

Production of Extracellular Surfactants as Biotechnologically Versatile Products by Fungal Isolates of Palm Oil Mill Effluent

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Abstract

This study employed untreated Palm oil mill effluent (POME) as both microbial source and fermentation substrate for the production of extracellular biosurfactant. POME samples were collected from an automated mill (NIFOR) and a semi-automated local mill (Iguye community) in Edo State, Nigeria. Physicochemical analysis of the POME samples showed acidic pH (3.8–5.1), elevated biochemical oxygen demand (22 300–36 600 mg/L), and high oil and grease content (3 439–7 104 mg/L), indicative of significant pollution. Fungal enumeration revealed total counts ranging from $1.43 \pm 1.12 \times 10^6$ to $6.51 \pm 1.88 \times 10^6$ cfu/mL at NIFOR and $2.26 \pm 0.93 \times 10^6$ to $8.70 \pm 1.58 \times 10^6$ cfu/mL at Iguye. Isolation and characterization using standard microbiological techniques and 28S rRNA (D1/D2) gene sequencing yielded 126 isolates across nine genera. *Penicillium oxalicum*, *Aspergillus flavus*, *Aspergillus niger*, *Geotrichum candidum*, *Candida utilis*, and *Rhodotorula glutinis* produced extracellular biosurfactants under submerged fermentation in POME-augmented mineral salt medium. Biosurfactant activity was confirmed through drop-collapse, oil displacement, emulsification index (E24), hemolytic activity, and surface tension reduction, with purified products from *G. candidum* and *C. utilis* reducing surface tension to <15 mN/m and exhibiting high emulsification. Activities were significantly different ($P < 0.05$) from the positive control (Tween-80). The findings highlight a sustainable strategy for valorizing agro-industrial waste while producing biotechnologically versatile fungal biosurfactants suitable for various applications.

Keywords: biosurfactant, palm oil mill effluent, fungi, waste valorization, surface tension

Резюме

В това проучване са използвани не-третиранни отпадъчни води от мелници за палмово масло (POME) както като микробен източник, така и като ферментационен субстрат за производството на извънклетъчен биоповърхностно-активно вещество. Пробите от POME са събрани от автоматизирана мелница (NIFOR) и полуавтоматизирана местна мелница (общност Игуе) в щата Едо, Нигерия. Физикохимичният анализ на пробите от POME показва киселинно pH (3.8–5.1), повишена биохимична потребност от кислород (22 300–36 600 mg/L) и високо съдържание на масла и мазнини (3 439–7 104 mg/L), което е показателно за значително замърсяване. Преброяването на гъбичките разкрива общ брой, вариращ от $1.43 \pm 1.12 \times 10^6$ до $6.51 \pm 1.88 \times 10^6$ cfu/mL в NIFOR и от $2.26 \pm 0.93 \times 10^6$ до $8.70 \pm 1.58 \times 10^6$ cfu/mL в Игуе. Изолирането и характеризирането с помощта на стандартни микробиологични техники и секвениране на 28S rRNA (D1/D2) гени дадоха 126 изолата от девет рода. *Penicillium oxalicum*, *Aspergillus flavus*, *Aspergillus niger*, *Geotrichum candidum*, *Candida utilis* и *Rhodotorula glutinis* продуцираха извънклетъчни биоповърхностно активни вещества при потопена ферментация в POME-добавена минерална солева среда. Активността на биоповърхностно активните вещества беше потвърдена чрез спадане на капките, изместване на маслото, индекс на емулгиране (E24), хемолитична активност и намаляване на повърхностното напрежение, като пречистените продукти от *G. candidum* и *C. utilis* намалиха повърхностното напрежение до <15 mN/m и показваха висока емулгация. Активността беше значимо различна ($P < 0,05$) от положителната контрола

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(Tween-80). Резултатите подчертават устойчива стратегия за оползотворяване на агро-индустриалните отпадъци, като същевременно се произвеждат биотехнологично гъвкави гъбични биоповърхностно активни вещества, подходящи за различни приложения.

Introduction

Surfactants are chemical compounds, characterized by the presence of amphipathic molecules consisting of hydrophobic and hydrophilic moieties, which are partitioned preferentially at the interface between fluid phases with different polarity and hydrogen bonds (Calvo *et al.*, 2009). The hydrophobic domain is made up of long chain fatty acids while the hydrophilic domain consists of Carbohydrates, amino acids and phosphate group (Nikhil *et al.*, 2016). Surfactants are capable of lowering the surface tension, interfacial tension and forming micro-emulsion to enable mixing of two immiscible solutions. Surfactants are used in agriculture for hydrophilization of heavy soils to sustain its nutrients, as antimicrobials in medicine, as emulsifiers, solubilizers, cleansers, foaming and wetting agents in manufacturing industries and in degradation of hydrocarbon within oil reservoirs in the petroleum industry (Perfumo *et al.*, 2010).

Most surfactants are obtained by chemical processes from petrochemical and oleochemical resources. The rising global demand for environmentally sustainable alternatives to synthetic surfactants has intensified research into microbial biosurfactants due to their biodegradability, low toxicity, and stability under extreme environmental conditions (Soares *et al.*, 2024; Ashamu *et al.*, 2025). Biosurfactants are amphiphilic compounds synthesized by microorganisms that reduce surface and interfacial tension, enhance emulsification, and facilitate hydrocarbon mobilization, making them valuable in environmental remediation, petroleum recovery, agriculture, pharmaceuticals, and food industries (Reis *et al.*, 2018; Tomilayo *et al.*, 2025).

While bacterial biosurfactants have traditionally dominated research, recent studies increasingly highlight molds and yeasts as promising producers. Fungal biosurfactants, often glycolipids such as sophorolipids and cellobiose lipids, exhibit superior stability across wide pH, salinity, and temperature ranges, characteristics essential for industrial applications (Silva *et al.*, 2021; Valkenburg *et al.*, 2025). Genera including *Aspergillus*, *Penicillium*, *Candida*, *Geotrichum*, *Rhodotorula*, and *Ustilago* have been reported to produce biosurfactants with significant emulsifying activity and surface-tension reduction when cultivated on renewable or waste substrates (Ekeogu, 2024; Tomilayo *et al.*, 2025; Soares *et al.*, 2024).

A major setback in commercial biosurfactant production is the high cost of conventional sub-

strates. Agro-industrial waste has therefore attracted attention as low-cost carbon sources. Palm oil mill effluent (POME), a by-product of palm oil processing, is produced in substantial volumes in Nigeria and is rich in carbohydrates, lipids, proteins, and minerals. Improper disposal of POME leads to environmental pollution due to its high biological and chemical oxygen demand (BOD, COD) and acidic nature; however, its nutrient-rich composition makes it an ideal substrate for microbial growth and biosurfactant production (Foo & Hameed, 2010; Ekeogu, 2024; Ashamu *et al.*, 2025).

Fungal isolates obtained from POME-contaminated environments has been shown to exhibit biosurfactant activity, as evidenced by drop-collapse, oil-spreading, and emulsification assays, and are capable of reducing surface tension and enhancing hydrocarbon solubilization (Ekeogu, 2024).

Studies evaluating fungal biosurfactant production using fungal isolates obtained from POME and directly using POME as a fermentation substrate particularly within Nigeria remain limited. This study therefore investigated biosurfactant production using untreated POME as both source of fungi and fermentation substrate and as well characterized the surface-active properties of the produced biosurfactant.

Materials and Methods

Sample collection

Samples were collected in 200 mL sterilized screw-capped glass bottles, in replicates, from two sites designated as Site A (NIFOR automated mill) and Site B (a semi-automated mill at Iguiye community). At each site, samples were collected from three spots: X, the discharge point where effluent runs off the press; Y, a point located 3–4 m away from spot X along the effluent runoff route; and Z, a pond of discharged effluent where effluents from spots X and Y converge.

Physicochemical analysis of POME samples

The physicochemical parameters analysed for the POME samples included pH, temperature, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total carbohydrate, total nitrogen, total phosphorus, total oil and grease, total suspended solids (TSS), and total ash, following standard methods (A.O.A.C., 2012).

Enumeration, isolation, characterization, and molecular identification of fungal isolates

The fungal load of the samples was determined using serial dilution and the spread plate

method. The collected POME samples were serially diluted in sterile normal saline, and aliquots (100 μ L) of the 10^{-3} dilution were aseptically plated onto Sabouraud Dextrose Agar (SDA) supplemented with ciprofloxacin (0.1 mg/mL). The plates were incubated at 27°C for 144 h.

After incubation, fungal colonies were enumerated to obtain the total fungal count, then characterized and identified based on cultural and microscopic characteristics. Molecular identification was carried out by genomic DNA extraction followed by polymerase chain reaction (PCR) amplification of the 28S rRNA gene. The nucleotide divergence at the 5' end of the large subunit (28S) ribosomal DNA (D1/D2 region) was amplified, and DNA homology was analysed using the Basic Local Alignment Search Tool (BLAST) against closely related taxa in the GenBank sequence database of the National Center for Biotechnology Information (NCBI). Phylogenetic analysis was conducted, and isolates were appropriately identified (Cheesbrough, 2000; Rodríguez *et al.*, 2016).

Screening for surfactant-producing isolates

Standard mineral salt medium composition (for screening) (g/L): $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.5; KH_2PO_4 : 1.0; Na_2HPO_4 : 1.0; NaNO_3 : 3.0; mycological peptone 0.6; Crude POME 10% v/v; Distilled water up to 1 L; pH 4.8 ± 0.3

Isolated colonies or spores preserved on PDA slants were sub-cultured into Sabouraud dextrose broth, vigorously shaken, and diluted in sterile normal saline. The resulting suspension was adjusted to a 1:100 dilution of the 0.5 McFarland turbidity standard, containing approximately 1×10^6 spores/mL. Thereafter, 1 mL of the standardized suspension was inoculated into 100 mL of mineral salt medium supplemented with 10% crude POME as the carbon source in a 250 mL Erlenmeyer flask. The cultures were incubated at 27°C for 144 h with continuous shaking at 150 rpm using a shaker incubator.

After incubation, the culture broth was allowed to stand for 24 h. Broths showing microbial growth and biosurfactant activity, indicated by oil emulsification and precipitate formation, were noted. Selected isolates were reinoculated onto slants and further screened using a haemolytic assay on basal blood agar to confirm biosurfactant production (Satpute *et al.*, 2010; Lanka and Pydipalli, 2018; Reis *et al.*, 2018).

Production and recovery of biosurfactants

Selected fungal isolates were inoculated into

sterilized POME (10%)-augmented mineral salt medium in 500 mL Erlenmeyer flasks and incubated in an orbital incubator at 27°C and 150 rpm for 144 h. Control flasks containing the same culture medium without fungal inoculation were also prepared. After incubation, the fermentation broth was filtered to remove visible biomass and centrifuged at 10 000 rpm for 20 min. The cell- and spore-free supernatant was gently decanted into sterile screw-capped bottles, and crude biosurfactant activity was evaluated using emulsification index (E_{24}), oil displacement, drop collapse, and surface tension assays. The supernatant was stored at 4°C for further analyses (Satpute *et al.*, 2010).

Purification of biosurfactants

Biosurfactants produced during fermentation were recovered and purified from the culture supernatant using centrifugation, acid precipitation, and solvent-solvent extraction, following a modified method of Reis *et al.* (2018). The fermented broth was first centrifuged at $9\,000 \times g$ for 15 min to remove microbial cells, and the resulting cell-free supernatant was transferred into sterile screw-capped bottles.

The supernatant was acidified to pH 2.0 using 6 N hydrochloric acid (HCl) under gentle stirring. Precipitate formation was visually monitored, after which the acidified supernatant was incubated at 4°C for 12–24 h to enhance biosurfactant precipitation.

Following incubation, the mixture was centrifuged at $9\,000 \times g$ for 15 min. The supernatant was carefully decanted, leaving behind a viscous precipitate containing the crude biosurfactant. The precipitate was resuspended in distilled water and subjected to solvent extraction using a chloroform-methanol mixture (2:1, v/v) in a separating funnel at a 1:1 ratio (aqueous phase: organic solvent). The mixture was gently shaken and allowed to stand until complete phase separation occurred. The organic phase containing the biosurfactant was collected, while the aqueous phase was discarded. The organic extract was concentrated under reduced pressure using a rotary evaporator at 40–45°C to prevent thermal degradation. Further drying to constant weight was carried out using a water bath maintained at 40°C.

The purified biosurfactant was stored at 4°C until further analysis. Biosurfactant activity was subsequently evaluated by determining surface tension reduction, emulsification index (E_{24}), and critical micelle concentration (CMC) (Jamal *et al.*, 2011; Reis *et al.*, 2018).

Data analysis

Data obtained was subjected to descriptive statistical analysis (mean and standard error of mean), and charts were generated using Microsoft Excel (2010). Inferential statistical analyses, including analysis of variance (ANOVA), Tukey's multiple comparison test, and *t*-test, were performed using GraphPad Prism (UK) software version 6 and SPSS version 20 (Chicago, USA) (Bert-hold & Hand, 2003; Ogbeibu, 2005).

A schematic representation of the experiments conducted is provided in Fig. 1.

Results

Physicochemical analysis of POME samples

The physicochemical properties of the POME samples from both sampled sites are presented in Table 1. The parameters measured included pH, temperature, biochemical oxygen demand, chemical oxygen demand, total carbohydrate, total nitrogen, total phosphorus, oil and grease, total suspended solids, and ash content. Variations were observed among samples collected from different sites and sampling points.

Enumeration, isolation, characterization and molecular identification of fungal isolates

The results of the total fungal counts (Table 2.0) revealed a range of 0.67 ± 0.33 to 7.34 ± 2.16

$\times 10^6$ cfu/mL at site A and a range of 1.59 ± 1.25 to $10.25 \pm 2.56 \times 10^6$ cfu/mL at site B. The mean fungal count from site B (2.26 ± 0.93 to $8.70 \pm 1.58 \times 10^6$ cfu/mL) represents higher values relative to A (1.43 ± 1.12 to $6.51 \pm 1.88 \times 10^6$ cfu/mL) but the difference was not significant. However, the difference between sampled spot x and z for both sites was significant ($p < 0.05$).

A total of 126 fungal isolates (Table 3.0) comprising nine (9) genera (*Penicillium*, *Aspergillus*, *Geotrichum*, *Fusarium*, *Candida*, *Rhododurula*, *Rhizopus*, *Microsporium* and *Mucor*) were isolated from both sites A and B.

Molecular characterization and identification (Plate 1, Figures 2, 3, and 4.) confirmed the fungal isolates to include *A. niger* with the highest percentage occurrence of 19.05% followed by *C. utilis* (14.28), *A. flavus* (11.90%), *G. candidum* (10.32%), *R. glutinis* (8.73%), *R. oryzae* (7.14%), *A. fumigatus* (6.35%), *P. oxalicum* (6.35%), *F. globosum* (5.56%), *M. mucedo* (5.56%) and the least occurring isolate *M. audouinii* (4.76%).

Screening for surfactant-producing isolates

The fungal isolates were screened for biosurfactant production and the results (Table 4.0) revealed biosurfactant producers to include *P. oxalicum*, *A. flavus*, *G. candidum*, *C. utilis*, *R. glutinis*, *R. oryzae*, *A. niger* and *M. mucedo* which were pos-

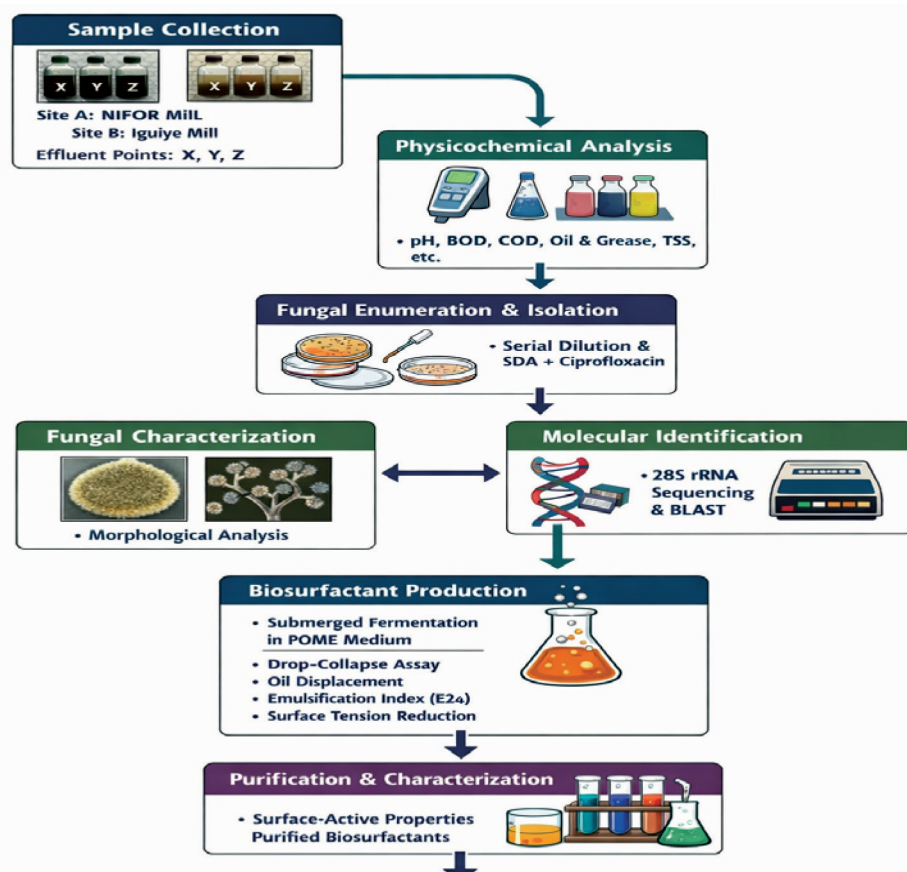


Fig. 1. Graphical diagram of experiment

Table 1. Physicochemical characteristics of Palm Oil Mill Effluent obtained from site A (NIFOR) and site B (Iguiye)

Parameters	Units	Site A			Site B		
		x	Y	Z	p	q	R
Ph	-	5.1±0.64 ^a	4.4±1.15 ^a	3.9±1.10 ^b	5.0±0.11 ^a	4.2±1.37 ^a	3.8±0.97 ^b
Temperature	°C	42±1.37 ^a	36±2.43 ^b	33±1.51 ^b	40±2.11 ^a	33±1.95 ^b	31±1.51 ^b
Biochemical							
Oxygen Demand (BOD)	(mg/L)	22300±1314 ^b	24230±1193 ^b	32931±2041 ^a	25491±1295 ^b	31317±1452 ^b	36600±1318 ^a
Chemical							
Oxygen Demand (COD)	(mg/L)	26800±1649 ^b	38315±2561 ^a	42800±1911 ^a	27528±1516 ^b	40578±2131 ^a	43815±2413 ^a
Total Carbo-hydrate (TC)	(mg/L)	16103±1431 ^a	11400±2413 ^b	10810±5414 ^b	15931±1161 ^a	9910±1781 ^b	6900±1171 ^b
Total Nitrogen	(mg/L)	480±29.58 ^b	510±38.52 ^b	760±42.45 ^a	497±18.47 ^b	790±10.71 ^a	811±14.83 ^a
Total Phosphorus	(mg/L)	43±5.41 ^b	71±3.82 ^a	83±7.91 ^a	46±4.71 ^b	81±5.15 ^a	96±4.81 ^a
Oil and grease	(mg/L)	6520±231 ^a	4904±149 ^b	3875±112 ^b	7104±183 ^a	5131±231 ^b	3439±143 ^b
Total Suspended Solids (TSS)	(mg/L)	21340±1214 ^b	42133±2712 ^a	42821±1971 ^a	28138±2115 ^b	47215±1343 ^a	51200±2103 ^a
Ash	(mg/L)	105±11.61 ^b	211±31.83 ^b	981±112.1 ^a	189±43.6 ^b	756±21.3 ^a	1456±165.5 ^a

Key: Results presented as Mean ± S.D. (standard deviation of means). Means on the same row bearing different alphabetical letters 'a - b' are significantly different at $P < 0.05$.

Table 2. Total Fungal Count (mean ± S. D x 10⁶sfu/mL) of the POME samples obtained from site A (NIFOR) and site B (Iguiye)

Sampled day	Site A			Site B		
	X	y	Z	P	Q	R
1	2.17±1.48	3.65±1.71	5.92±1.25	2.98±1.51	5.11±2.53	9.15±2.15
2	1.45±1.10	6.14±2.49	6.26±1.81	2.47±1.63	7.82±1.74	10.25±2.56
3	0.67±0.33	4.81±1.21	7.34±2.16	1.59±1.25	3.46±1.81	6.71±3.11
Mean of Means	1.43±1.12 ^a	4.87±2.16 ^b	6.51±1.88 ^b	2.26±0.93 ^a	5.46±2.13 ^b	8.70±1.58 ^c

Key: S.D (standard deviation of means). Means on the same row bearing different letters 'a-c' are significantly different at $P < 0.05$. Post Hoc: $P(T_c \leq t)$ -Value = 0.43026 (No significant difference in fungal count between the two sites, $P > 0.05$) using paired t-test to compare mean of means at 0.05 significance level. However, site A (P of $T \leq t$ value 0.016048) and site B (P of $T \leq t$ value 0.032096) are significantly different ($P < 0.05$) from Hypothesized mean count ($\sim 1.5 \times 10^3$) of treated POME.

itive for emulsification test indicated by precipitate formation and were also positive for hemolysis of blood agar indicated by the production of clearance zone in inoculated blood agar.

Accordingly, *G. candidum* recorded the highest clearance zone diameter (CZD) of 10.6 ± 2.3 mm, followed by *C. utilis* (9.6 ± 1.5 mm), *P. oxalicum* (8.5 ± 1.5 mm), *A. niger* (6.5 ± 0.5), *R. glutinis* (6.1 ± 1.3 mm), *A. flavus* (4.0 ± 1.0 mm), *A. fumigatus* (3.0 ± 1.0 mm), *F. globosum* (1.8 ± 0.4 mm), *M. mucedo* (0.9 ± 0.3 mm) and the least was a

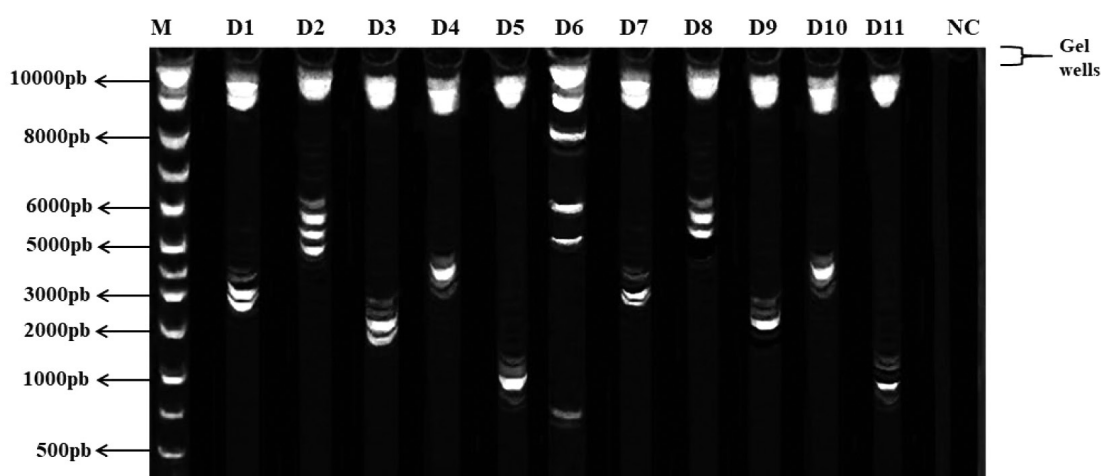
negligible clearance zone recorded by *M. audiounii* (0.2 ± 0.1 mm).

Production and recovery of biosurfactants

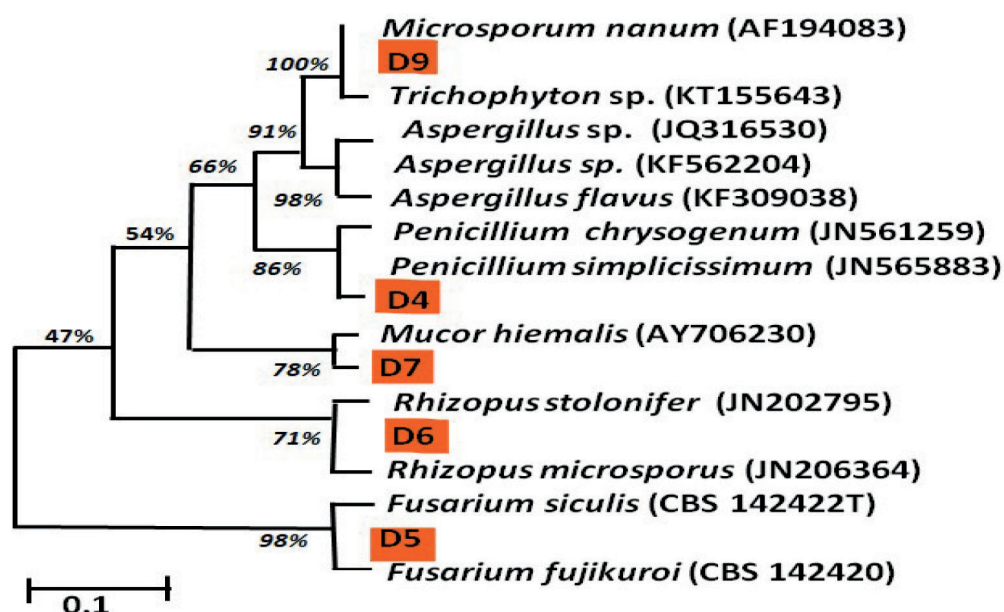
The results of biosurfactants activity assay which includes, Drop collapse test (DCT), Emulsification index (EI), oil displacement and surface tension (ST) (Table 5.0) revealed that the oil drop collapsed faster with reference to time for *G. candidum*, followed by *C. utilis*, *P. oxalicum*, *Aspergillus niger*, *R. glutinis* and *A. flavus*.

Table 3. Percentage occurrence of POME fungal isolates obtained from site A and B

Organisms	Site A (NIFOR)	Site B (Iguiye)	No. of isolates	% frequency of occurrence
<i>Penicillium oxalicum</i>	3	5	8	6.35
<i>Aspergillus flavus</i>	9	6	15	11.90
<i>Geotrichum candidum</i>	6	7	13	10.32
<i>Fusarium globosum</i>	-	7	7	5.56
<i>Candida utilis</i>	9	9	18	14.28
<i>Rhodotorulla glutinis</i>	6	5	11	8.73
<i>Rhizopus oryzae</i>	3	6	9	7.14
<i>Aspergillus niger</i>	11	13	24	19.05
<i>Microsporium audouinii</i>	2	4	6	4.76
<i>Aspergillus fumigatus</i>	4	4	8	6.35
<i>Mucor mucedo</i>	3	4	7	5.56

**Plate 1.** Agarose gel electrophoresis of PCR amplification product of 28S rRNA gene band of the isolates

Key: M = 1000 bp DNA size marker, NC = Negative control, D1 = *A. flavus* (JN938926), D2 = *A. fumigatus* (JN179606), D3 = *A. niger* (JN938934), D4 = *P. oxalicum* (KF233758), D5 = *F. globosum* (AF160285), D6 = *R. oryzae* (CBS108934), D7 = *M. mucedo* (CBS108943), D8 = *G. candidum* (JN9392021), D9 = *M. audouinii* (AF194085), D10 = *C. utilis* (AF218921), D11 = *R. glutinis* (HM190321).

**Fig. 2.** Phylogenetic tree based on the gene sequence of D4, D5, D6, D7 and D9 fungal isolates

Keys: D9 = *M. audouinii* (AF194085), D4 = *P. oxalicum* (KF233758), D7 = *M. mucedo* (CBS108943), D6 = *R. oryzae* (CBS108934), D5 = *F. globosum* (AF160285).

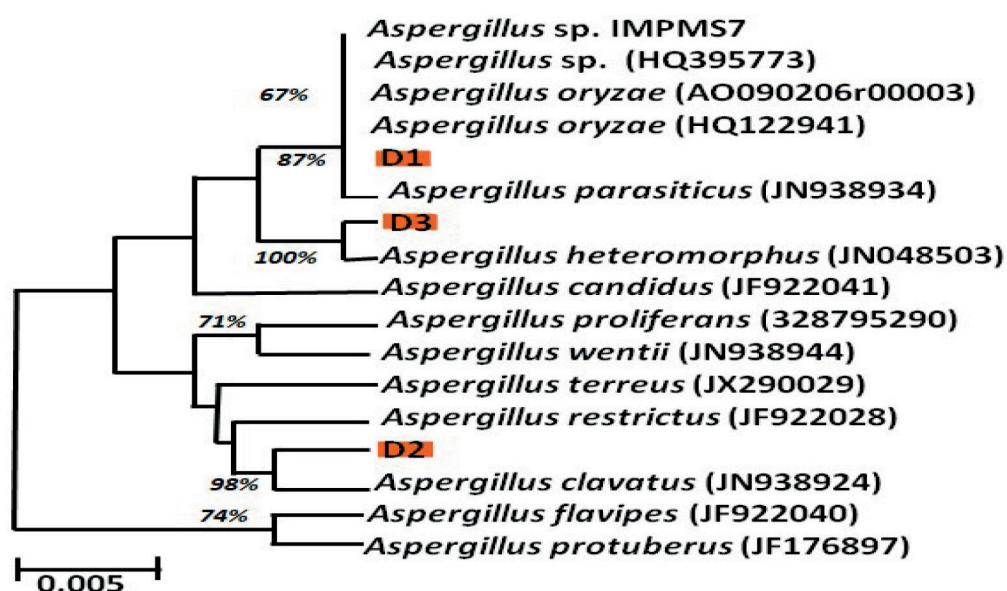


Fig. 3. Phylogenetic tree based on the gene sequence of D1, D2 and D3 fungal isolates.
Keys: D1 = *A. flavus* (JN938926); D3 = *A. niger*(JN938934), D2 = *A. fumigatus* (JN179606).

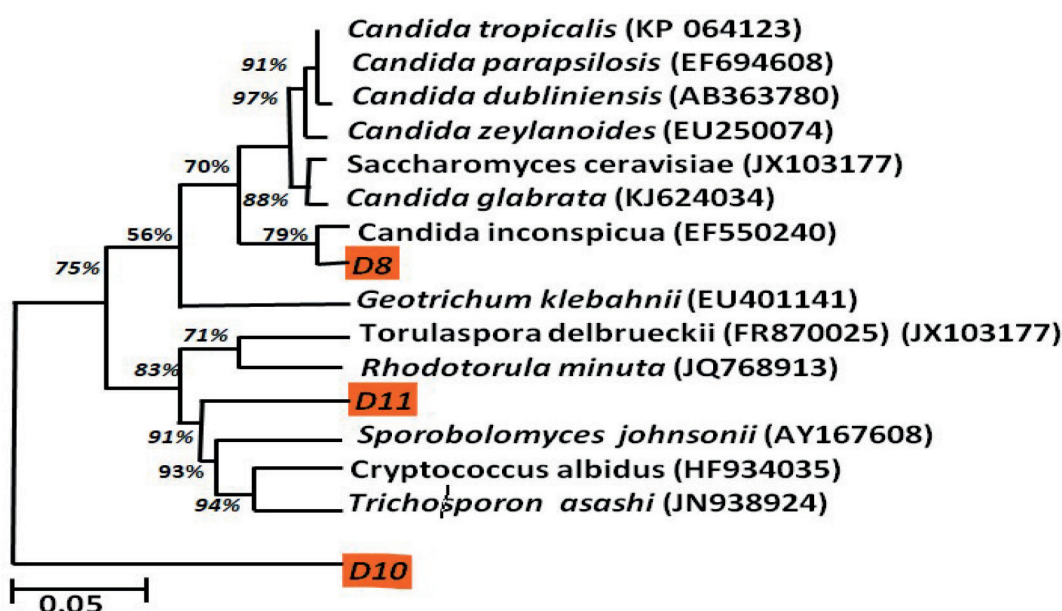


Fig. 4. Phylogenetic tree based on the gene sequence of D8, D10 and D11 fungal isolates
Keys: D8 = *G. candidum* (JN9392021); D11 = *R. glutinis* (HM190321), D10 = *C. utilis*(AF218921).

G. candidum recorded the highest displacement zone diameter (DZD) of 36.0 ± 0.1 mm, followed by *C. utilis* (32.1 ± 0.3 mm), *P. oxalicum* (27.3 ± 0.7 mm), *R. glutinis* (26.5 ± 0.5 mm), *A. niger* (24.6 ± 0.4 mm) and the least DZD was recorded by *A. flavus* (20.5 ± 1.5 mm). Similar pattern was observed for the EI and ST with each indicating higher biosurfactant activity among the yeasts relative to most of the molds except for *P. oxalicum* which showed higher surfactant activity among the molds.

Purification of biosurfactants produced

The results in Figure 5. and Figure 6. show the effect of purification on the produced biosurfactants activity which shows the product by *G. candidum* to be higher as revealed by the emulsi-

fication Index ($41.5 \pm 1.5\%$) and also remarkable in lowering the surface tension (ST) between water and hexadecane with a record of 11.0 ± 1.5 mN/m. This was followed by *A. niger* with EI ($38.5 \pm 0.5\%$) slightly higher than $38.2 \pm 0.1\%$ obtained for *C. utilis* that had the next lower ST 11.8 ± 1.2 mN/m respectively. *A. flavus* product had EI of $38.0 \pm 1.8\%$ with ST of 18.3 ± 1.6 mN/m. *R. glutinis* recorded EI of $37.6 \pm 0.7\%$ with ST of 14.3 ± 0.3 mN/m whereas *P. oxalicum* recorded EI of $26.7 \pm 0.3\%$ with ST of 16.4 ± 1.5 mN/m. The ST followed a similar but slightly different pattern.

Discussion

This study investigated the potential of palm oil mill effluent (POME) obtained from the Nigeri-

Table 4. Screening tests for biosurfactant producers among POME fungal isolates

Fungi	Emulsification layer/ppt	Haemolysis of blood agar (mm)
<i>P. oxalicum</i>	+	8.5 ± 1.5
<i>A. flavus</i>	+	4.0 ± 1.0
<i>G. candidum</i>	+	10.6 ± 2.3
<i>F. globosum</i>	-	1.8 ± 0.4
<i>C. utilis</i>	+	9.6 ± 1.5
<i>R. glutinis</i>	+	6.1 ± 1.3
<i>R. oryzae</i>	+	0.2 ± 0.3
<i>A. niger</i>	+	6.5 ± 2.5
<i>M. audouinii</i>	-	0.2 ± 0.1
<i>A. fumigates</i>	-	3.0 ± 1.0
<i>M. mucedo</i>	+	0.9 ± 0.3

Key: +/- = positive/negative for emulsification layer or precipitate formation. Results of Blood agar β -hemolyses were represented in mean $\pm$ SD (Standard deviation of mean)

an Institute for Oil Palm Research (NIFOR) and a local mill at Iguiye community in Edo State, Nigeria, as substrates for fungal biosurfactant production using fungal isolates recovered from the POME.

The physicochemical characteristics of the POME samples (Table 1.) indicated slight variations between the two sampling sites—Site A (NIFOR) and Site B (Iguiye); however, these differences were not statistically significant. The observed values were outside the permissible limits prescribed by the World Health Organization (WHO) for treated effluents considered safe for environmental discharge, as reported by Akhabari *et al.* (2020). The POME samples were characterized by high biological oxygen demand (BOD) and chemical oxygen

demand (COD), low pH, and a colloidal nature with high suspended solids. These properties indicate a high pollution load at the receiving ponds. This finding agrees with the report of Ohimain *et al.* (2015), who documented similar physicochemical characteristics for POME from automated and semi-automated processing mills in Rivers State, Nigeria, and concluded that such effluents are unsuitable for discharge without treatment.

The physicochemical results of the study further confirm the suitability of POME as a low-cost substrate for biosurfactant production, in agreement with earlier findings highlighting its nutrient-rich composition and potential as a carbon and energy source for biosurfactant-producing microorganisms (Ashamu *et al.*, 2025; Ekeogu, 2024).

The fungal load at Site A (automated processing) was lower than that recorded at Site B (semi-automated processing) (Table 2); however, the difference was not statistically significant. The high fungal population observed at both sites further confirms that POME provides a favourable ecological niche for microorganisms capable of surviving under acidic, oily, and oxygen-limited conditions. Similar observations were reported by Ohimain *et al.* (2015), who documented higher microbial loads in semi-automated mills compared to automated mills in Rivers State, Nigeria. The results are also comparable to the relatively higher microbial counts reported by Okechalu *et al.* (2014) and Izah and Ohimain (2016). These findings affirm the presence of abundant microbial populations in POME due to its high content of degradable organic matter (Yossan *et al.*, 2012; Kamyab *et al.*, 2018).

A total of 126 fungal isolates were character-

Table 5. Oil drop-collapse, emulsification index, oil displacement and surface tension tests of biosurfactant products of screened and selected POME fungal isolates

Fungi	Drop-collapse (seconds)					EI (24) (%)	Oil disp. (mm)	ST (mN/m)
	0	2	10	30	60			
<i>P. oxalicum</i>	---	---	--+	+++	+++	17.5 $\pm$ 2.8bc	27.3 $\pm$ 1.7ab	24.3 $\pm$ 1.4b
<i>A. flavus</i>	---	---	---	---	-++	7.3 $\pm$ 1.4c	20.5 $\pm$ 1.5b	35.7 $\pm$ 2.6b
<i>G. candidum</i>	---	-++	+++	+++	+++	36.3 $\pm$ 1.7ab	36.0 $\pm$ 3.6 a	18.5 $\pm$ 1.5ab
<i>C. utilis</i>	---	---	-++	+++	+++	25.5 $\pm$ 2.5b	32.1 $\pm$ 2.3ab	20.1 $\pm$ 1.8ab
<i>R. glutinis</i>	---	---	---	---	+++	15.0 $\pm$ 1.1bc	26.5 $\pm$ 1.5 b	31.9 $\pm$ 1.3b
<i>A. niger</i>	---	---	---	-++	+++	12.3 $\pm$ 1.6c	24.6 $\pm$ 3.4 b	32.1 $\pm$ 2.4b
Blank Medium	---	---	---	---	---	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	72.3 $\pm$ 2.8c
Tween-80 (20%)	--+	+++	+++	+++	+++	52.5 $\pm$ 1.5 a	41.3 $\pm$ 1.1 a	4.4 $\pm$ 1.3 a

Key: +++ (Highly positive i.e in triplicates), -++ (Moderately positive i.e in two of triplicates), -+ (Weakly positive i.e in one of replicates), --- (Completely negative i.e in triplicates). The results are in Mean $\pm$ SD (standard deviation of mean).

Significance at $p < 0.05$ with respect to positive control (Tween-80). Means on the same row bearing different letters 'a - c' are significantly different at $P < 0.05$.

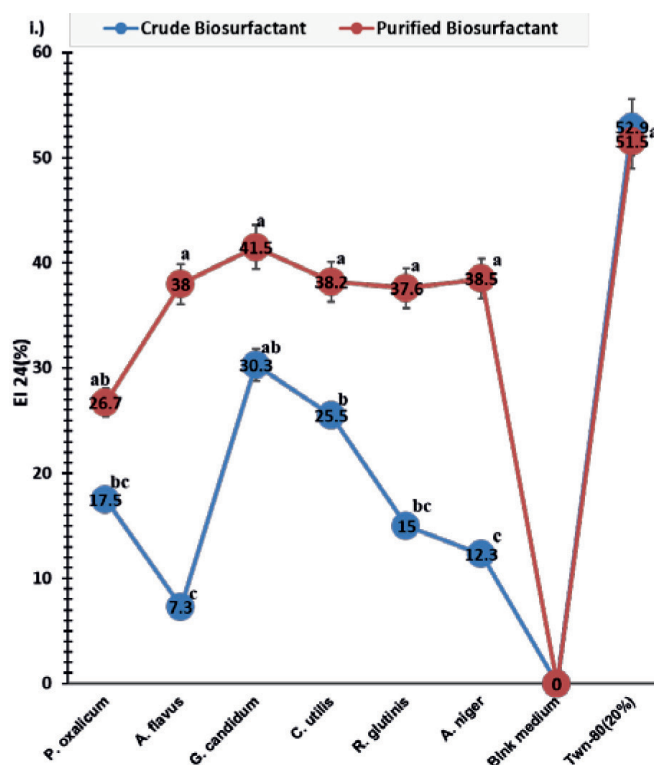


Fig. 5. Effect of purification on biosurfactant activity using emulsification index (EI24) assay
Key: Blnk (Blank) medium without test organism and corresponding product. Significance at $P < 0.05$ with respect to positive control (Tween-80). Means bearing different letters 'a - c' are significantly different at $P < 0.05$.

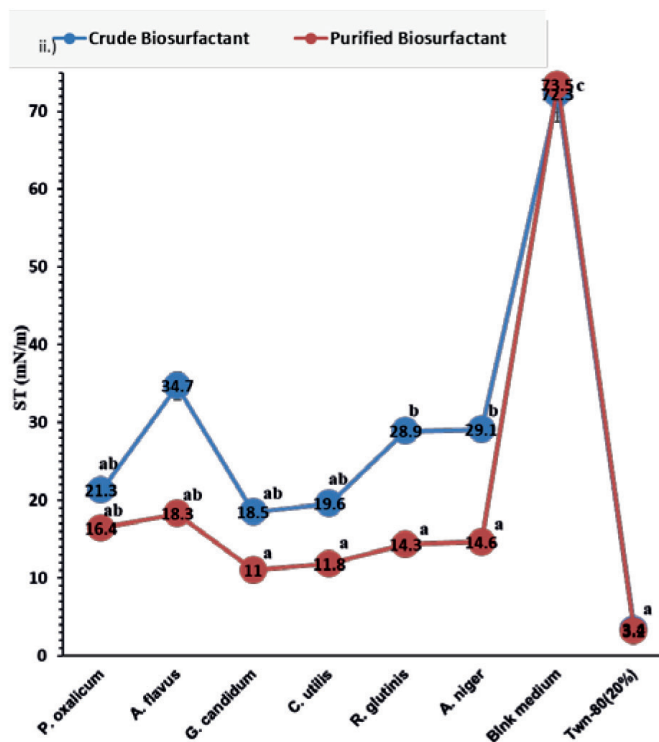


Figure 6. Effect of purification on the surface tension (ST) lowering properties between water and hexadecane at 25°C
Key: Blnk (Blank) medium without test organism nor corresponding product. Significance at $p < 0.05$ with respect to positive control (Tween-80). Means bearing different letters 'a - c' are significantly different at $P < 0.05$.

ized, including *A. niger*, *C. utilis*, *A. flavus*, *G. candidum*, *R. glutinis*, *R. oryzae*, *A. fumigatus*, *P. oxalicum*, *F. globosum*, *M. mucedo*, and *M. audouinii*. These results corroborate the findings of Okechalu *et al.* (2014) and Ohimain *et al.* (2015), who independently reported *Aspergillus* spp. and *Candida* spp. as dominant fungal isolates in POME. These organisms contribute to the deterioration, acidity, and toxicity of untreated POME. There are also reports indicating that these molds and yeasts produce metabolites that enhance their survival in POME (Hii *et al.*, 2012; Ohimain *et al.*, 2015). Such metabolites include lipases, biosurfactants, antibiotics, and other bioactive compounds that provide competitive advantages and facilitate nutrient uptake in oily and oxygen-reduced environments (Sheil *et al.*, 2009; Nwuche & Ogbonna, 2011).

For an organism to be selected as biosurfactant producer, it must be positive for both emulsification test and hemolysis of blood agar test and must also produce a clearance zone of ≥ 4.0 mm. Similar benchmark for biosurfactant producer strains selection was established by Yossan *et al.* (2012) and Reis *et al.* (2018) who independently established a benchmark of ≥ 3.0 mm clear zone on blood agar together with positive results for emulsification layer assay. Therefore, from this study (Table 4.0), biosurfactant producers in detectable or appreciable quantities based on the existing benchmark include: *P. oxalicum*, *A. flavus*, *G. candidum*, *C. utilis*, *R. glutinis* and *A. niger*. This result corroborates the report of Saptute *et al.* (2010) and Bhardwaj *et al.* (2013) who independently isolated *C. bombicola*, *A. ustus*, *Ustilagomaydis*, *G. candidum*, *R. glutinis*, *Fusarium* spp. and *Penicillium* sp. as surfactants producers. The production of biosurfactants by fungal isolates is not as popular as bacterial exploration probably owing to the longer generation time of fungal cells relative to their bacterial counterpart. However, quality is sometimes as important as quantity when it comes to biotechnological exploration due to the fact that once a product of quality has been established; biotechnological improvement or versatility tends to easily provide faster mechanism and vehicle for increasing the quantity of products. In agreement with this line of thought, *C. bombicola*, *F. fujikuroi*, *Pleurotus ostreatus* and *Aspergillus* spp. were among isolates reported to produce potent, active or high quality biosurfactant (Soberón-Chávez and Maier 2011; Soleimaninanadegani and Manshad, 2014; Velioglu and Ürek, 2015).

The results of biosurfactants activity assay

which includes, Drop collapse test (DCT), Emulsification index (EI), Oil displacement and surface tension (ST) (Table 5.0) revealed that the oil drop collapsed faster with reference to time for *G. candidum*, followed by *C. utilis*, *P. oxalicum*, *Aspergillus niger*, *R. glutinis* and *A. flavus*. The drop-collapse test was considered positive when within 60 seconds; the drop flattens and collapsed on the surface. A false positive oil-drop collapse occurring well over one minute is suggestive that the product may have been produced only in small quantity or that the bulk of the biosurfactant products remained intracellular. Similar pattern was reported for oil displacement zone diameter (DZD) as *G. candidum* recorded the highest DZD of 36.0 ± 0.1 mm, followed by *C. utilis* (32.1 ± 0.3 mm), *P. oxalicum* (27.3 ± 0.7 mm), *R. glutinis* (26.5 ± 0.5 mm), *A. niger* (24.6 ± 0.4 mm) and the least DZD was recorded by *A. flavus* (20.5 ± 1.5 mm). These results are comparable to the positive control (20% Tween 80) which showed the highest DZD even at a very high dilution which is a characteristic of highly refined and industrial grade surfactant as opposed to the negative control (Blank) that showed no activity. Similar pattern was observed for the DCT, EI and ST with each indicating higher biosurfactant activity among the yeasts relative to most of the molds except for *P. oxalicum* which showed higher surfactant activity among the molds. These results corroborate the report of Desai and Fisher, (2007) who reported higher biosurfactant production quantity per time among yeasts relative to molds, a situation that tends to favour activity when screening isolates for biosurfactant production, i.e the higher the biosurfactant produced, the higher the activity. The reasons for the relatively higher activity among the yeasts than molds may be attributed to their generation time, cell structure in terms of nutrient absorption ability as well as surfactant types produced (Rahman and Gakpe, 2008). Most Yeasts generally can grow as a single cell, multiplying with shorter generation time relative to molds which are fungi that grow in multicellular filaments known as hyphae that are tubular with multiple nuclei forming a single organism; a sort of complex arrangement that lengthens molds generation time. The shorter the generation time, the faster the rate of production of secondary metabolites such as biosurfactants and vice versa (Yossan *et al.*, 2012; Kostakioti *et al.*, 2013).

The effect of purification on the produced biosurfactants activity suggests that there is a directly proportional relationship between the quantity of biosurfactant produced and biosurfactant

activity. The results also showed that purification increases the activity of biosurfactants and it could be deduced also that the activity of biosurfactants produced is concentration dependent.

A good surfactant can lower surface tension of water from 75 to 35mN/m and the interfacial tension water/hexadecane from 40 to less than 20mN/M (Krishnaswamy *et al.*, 2008; Fakruddi 2012), therefore this study recorded lower ST values than 20mN/M showing that the activities of the biosurfactant products are good enough. Tomilayo *et al.* (2025), reported effective biosurfactant production by fungi grown on solid agricultural wastes. The present study extends this knowledge by demonstrating that liquid industrial waste (POME) can function both as a growth medium for microorganisms and as a substrate capable of supporting biosurfactant production.

In a related study, Safi *et al.* (2014), reported production of a glycolipid with emulsifier properties during cultivation of *P. citrinum* on mineral medium with 1% olive oil as carbon source. The growth associated emulsifier production reached maximal activity at 120 h at $30.5 \pm 2^\circ\text{C}$ and pH 4-6 of cultivation. Also, *Geotrichum* spp., *Aspergillus* spp., *Fusarium* spp., yeasts and all other fungal species associated with POME play significant role in lipid degradation, not only attributed to lipase enzyme, but also the secretion of biosurfactants by the associated species (Coulibaly *et al.*, 2004; Cai *et al.*, 2007; Foo and Hameed, 2010; Ponraj and Din, 2013). The lowering of surface tension are indications of high surfactant activity as opposed to the blank medium (negative control) with surface tension (ST) as high as 73.5 ± 1.5 mN/m. Biosurfactant minimizes the surface tension and interfacial tension between water and lipid phase, facilitating the breakdown of lipids. According to Luna *et al.* (2012), surfactants are soluble to some degree in aqueous solution and only aggregate into micelles when they reach a sufficient concentration referred to as the critical micelle concentration (CMC). When comparing surfactants activity, the lower the ST and CMC, the more active the biosurfactant produced and conversely, the higher the ST and CMC, the lesser the surfactant in activity evaluation (Luna *et al.*, 2012; Liu *et al.*, 2013; Chakraborty *et al.*, 2015). Biosurfactants with low CMC values require less quantity in order to form micelles and solubilize lipids or associated proteins easily (Soleimaninanadegani and Manshad, 2014). *G. candidum* is known to hydrolyze phenols and secrete a peroxidase enzyme that breaks down a

wide range of colour dyes, an ability that may also be attributed to POME degradation (Coulibaly *et al.*, 2004). The same POME colour removal ability is observed on *Aspergillus* sp. though it takes place through a different mechanism found to be related to bio-adsorption and biosurfactant metabolites production by the organism (Neoh *et al.*, 2013; Soleimaninanadegani and Manshad, 2014)

Conclusion

This study demonstrated that POME can serve both as a reservoir of biosurfactant-producing fungi and as an effective, low-cost substrate for extracellular biosurfactant production. The physicochemical characteristics of the POME samples are indicative of pollutants and there was no significant difference between POME samples obtained from site A (NIFOR) and B (Iguiye). The mean fungal count from site B (2.26 ± 0.93 to $8.70 \pm 1.58 \times 10^6$ cfu/mL) represents higher values relative to A (1.43 ± 1.12 to $6.51 \pm 1.88 \times 10^6$ cfu/mL) but the difference was not statistically significant. However, the difference between sampled spot X and Z for both sites was significant ($p < 0.05$). A total of 126 fungal isolates were characterized including *A. niger*, *C. utilis*, *A. flavus*, *G. candidum*, *R. glutinis*, *R. oryzae*, *A. fumigatus*, *P. oxalicum*, *F. globosum*, *M. mucedo* and *M. audouinii*. The screening tests showed biosurfactant producers in detectable quantities to include *P. oxalicum*, *A. flavus*, *G. candidum*, *C. utilis*, *R. glutinis* and *A. niger*. Purification improved surfactant activity, However, the positive control (20%Tween-80) maintained overall higher activity.

The findings highlight a sustainable approach for environmental management and industrial biosurfactant production.

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