

Comparative Evaluation of Potential Use of Agro Wastes for Biosurfactant Production by *Bacillus thuringiensis* and *Yarrowia lipolytica*

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ABSTRACT

Biosurfactants have several industrial, environmental, agricultural, and medical applications because they can emulsify oils, enhance the removal of contaminants, and improve the interaction between different substances. The production of biosurfactants from agricultural wastes provides an eco-friendly and sustainable alternative to synthetic surfactants. This study evaluated the use of cassava mill effluent (CME), palm oil mill effluent (POME), and spoilt fruits extract (SFE) as substrates for biosurfactant production using *Bacillus thuringiensis* and *Yarrowia lipolytica*. Soil and river water samples used for microbial isolation were obtained from the Rivers State University farm, while agricultural waste substrates were collected from a fruit garden market in Rivers State, Nigeria. Standard microbiological and analytical methods were employed for isolation, screening, identification, and biosurfactant production studies. Biosurfactant activity was assessed using surface tension, oil spread, and drop collapse tests. Results showed high microbial loads in the agricultural wastes. Total heterotrophic bacterial counts ranged from $1.84 \pm 0.02 \times 10^7$ CFU/ml in POME to $2.31 \pm 0.41 \times 10^7$ CFU/ml in SFE, while total fungal counts ranged from $4.6 \pm 0.12 \times 10^5$ CFU/ml in SFE to $8.3 \pm 0.17 \times 10^5$ CFU/ml in CME. *Bacillus thuringiensis* and *Yarrowia lipolytica* were successfully isolated, identified, and screened for biosurfactant production. Nutrient analysis revealed that SFE contained the highest nitrate and phosphorus concentrations, whereas POME had the lowest nitrate level. Surface tension analysis showed that *Bacillus* with CME produced the lowest surface tension, 31.6 N/m, followed closely by *Bacillus* grown with POME at 32.1 mN/m. *Yarrowia* recorded relatively similar values of 33.1 mN/m, 33.6 mN/m, and 33.7 mN/m when grown with CME, POME, and SFE, respectively. Conversely, the highest surface tension, 60 N/m, was recorded for *Bacillus* with SFE, indicating poor biosurfactant activity under these conditions. Oil spread results showed that SFE with *Bacillus thuringiensis* produced a 20 mm clearing zone, while SFE with *Yarrowia lipolytica* produced the least (15 mm). The drop collapse test further confirmed that *Yarrowia lipolytica* produced more biosurfactant in CME. Overall, the findings revealed that, among the tested substrates, sterilized Cassava Mill Effluent has potential for biosurfactant production using *Bacillus thuringiensis*, while Spoilt Fruit Extract has potential for biosurfactant production using *Yarrowia lipolytica*.

Keywords: Biosurfactant, Cassava Mill Effluent, Palm Oil Mill Effluent, Spoilt Fruits Extract.

Introduction

The growing demand for environmentally friendly and sustainable industrial products has increased interest in biosurfactant production using renewable and low-cost substrates. Biosurfactants are surface-active compounds produced by microorganisms that reduce surface and interfacial tension between liquids, solids, and gases (Joanna et al., 2018). Compared to synthetic surfactants, they offer key advantages including biodegradability, low toxicity, environmental

compatibility, high specificity, and stability under extreme conditions such as temperature, pH, and salinity (Adeyemi et al., 2019). These properties support their application in petroleum, agriculture, food processing, pharmaceuticals, cosmetics, and environmental bioremediation (Joshi et al., 2019). Biosurfactants are produced by bacteria, yeasts, and fungi. Among yeasts, *Yarrowia lipolytica* is notable for its metabolic versatility and ability to utilize diverse and unusual carbon sources for biosurfactant production.

Among bacteria, *Bacillus thuringiensis* is widely known for its biopesticide role due to insecticidal crystal (Cry) proteins, but it also produces valuable biosurfactants with industrial relevance (Adeyemi *et al.*, 2019). Structurally, biosurfactants are classified into lipopeptides, glycolipids, phospholipids, fatty acids, neutral lipids, and polymeric or particulate compounds (Kumar *et al.*, 2021). Low molecular weight biosurfactants (e.g., glycolipids and lipopeptides) are effective in reducing surface tension, while high molecular weight biosurfactants (e.g., emulsan, liposan) are more effective in emulsification and stabilization of oil droplets (Makkar, *et al.*, 2011). The former acts by reducing interfacial tension, whereas the latter prevents coalescence of oil droplets through strong surface binding (Nelson-Smith, 2018). Biosurfactants have broad industrial applications due to their multifunctional properties, including use in agriculture (Singh and Cameotra, 2004a.), food processing (Makkar *et al.*, 2011), soil and water remediation (Masaaki *et al.*), microbial enhanced oil recovery (Nengi-Benwari *et al.*, 2025), biomedical applications (Singh and Cameotra, 2004.), nanotechnology (Singh *et al.*, 2017.), and detergent formulations (Kourmentza *et al.*, 2017). Despite these advantages, commercial biosurfactant production remains limited due to high production costs, especially substrate and media expenses, which account for 30–50% of total costs (Adeyemi *et al.*, 2019). This has driven interest in agricultural wastes as alternative substrates. These wastes are inexpensive, renewable, and rich in carbohydrates, lipids, proteins, and minerals required for microbial growth and metabolite synthesis (Adeyemi *et al.*, 2019). Their utilization also contributes to waste valorization and environmental sustainability (Mostafapour *et al.*, 20214). Agricultural wastes such as cassava peels, palm oil mill effluent, cassava mill effluent, fruit wastes, molasses, whey, sugarcane bagasse, and waste frying oils have been widely studied for biosurfactant production (Naughton *et al.*, 2019). These substrates support microbial growth and improve economic feasibility of production processes (Rawat *et al.*, 2020), while reducing environmental pollution caused by agro-waste disposal.

Among bacterial producers, *Bacillus thuringiensis* and other *Bacillus* species are efficient biosurfactant producers due to rapid growth, adaptability, and ability to utilize diverse substrates, including agro-wastes

(Sang-Cheol *et al.*, 2010). Their biosurfactants exhibit strong emulsifying and antimicrobial properties and are useful in bioremediation, enhanced oil recovery, and industrial cleaning (Santos *et al.*, 2016).

Similarly, *Yarrowia lipolytica* is a non-conventional yeast that can produce biosurfactants and bioemulsifiers from both hydrophobic and hydrophilic substrates (Rufino *et al.*, 2014). Its ability to metabolize oils, fats, and agricultural residues efficiently (Silva *et al.*, 2020) makes it valuable in biotechnology for producing enzymes, lipids, organic acids, and biosurfactants (Zarinviarsaghi *et al.*, 2017). Biosurfactants from *Y. lipolytica* are also noted for stability and industrial relevance (Sang-Cheol *et al.*, 2010). The use of agricultural wastes for biosurfactant production using *B. thuringiensis* and *Y. lipolytica* offers a dual benefit of reducing production cost and managing agricultural waste pollution (Santos *et al.*, 2016). However, biosurfactant yield depends on substrate type, composition, and microbial metabolic capacity (Silva *et al.*, 2020). Therefore, evaluating different agricultural wastes is essential for identifying optimal substrates.

This study therefore investigates the use of selected agricultural wastes for biosurfactant production using *Bacillus thuringiensis* and *Yarrowia lipolytica*, aiming to identify low-cost substrates and evaluate microbial biosurfactant-producing potential. The findings will contribute to sustainable biosurfactant production and environmental biotechnology development.

Materials and Methods

Sample Collection

Soil sample was collected from the school farm of River State University, Nigeria using polythene bag and hand trowel, River water samples were collected from a river at Slaughter, Trans-Amadi, Port Harcourt while agricultural wastes such as Palm oil mill effluent was collected from an Oil Mill Industry at Elele, using a sterile 10 litres Jerrycan. Cassava mill effluent was collected from a cassava mill in Oginigba, Trans Amadi, Rivers State. Spoilt fruits (SFEs comprising of orange, lemon, pineapple, pawpaw, banana, and pear) were collected from Fruits Garden market in Port Harcourt, River state using a sack bag sterilized with 70% ethanol.

These wastes were transported to the Microbiology laboratory of the Rivers State University, Nigeria for experimentation in biosurfactant production.

Isolation of Bacteria from the Soil Sample

Bacillus thuringiensis was isolated as described by Prescott *et al.* (2011), enrichment technique was used in the isolation of this bacteria. A flask containing 90ml each of nutrient broth was prepared and sterilized in an autoclave. Ten grams (10g) of the soil sample was inoculated into each of the flasks and both flasks were incubated at 37.2°C for 24 hours. After incubation, ten-fold serial dilution was carried out using normal saline to a dilution of 10^{-5} . Aliquot of 0.1 ml of the different dilutions were spread inoculated in triplicates on plates of nutrient agar (NA). The inoculated NA plates were incubated at 37.2°C for 48 hours (Silva *et al.*, 2020).

Isolation of Yeasts from the River Water Sample

Isolation of Yeast species were achieved according to Kurtzman *et al.* (2011). The river water samples were allowed to settle at room temperature (28.5°C) for 48 hours, and then used for isolation using enrichment technique. A flasks containing 90 ml of potato dextrose broth whose pH has been adjusted to 5.5 using 0.1 M H₂SO₄ (aq.) were prepared and sterilized in an autoclave. About 10 ml of the river water sample was inoculated into the flask and incubated at ambient temperatures for 4 days. After incubation, ten-fold serial dilution was carried out using normal saline to a dilution of 10^{-5} . Aliquot of 0.1 ml of the different dilutions were then spread inoculated in triplicates on plates of sabouraud dextrose agar (SDA). Inoculated plates were incubated at ambient temperatures for up to 5 days. After incubation period, cream and pink coloured round glistening or dull colonies (characteristic of yeast colonies) were sub-cultured onto fresh SDA plates so as to obtain pure yeast isolates (Zarinviarsaghi *et al.*, 2017).

Microbiological Analysis of Agricultural Wastes

The respective Agricultural wastes were diluted serially by transferring 25 grams into 250ml of sterile 1% peptone water, after shaking vigorously, 1ml of the suspension was transferred to 9ml of the 1% peptone water in a test tube serially.

An aliquot (0.1 ml) of 10^{-1} dilution from the respective samples were inoculated separately to surface dried

Nutrient agar in duplicates for enumeration of total heterotrophic bacterial population and Potatoes dextrose agar for isolation of total fungi. The plates were incubated at 37° C for 24 hours except for PDA plates that were incubated at room temperature 28.5° C (Kpormon and Douglas, 2018).

Total heterotrophic Bacteria were enumerated as described by Prescott *et al.* (2011). An aliquot (0.1 ml) of 10^{-1} dilution from the respective samples were inoculated separately in duplicate on surface dried Nutrient agar plates and spread evenly before incubation at 37.2°C for 24 hours. Bacterial colonies that grew on the nutrient agar plates (NA) were counted as total heterotrophic bacteria while, colonies that grew on the potatoes dextrose agar plates (PDA) were counted as total fungi. The enumerated colonies expressed as colony forming unit per gram (CFU/g) for the respective samples (Silva *et al.*, 2020) using the formula below;

$$\text{CFU/g} = \frac{\text{number of colonies}}{\text{Dilution} \times \text{volume plated}} \dots\dots\dots \text{Equation 1}$$

Identification of Microbial Isolates

Identification of *Yarrowia lipolytica*

The *Yarrowia lipolytica* isolates were identified based on their colonial and microscopic characteristics. Microscopic examination was carried out using Gram staining and observation under a bright-field microscope with a 40× objective lens (Dhawale & LaMaster, 2003.). The isolates were subjected to catalase, oxidase, and carbohydrate utilization tests (Begum *et al.*, 2020.), while molecular identification techniques were performed according to the methods described by Nisiotou and Gibson (2005).

Identification of *Bacillus thuringiensis*

Colonial morphology of the bacterial isolates were described. The isolates were then subjected to Gram staining & microscopic examination, and biochemical tests including Starch hydrolysis, Mannitol, Sucrose, Lactose, Glucose. Citrate, Maltose, H₂S, Lecithinase.

Gram staining and biochemical tests were carried out as described in Peekate, (2022) and biomolecular characterization of isolates was carried out using standard analytical methods according to Felsenstein (1985).

Determination of Nitrate, and Phosphate Composition of the Agro-Wastes

Nitrate Concentration

Nitrate concentration in the respective experimental set-ups was determined using a modified indophenol blue method (modification of APHA method 4500-NH₃ F; (Neboh *et al.*, 2013). In the modified method, 50ml of the sample and 1g of Aluminum pieces were placed in 250 ml Buchner flask. The side arm of the flask was connected into an extracting solution (98 ml of 1.0 M HCl, 1 ml Phenol-nitroprusside solution, and 1 ml alkaline hypochlorite solution) in a 250 ml beaker through a rubber hose. Ten millimeters (10 ml) of 0.5 M NaOH was added into the Buchner flask, and the flask immediately sealed and allowed for 1 hour for ammonia gas production and blue colour development in the extracting solution (ES). On expiration of the time, the absorbance of the ES was measured at 630 nm using a 721 VIS Spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China). The absorbance was used to determine the concentration (mg/L) of ammonium (NH₄⁺) in the sample through extrapolation from a previously calibration graph. The obtained NH₄⁺ concentration was then used to derive the nitrate concentration through multiplication with a stoichiometric factor.

Phosphate Concentration

Phosphate concentration was determined using the ascorbic acid method (Neboh *et al.*, 2013).). In this method, 40 ml of the sample was filtered through a Whatman No. 1 filter paper. The filtrate was transferred into a 50 ml capacity volumetric flask, and 6.5 ml of the analyzing reagent (373.2 ml distilled water, 0.06 g Potassium antimony tartrate, 2.4 g Ammonium molybdate, 2.12 g Ascorbic acid, and 26.8 ml concentrated Sulphuric acid) added. The content of the flask was then made up to the 50 ml mark with distilled water, and allowed for about 30 minutes for blue colour development.

The absorbance of the processed filtrate was measured at 880 nm using a Spectrophotometer. The absorbance was then used to determine the phosphate concentration (mg/L) in the sample through extrapolation from a previously obtained calibration graph.

Preparation of Isolated Microorganisms for Biosurfactant Production

Inoculums from stock cultures of identified bacteria and yeasts were re-inoculated on freshly prepared NA and SDA plates respectively. The inoculated plates were incubated at ambient temperatures (27 °C) for 48 hours (Peekate and Abu, 2017). After incubation, the bacteria and yeasts were streaked in a confluent manner on 3 sterile NA and potato dextrose agar (PDA) plates. The inoculated NA and PDA plates were incubated at ambient temperatures (27 – 32 °C) for 48 hours. After incubation, the ensuing bacterial and yeast colonial growths were scrap using sterile spatula, and transferred into sterile 100 ml mineral salt broth (MSB) separately. The composition (g/L) of the MSB is as follows: MgSO₄·7H₂O-0.42, KH₂PO₄ - 0.83, NaCl - 10.0, KCl - 0.29, Na₂HPO₄ - 1.25, NaNO₃ - 0.42 (Adomi, 2020). The cell population of the isolates in the MSB was determined through turbidity measurement using a spectrophotometer.

Determination of cell Population in MSB

The absorbance of the cell-MSB suspension and a prepared 0.5 McFarland standard were measured with the aid of a 721 VIS spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China) set at 600 nm, using sterile MSB as the reference blank. The 0.5 McFarland standard was prepared as stated in the Clinical and Laboratory Standards Institute Manual (Peekate, 2019). The turbidity of 0.5 McFarland standard is equivalent to the turbidity of a broth culture containing 1.5×10⁸ CFU/ml. Based on this, the absorbance readings of the cell-MSB suspension and the prepared 0.5 McFarland standard were supplied into the arithmetic equation below (Eq. 1) so as to determine the cell population (*P*) in the cell-MSB suspension.

$$P = \frac{\text{Asorbance of cell MSB suspension} \times 1.5 \times 10^8 \text{ CFU/ml}}{\text{Absorbance of 0.5 McFarland standard}}$$

... E q. 2

Experimental Set-up: The experimental setup was prepared as presented in Table 1. The wastes were sterilized at 121 °C at 15 PSI for 15 minutes before use in the experimental setups. Inoculated setups were incubated on a shaker operated at 400 rpm for a period of 10 days. After incubation, the fermented broths were screened for biosurfactant production (Peekate and Abu 2017).

Table 1: Experimental setup

Treatment	Microorganism	Inoculum (ml)	Substrate	Substrate volume (ml)	Total volume
T1	<i>Yarriowia</i> culture	5	Cassava mill effluent	490	495
T2	<i>Yarriowia</i> culture	5	Palm oil mill effluent	490	495
T3	<i>Yarriowia</i> culture	5	Spoilt fruits extract	490	495
T7	<i>Bacillus</i> culture	5	Cassava mill effluent	490	495
T8	<i>Bacillus</i> culture	5	Palm oil mill effluent	490	495
T9	<i>Bacillus</i> culture	5	Spoilt fruits extract	490	495
T13	Control (Klin- detergent)	-	-	495	495

Screening for Biosurfactant Production

The fermented waste media were screened for the presence of biosurfactant using the following methods: surface tension reduction, foaming capacity, oil spread diameter, and drop collapse activity as described by Peekate and Abu (2017).

Oil-spreading Test

About 40ml of distilled water was added to a Petri dish, followed by the addition of 0.5ml of diesel oil to the surface of the water, and then a drop of the fermented broth was placed on the oil surface. The diameter of the clear zone on the oil surface was measured using a centimeter rule, and the time taken to achieve the spread was also noted (Ali *et al.*, 2018).

Reduction in Surface Tension

The capillary rise method was used in measuring the surface tensions of the fermented broth. A sterile capillary tube of about 0.2cm diameter was used to measure the rise in height of the fermented broth, which was then used to calculate the surface tension (Peekate and Abu, 2017).

Drop Collapse Test

The Drop Collapse test involves coating each well in a ceramic well plate with a drop of engine oil, followed by incubation at 35°C for approximately an hour. After incubation, it was allowed to cool for three minutes. Two drops of each of the test solution was added to the oil-coated wells. The shapes of the drops were observed after a minute using a magnifying lens. If the drops collapse or become flat, the test solution is considered positive for the inherent presence of surfactant.

Conversely, if the drops remain intact and rounded, the test solution is considered negative for the presence of surfactant. This method was used to detect the presence of surfactants in a solution (Ali and Al-Hamdani 2018).

Statistical Analyses

The data were analysed using descriptive statistics tools in the form of mean, standard deviation and percentages. The null hypothesis was tested with the aid of some selected inferential statistical tools such as one-way ANOVA and T. test.

Results

Cassava mill effluent (CME), palm oil mill effluent (POME) and spoilt fruit extract (SFE) contained appreciable populations of heterotrophic bacteria and fungi, as presented in Figure 1 (expressed in Log10). The total heterotrophic bacterial counts ranged from 1.84×10^7 CFU/ml in POME to 2.31×10^7 CFU/ml in SFE, while total fungal counts ranged from 4.6×10^5 CFU/ml in SFE to 8.3×10^5 CFU/ml in CME. POME recorded the lowest bacterial population among the substrates examined.

Nitrate and phosphorus concentrations as presented in Table 2 varied among palm oil mill effluent (POME), spoilt fruit mixture (SFM) and cassava mill effluent (CME). Nitrate concentrations ranged from 0.1 ± 0.0 mg/L in POME to 3.2 ± 0.2 mg/L in SFM, while phosphorus concentrations ranged from 1.3 ± 0.2 mg/L in CME to 4.6 ± 0.4 mg/L in SFM. The differences were statistically significant ($P < 0.05$). SFM had significantly higher nitrate and phosphorus concentrations than POME and CME, which did not differ significantly from each other.

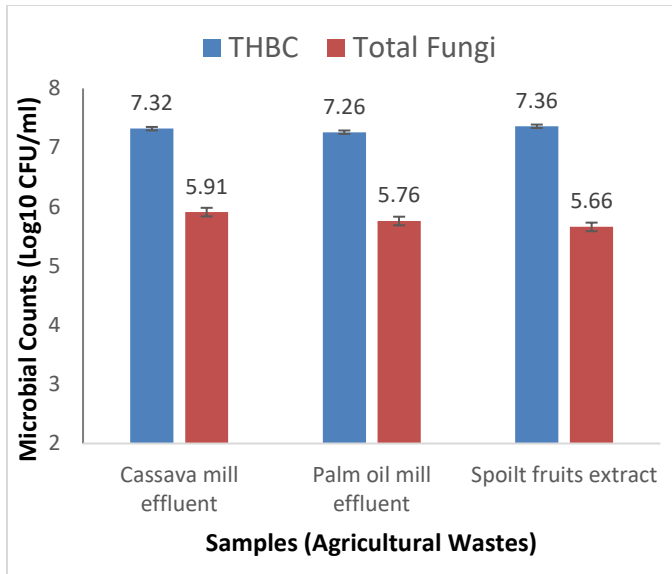


Figure 1: Microbial Population of the Agricultural Waste

Biosurfactant production by *Bacillus thuringiensis* and *Yarrowia lipolytica* was screened on CME, POME and SFE using the oil spread test, the drop collapse test and surface tension measurement. Activity varied with both substrate and organism.

Table 3 presents the Oil spread activity and Drop collapse results. Oil spread activity was positive in most setups. *Bacillus thuringiensis* cultured in CME, POME and SFE each gave an oil displacement diameter of 20 mm. *Yarrowia lipolytica* cultured in SFE gave the highest diameter (25 mm), with a "burst and cleared" reaction. *Yarrowia lipolytica* grown in CME and POME showed no oil displacement and the oil droplet sank (Table 3). The control (commercial detergent) gave 45 mm. Positive collapse reactions were observed in CME + *Bacillus*, CME + *Yarrowia*, POME + *Bacillus* and POME + *Yarrowia*, as well as in the control. Negative reactions were observed in SFE + *Bacillus* and SFE + *Yarrowia* (Table 3).

Table 2: Nitrate and Phosphorus Composition of the Agricultural Wastes

Nutrient composition	Palm-Oil Mill Effluent (PME)	Spoilt Fruit Mixture (SFM)	Cassava Mill Effluent (CME)	P. Value
Nitrate (mg/L)	0.1±0.0 ^a	3.2±0.2 ^b	0.7±0.2 ^a	0.002
Phosphorus (mg/L)	1.7±0.1 ^a	4.6±0.4 ^b	1.3±0.2 ^a	0.003

Note: Means with similar superscripts show no significant difference at $P \geq 0.05$

Table 3: Oil Spread (OS) Activity Test Result

Coded Isolate	Oil Spread (OS) Activity	Drop Collapse
	After Spread Diameter	Reaction
CME + <i>Bacillus</i>	20 mm	+
CME + <i>Yarrowia</i>	-	+
POME + <i>Bacillus</i>	20 mm	+
POME + <i>Yarrowia</i>	-	+
SFE + <i>Bacillus</i>	20 mm	-
SFE + <i>Yarrowia</i>	25 mm	-
Control (Klin)	45 mm	+

Key: CME: Cassava mill effluents, POME: Palm oil mill effluents, SFEs: spoilt fruit extract, Control (Klin) = commercial detergent.

Figure 2 presents the surface tension values for *Bacillus* and *Yarrowia* grown on CME, POME and SFE. *Bacillus* with CME produced the lowest surface tension (31.6 mN/m), followed by *Bacillus* with POME (32.1 mN/m). *Yarrowia* recorded similar values of 33.1, 33.6 and 33.7 mN/m on CME, POME and SFE, respectively.

The highest surface tension (60 mN/m) was recorded for *Bacillus* with SFE. CME gave the greatest reduction in surface tension for both organisms, particularly *Bacillus*. *Yarrowia* values were relatively stable across substrates, while *Bacillus* values varied more.

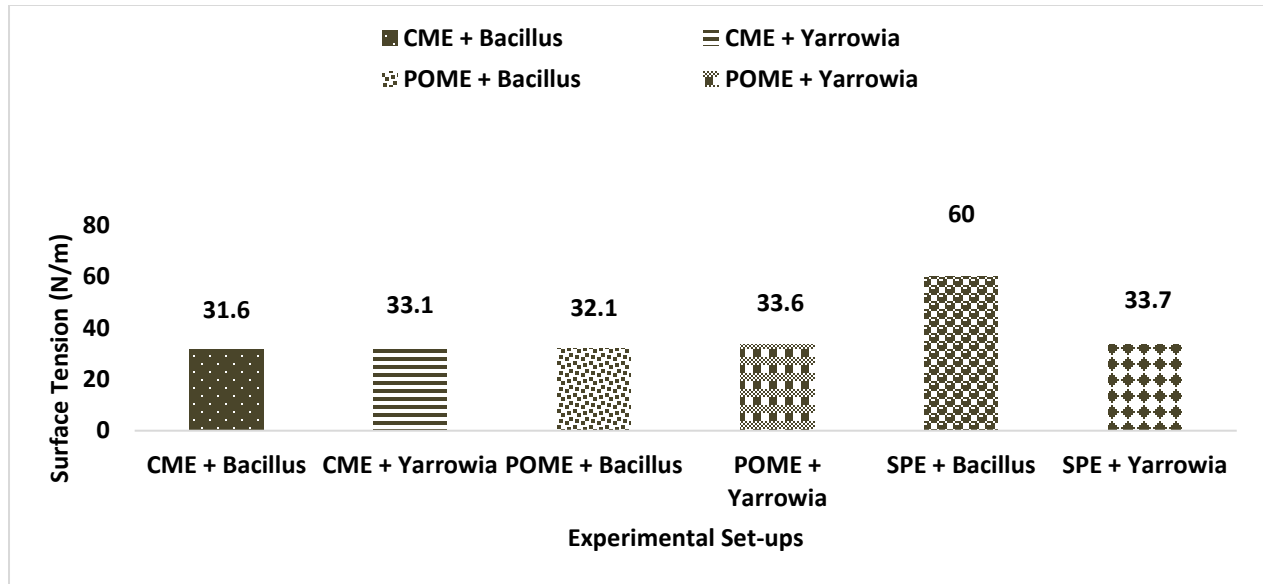


Figure 2: Surface Tension Results of Biosurfactant Production Using *Bacillus* and *Yarrowia*

Keys: CME = Cassava Mill Effluent, POME = Palm Oil Mill Effluent, SFE = Spoilt Fruit Extract

Discussion

The appreciable bacterial and fungal populations in CME, POME and SFE indicate that these substrates are rich in organic nutrients capable of supporting microbial growth and metabolic activity, and may therefore serve as suitable substrates for biosurfactant production.

The high bacterial population in spoilt fruit extract may be attributed to its rich carbohydrate and sugar content, which provides readily available carbon and energy for microbial metabolism. Spoiling fruits support rapid microbial growth because of their high moisture content and abundance of simple sugars and organic compounds (Kpormon and Douglas 2018). The elevated bacterial count in SFE therefore indicates its strong potential as a fermentation substrate for biosurfactant-producing microorganisms such as *Bacillus thuringiensis* and *Yarrowia lipolytica*. Carbon-rich substrates have been reported to enhance biosurfactant synthesis by supporting active cellular metabolism and metabolite production (Adeyemi *et al.*, 2019).

Similarly, the high fungal population in CME may be linked to the starch-rich nature of cassava residues and the acidic condition commonly associated with cassava processing wastes.

Such environments favour fungi and yeasts capable of utilizing complex carbohydrates (Joshi *et al.*, 2019). The high fungal count in CME suggests that the substrate may be particularly suitable for yeast-based biosurfactant production, especially by *Yarrowia lipolytica* (Neboh *et al.*, 2013).

The lowest bacterial population in POME may be due to residual oil components and phenolic compounds that can inhibit the growth of some microorganisms (Adeyemi *et al.*, 2019). Nevertheless, the counts in POME were still considerably high, indicating adequate nutrients to support microbial growth and biosurfactant production. Previous studies report that POME contains substantial quantities of lipids, nitrogen and organic matter that can serve as carbon sources for biosurfactant-producing microorganisms (Neboh *et al.*, 2013).

The microbial populations in all the wastes further emphasize their biodegradability and nutrient richness. Agricultural wastes with high organic loads are considered an economically viable alternative to expensive synthetic media in industrial biotechnology, including biosurfactant production (Adeyemi *et al.*, 2019). The presence of indigenous microbial populations also indicates that these wastes naturally support the microbial adaptation and enzymatic activities required for fermentation.

The significant differences in nutrient composition indicate that the wastes differ considerably in nutrients and may influence microbial growth and biosurfactant production. The significantly higher nitrate concentration in SFM suggests that it contains more nitrogenous compounds capable of supporting microbial metabolism and biosurfactant synthesis. Nitrogen is essential for microbial cell growth, protein synthesis and enzyme production during fermentation (Adeyemi *et al.*, 2019).

These variations may have significant implications for production efficiency. Biosurfactant synthesis is strongly influenced by the carbon-to-nitrogen ratio and the availability of essential minerals in the substrate (Adeyemi *et al.*, 2019). Substrates with balanced nutrient composition generally promote higher microbial biomass and metabolite production. The high nitrate and phosphorus contents in SFM may therefore explain its suitability as a promising low-cost substrate for biosurfactant production using *Bacillus thuringiensis* and *Yarrowia lipolytica*.

The screening results show that the type of agricultural substrate significantly influenced biosurfactant production by the test microorganisms.

The oil spread method is widely regarded as a rapid and reliable screening technique because biosurfactants reduce oil-water interfacial tension, thereby displacing oil on the water surface (Joanna *et al.*, 2018). Larger oil displacement diameters generally indicate higher biosurfactant activity and concentration. The strong activity of *Yarrowia lipolytica* in SFE may be attributed to the high nutrient content of spoiled fruit extract, especially its elevated nitrate and phosphorus levels, which likely enhanced microbial metabolism and biosurfactant synthesis. Fruit wastes are rich in fermentable sugars and organic compounds capable of stimulating biosurfactant production by yeasts and bacteria (Adeyemi *et al.*, 2019).

The positive oil spread activity of *Bacillus thuringiensis* in CME also suggests that cassava mill effluent supported lipopeptide biosurfactant production. *Bacillus* species are well known for producing potent biosurfactants such as surfactin, iturin and fengycin, which have excellent surface-active properties.

The ability of *Bacillus thuringiensis* to utilize cassava-based substrates may be associated with its adaptability to carbohydrate-rich agro-industrial wastes (Ali and Al-Hamdani 2018).

The drop collapse test is based on the principle that biosurfactants reduce surface tension, causing liquid droplets to spread or collapse on hydrophobic surfaces (Peekate and Abu 2017). Positive collapse reactions therefore indicate the presence of surface-active compounds in the culture broth.

The discrepancy between oil spread and drop collapse results in some treatments may be due to differences in sensitivity between the screening methods. Some biosurfactants may show better oil displacement while showing weak drop collapse activity, depending on their molecular composition and concentration (Kumar *et al.*, 2021). For instance, although SFE + *Yarrowia* produced strong oil spread activity, its drop collapse result was negative, suggesting that the biosurfactant produced may possess stronger emulsifying ability than droplet-spreading characteristics.

Surface tension reduction is an important indicator of biosurfactant production because effective biosurfactants reduce the cohesive forces between liquid molecules. Lower surface tension values therefore indicate greater biosurfactant activity and better substrate suitability (Kumar *et al.*, 2021).

CME was the preferred substrate for biosurfactant production. The small difference between CME and POME for *Bacillus* notwithstanding, the consistently lower values with CME indicates its greater suitability under the experimental conditions. This may be attributed to the high carbohydrate and organic nutrient content of cassava-processing effluent, which provides readily available carbon and energy for microbial growth and biosurfactant synthesis (Neboh *et al.*, 2016; Adeyemi *et al.*, 2019).

POME showed appreciable potential as an alternative substrate, especially for *Bacillus*, whereas SFE was the least suitable substrate. The relatively stable performance of *Yarrowia* contrasted with the greater variation shown by *Bacillus*. These results demonstrate the potential of locally available agro-industrial wastes, particularly CME, for sustainable and cost-effective biosurfactant production and waste valorization.

Conclusion

The findings of the research revealed that among the tested substrates there exist a potential for producing biosurfactant from sterilized Cassava Mill Effluent using *Bacillus thuringiensis*, while Spoilt Fruit Extract among the tested substrates showed potential for biosurfactant production using *Yarrowia lipolytica*. Further quantitative production studies and biosurfactant characterization is suggested for future studies in identifying a universally optimal substrate because screening assays can produced discordant results.

References

- Adeyemi, J. O., Onajobi, I. B., Adebajo, L. O., Orji, F. A., Ilusanya, O. A. F., & Samson, O. J. (2019). Biosurfactant production potentials of some bacteria recovered from agro-industrial wastes. *FUW Trends in Science & Technology Journal*, 4(3), 870–873.
- Adomi, P., Montoneri, E., Biasizzo, M. Quaglitto, P., Viscardi, G. & Boffa, V. (2020). Upgrading biomass wastes in chemical technology. Humic acid-like matter isolated from compost as chemical auxillary for textile dyeing. *Journal Chemical Technology Biotechnology*, 82, 939-948.
- Ali, A. M. & Al-Hamdani, A. A. (2018). Application of Drop Collapse Method for Evaluating Surface Tension of Synthetic Surfactant. *Journal of Physics: Conference Series*, 1007(1), 23-48.
- Begum, K., Shahid, M. S., Ferdoush, S. & Nur, F. (2020). Phenotypic identification and antifungal susceptibility of two *Rhodotorula* species isolated from dandruff samples. *Dhaka University Journal of Pharmaceutical Science*, 19 (2), 185-190.
- Dhawale, S and LaMaster, A. (2003). *Microbiology Laboratory manual*. The mcHill Company. Inc., USA.12 Pp 18.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39(4), 783–791.
- Joanna, C., Marcin, L., Ewa, K. & Grazyna, P.A. (2018). nonspecific synergistic effect of biogenic silver nanoparticules and biosurfactant towards environmental bacteria and fungi. *Ecotoxicology*, 27(3), 352-9.
- Joshi, S., Bharucha, C., Jha, S., Yadav, S., Nerurkar, A., & Desai, A. J. (2019). Biosurfactant production using agro-industrial wastes by *Bacillus* spp. and its application in bioremediation. *Bioresource Technology Reports*, 7, 100216.
- Kourmentza, H., Hirai, A., Minabe, T. & Obata, H. (2017). Stabilization of astaxanthin by a novel biosurfactant produced by *Rhodotorula mucilaginosa* KUGPP-1. *Biocontrol Science*.18(1), 23-42
- Kpormon, L. B. & Douglas, S. I. (2018), Comparative Ecotoxicological Assay of E- waste (Phone Batteries) on Some Aquatic Micro Flora. *Journal of Advances in Biology & Biotechnology*, 17(2), 1-10.
- Kumar, S., Kaushik, P., & Kumar, R. (2021). Advances in the use of *Bacillus thuringiensis* in sustainable agriculture: Current status and future prospects. *Egyptian Journal of Biological Pest Control*, 31, 1–12.
- Kurtzman, C., Fell, J. & Boekhout, T. (2011). The Yeasts: a taxonomic study definition, classification and nomenclature of the yeasts. The Yeasts a Taxonomic Study. 3-9.
- Makkar, R., Cameota, S. & Banat, I. (2011) Advances in utilization of renewable substrates for biosurfactant production. *AMB Express*; 1:5.
- Masaaki, M., Daido, H., Toshifumi, T. & Tsuneo, I. (2000). A new lipopeptide biosurfactant produced by *Arthrobacter* species. *Biochimica et Biophysic Acta*, 1488(3), 211-8.
- Mostafapour, M. J & Saffari M. (2014). Isolation and identification of biosurfactant producing strains from the genus *Acinetobacter* spp and antibacterial effects of biosurfactant produced on some of the negative and gram-positive bacteria *in vitro*. *New Cell Molecular Biotechnology Journal*, 4(14), 79-19.

- Naughton, P.J. Marchant, R. Naughton, V. & Banat I.M., (2019) Microbial biosurfactants: current trends and applications in agricultural and biomedical industries. *Journal of Applied Microbiology*, 127, 12–28.
- Neboh, G., A. Dhasmana, V. & Kumar, H. (2016). Biosurfactants: the next generation biomolecules for diverse applications, *Environ. Sustain*, 3, 353–369,
- Neboh, H.A., Ilusanya, O.A., Ezekoye, C.C. & Orji, F.A. (2013) Assessment of Ijebu-Igbo Abattoir Effluent and Its Impact on the Ecology of the Receiving Soil and River. *Journal of Environmental Science, Toxicology and Food Technology*, 7, 61-67.
- Nengi-Benwari, A. O., Aleruchi, O., Longjohn, I. R., & Opara, C. F. (2025). Isolation and identification of zbiosurfactant-producing bacteria from hydrocarbon-contaminated mechanic workshop soils in Port Harcourt, Nigeria. *Microbiology Research International*, 13(1), 23–29.
- Nisiotou, A. A., & G. R. Gibson, H. (2005). Isolation of culturable yeasts from market wines and evaluation of the 5.8S-ITS rDNA sequence analysis for identification purposes. *Lett. Appl. Microbiol*, 41, 454-463.
- Peekate P. L. & Abu G. O. (2017). Optimizing C:N Ratio, C:P Ratio, and pH for Biosurfactant Production by *Pseudomonas fluorescens*. *Journal of Advances in Microbiology*, 7(2), 1-14.
- Peekate, L. P (2019) Screening selected detergents for use as positive control in assessing for biosurfactant production. *Journal of Applied Sciences* 19(8)
- Peekate, L. P. (2022). Deciphering the identity of bacterial isolates through conventional means: A practical guide. Edese Printing & Publishing Company, Port Harcourt, Nigeria, 21-48.
- Prescott, L.M., Harley, J.P. & Klein, D.A. (2011) Microbiology. Sixth International Edition. Mcgraw-Hill Publishing Company, UK, 652-668.
- Rawat G., Dhasmana, A. & Kumar, V. (2020). Biosurfactants: the next generation biomolecules for diverse applications, *Environ. Sustain*, 3, 353–369,
- Rufino, L., Banat, M.I., Teixeira, J. & Oliveira, R. (2014). Biosuractant: potential application in medicine. *J. Antimicrobial. Chem*, 57, 609-618.
- Sang-Cheol, K. S. H., Park, I. H., Chung, S.Y, Chandra, M. S. & Choi, Y.L. (2010). Isolation, purification and characterization of novel fengycin S from *Bacillus amyloliquefaciens* LSC04 degrading-crude oil. *Biotechnology Bioprocess Engineering*, 15, 246-253.
- Santos, D.K.F., Rufino, R.D. Luna, J.M. Santos, V.A. & Sarubbo, L.A. (2020). Biosurfactants: multifunctional biomolecules of the 21st century, *International Journal Molecular Science*, 17, 1–31,
- Silva, L.A, Veras, B.O. Ribeiro, B.G. Aguiar, J.S. Campos J.M. Guerra, J.M. Luna, L. & Sarubbo. A. (2020). Production of cupcake-like dessert containing microbial biosurfactant as an emulsifier. *Peer Journal*, 1–23.
- Singh, A., Van Hamme, J. D. & Ward, O.P. (2017). Surfactants in microbiology and biotechnology: Part 2. Application aspects. *Biotechnology Advances*, 25(1), 99-121.
- Singh, P. & Cameotra, S.S. (2004). Potential application of microbial surfactants in biomedical sciences. *Trends Biotechnology*, 22,142-146.
- Zarinviarsagh, M., Ebrahimipour, G. & Sadeghi, H. (2017). Lipase and biosurfactant from *Ochrobactrum intermedium* strain MZV101 isolated by washing powder for detergent application, *Lipids Health Dis*, 16 -177.