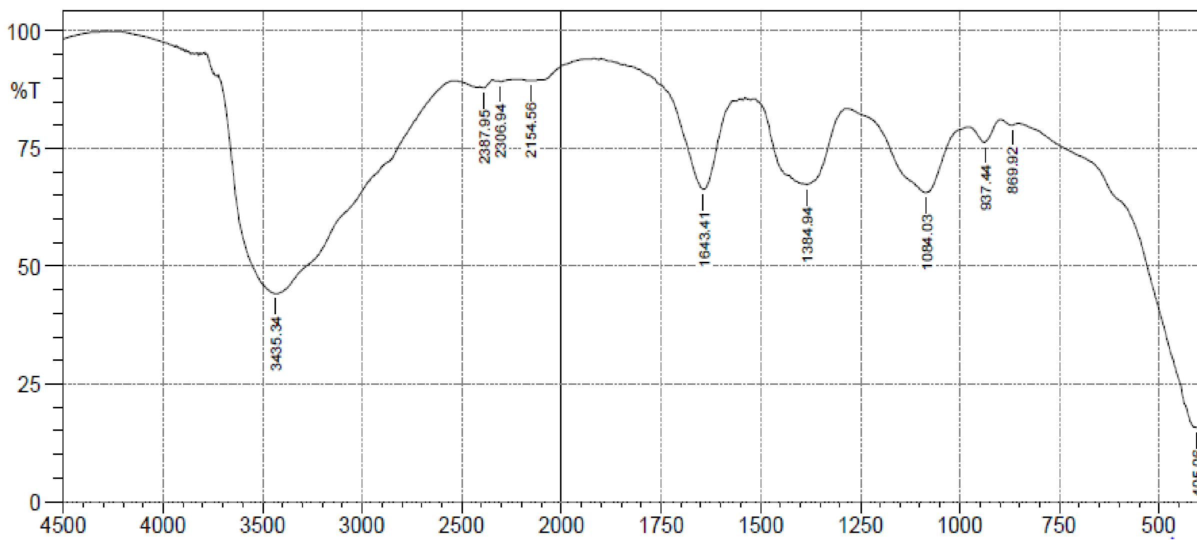

Figure 4. Infra-red spectra for the biosurfactant Bios-H15 from *Rhodotorula bogoriensis* H15
The various numbers attached to peaks are the wavelength (cm) corresponding to each functional group

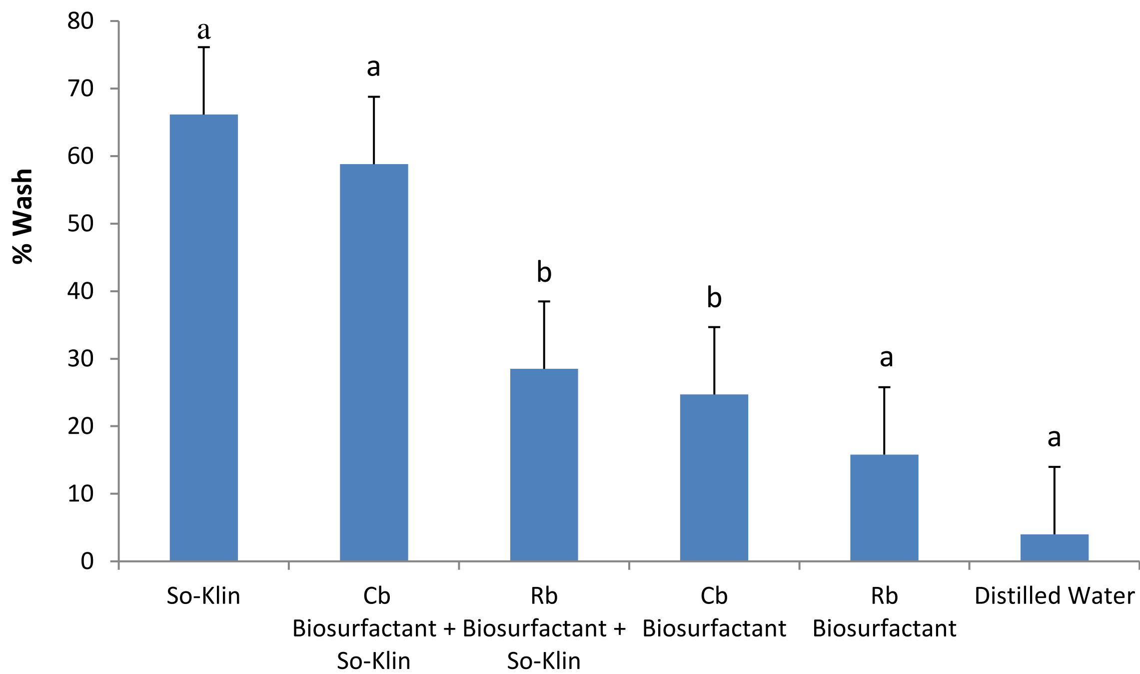

Figure 5. Detergency potential of biosurfactants produced by *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15

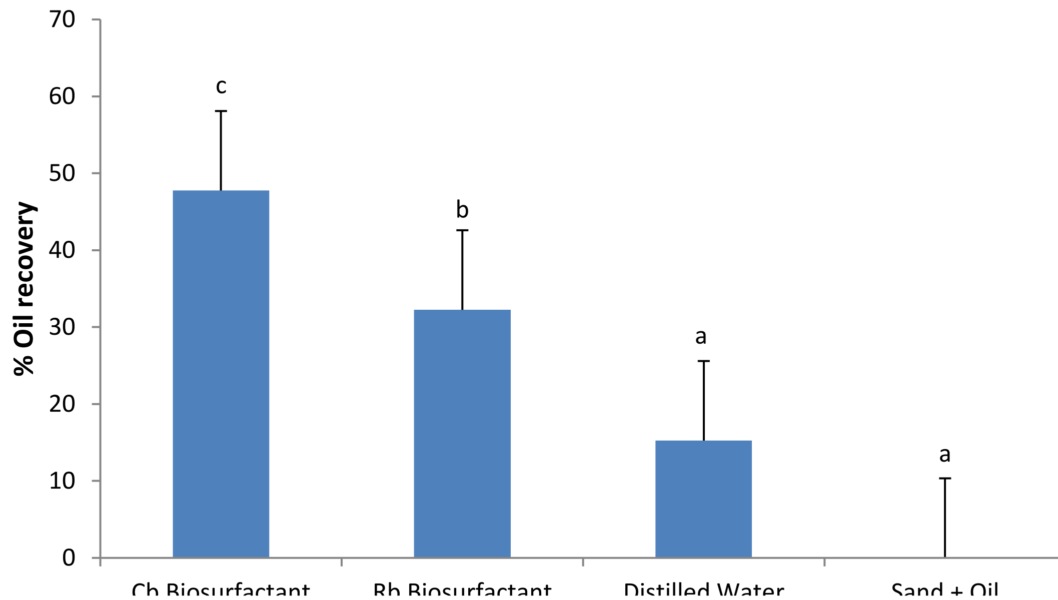

Figure 6. Enhanced oil recovery of biosurfactants produced by yeast

When used separately as a detergent, the biosurfactant produced by *Candida boleticola* H09 was able to remove 24.7% of oil which was significantly different from 15.8% of oil removed by biosurfactant produced by *Rhodotorula bogoriensis* H15. This study suggests that since the quantity of oil removed from the oil contaminated fabrics by biosurfactants alone was significantly different from the control (Distilled water); the biosurfactants could serve as a detergent. In a similar study conducted by Sobrinho *et al.* [34], biosurfactant produced by *Candida sphearica* removed 41% of oil from fabrics while on the other hand, biosurfactants produced by *Klebsiella* sp. showed excellent performance as it removed 100% of oil from fabrics [23].

The recovery of crude oil of 47.75% obtained from biosurfactants produced by *Candida boleticola* H09 is similar to the 50% recovery achieved by biosurfactants produced by *Candida sphaerica* in a study conducted by Sobrinho *et al.* [34]. The biosurfactant produced by *Rhodotorula bogoriensis* H15 was able to enhance the recovery of 32.25% of crude oil (Figure 6) as compared to the control (distilled water) which was able to recover only 5.23% of the crude oil and the statistical analysis revealed a significant difference ($p<0.05$) between the amount of biosurfactant produced by *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15 when compared with the control. When compared to the study conducted by Elshafie *et al.* [30], where 27.27% of residual oil was recovered when the potential of *Candida bombicola* ATCC 22214 Sophorolipids (SPLs) in enhancing oil recovery was tested using core-flooding experiments under reservoir conditions, the sophorolipids from *Rhodotorula bogoriensis* H15 performed better and this confirmed the potential of these SPLs for applications in microbial enhanced oil recovery[30]

In a study conducted by Seema [35] to investigate enhanced oil recovery potential of biosurfactant produced by *Pseudomonas* sp., the result showed that the biosurfactant was a rhamnolipid and had a percentage recovery of 60% which was higher than the results of this study while that of distilled water which was the control had a percentage recovery of 10%. In another study conducted by Rodriguez *et al.* [44], biosurfactant produced by *Bacillus subtilis* was able to recover 31% of crude oil in a simulated reservoir condition of 40^0C and a pressure of 398-440 psi. In comparison, the results of the present study performed better.

CONCLUSIONS

The yeasts screened for biosurfactant production were able to cause the collapse of oil drops, spreading and emulsification of oils, indicating that they could produce biosurfactants. They grew and utilize various agro-wastes as carbon and energy source, indicating they could utilize unconventional carbon substrates for growth as well as in synthesis of bioproducts such as biosurfactants. However, the yeasts, *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15 were able to produce lactonic sophorolipid Bios-H09 and acidic sophorolipid Bios-H15 respectively, which displayed moderate stability against extreme environmental conditions, indicating that they could thrive in the oil well. The biosurfactants had potential for use in enhanced oil recovery.

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