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BIO-SURFACTANTS FROM THE PALM OIL INDUSTRY: AN OVERVIEW

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ABSTRACT

Bio-surfactants are surface-active agents that are produced by plants and microorganisms. Unlike chemical surfactants, they are biodegradable, less toxic, and have many applications in many industries, including the pharmaceutical and food industries. Bio-surfactants are structurally diverse owing to the diversity of pathways and microorganisms involved in their biosynthesis. Research on microbial bio-surfactants has increased over the years because of their multiple features and applications, such as detergency, foaming, emulsifying, and solubilisation of hydrophobic compounds. However, the high cost of production remains a challenge for the large-scale production of bio-surfactants. The use of cheap agricultural waste as substrates for bio-surfactant production can significantly reduce the cost of production. The refining of palm oil generates a large amount of waste, including palm fatty acid distillate, palm oil mill effluent, and palm oil decanter cake. These wastes and their disposal pose a significant challenge to the environment. Several studies have described the use of palm oil and the waste products of palm oil refineries as substrates for the production of bio-surfactants. Beyond providing substrates for bio-surfactant production, the soil and effluent from the palm oil industry are a very rich source of bio-surfactant-producing microorganisms. This review evaluated the literature on bio-surfactant production using palm oil and wastes from palm oil processing to determine their suitability for bio-surfactant production. It identified the role of bio-surfactant production in the management of waste from the palm oil industry and discusses the challenges and prospects of bio-surfactant production in the palm oil industry.

INTRODUCTION

Bio-surfactants are surface-active and emulsifying compounds produced by plants and microorganisms (Akbari *et al*., 2019). Similar to chemical surfactants, they are amphipathic molecules that have both hydrophobic and hydrophilic moieties (Santos *et al*., 2016). This surface-active property allows bio-surfactants to minimize the interfacial tension between surfaces and fosters their application in diverse industries, including agriculture, pharmaceuticals, cosmetics, food, health, petroleum, and textiles (Markande *et al*., 2021). However, unlike chemical surfactants, they are less toxic and more environmentally friendly because of their degradability and stability over a wide range of environmental conditions, such as pH, temperature, and salinity (Franzetti *et al*., 2018). As a result, there has been growing interest in the study of bio-surfactants over the past three decades.

Although bio-surfactants are produced by both plants and microorganisms, microbial bio-surfactants have received more attention because of their multiple features and applications, such as detergency, dispersion, wetting, foaming, emulsifying, and solubilisation of hydrophobic compounds (Karnwal *et al*., 2023). Microbial bio-surfactants are produced extracellularly by microorganisms that grow on water-immiscible substrates, such as crude oil, cooking oil, olive oil, and palm oil (Ron and Rosenberg, 2002; Shah *et al*., 2016). Microbial bio-surfactants are economical because they can be produced from waste materials (Henkel *et al*., 2012; Hasanizadeh *et al*., 2017). One of the main challenges in the production of microbial bio-surfactants is the high cost of production, and the cost of carbon sources accounts for approximately half of the cost of production (Kee *et al*., 2022). Globally, over 30% of the food produced annually ends up as agricultural waste (Kee *et al*., 2021). Therefore, the use of cheap carbon sources, particularly agricultural wastes, for the production of microbial bio-surfactants has been a subject of research interest.

Palm oil and waste from processing have been used in the production of microbial bio-surfactants (Kee *et al*., 2022). The refining process of palm oil generates large amounts of waste and by-products, including empty fruit bunches (EFB), palm kernel shells (PKS), Palm Fatty Acid Distillate (PFAD), and Palm Oil Mill Effluent (POME) (Kee *et al*., 2021). These low-value by-products of palm oil processing have also been used as substrates for bio-surfactant and biofuel production (Mahlia *et al*., 2019; Kee *et al*., 2022).

This review evaluated the literature on bio-surfactant production using palm oil and the wastes or by-products of this process to determine the suitability of these substrates for bio-surfactant production.

Palm Oil Industry

The African Oil palm (Elaeis guineensis), originally from West Africa, is an economically important cash crop and a source of the most consumed vegetable oil globally (Shahbandeh, 2022). The vegetable oil produced from oil palm (palm oil) is used for both food and non-food purposes, such as cosmetics and drug production, agrochemicals, surfactants, and industrial chemicals, amongst others (Chaparro *et al*., 2023). In Nigeria, the oil palm grows along the coastline of the southern part of the country and is cultivated on about 3.0 million hectares of land (Shehu *et al*., 2021). According to Food and Agriculture Organization Statistics (FAOSTAT) -2021, after Indonesia, Malaysia, Thailand, and Colombia, Nigeria ranks the fifth-largest producer of palm oil globally. In 2023, Nigeria produced about 1.4 million metric tons and consumed 1.8 million metric tons of palm oil in the preceding year (Sasu, 2023).

Palm Oil Processing

Palm oil is produced from the mesocarp of oil palm fruit (Achoja *et al*., 2019). Different by-products or wastes are generated during palm oil processing. As described by Mahlia *et al*. (2019), the processing of palm oil begins with the separation or stripping of the oil palm fruit from the fresh fruit bunch. The fruit is then heated and pressed, resulting in a mixture of crude palm oil (CPO), water, fibre, and nuts. The CPO was separated from the mixture and further purified to yield pure palm oil and palm oil effluent (POME), a waste product released in the process. The nuts and fibre are further processed to produce palm kernel oil (PKO), releasing palm kernel cake, fibre, and shells (Figure 1).

The two main products of the palm oil mill are CPO and PKO, which are produced from the mesocarp and endosperm of oil palm fruit, respectively (Achoja *et al.*, 2019). However, the process generates different by-products or waste from POME, empty fruit bunch (EFB), fibre, shell, and palm kernel cake (Sundalian *et al.*, 2020). The CPO from the palm oil mill can either be used as it is or it is further processed in the palm oil refinery to eliminate impurities using either physical or chemical refining techniques, thereby producing refined palm oil (RPO) (Chew and Nyam, 2020). The steps involved in refining CPO include degumming, bleaching, and deodorisation (Chew & Nyam, 2020). This process generates significant amounts of waste, including soapstock (palm acid oil), spent bleaching earth, and PFAD (Chew & Nyam, 2020). Figure 2 shows the process and by-products of the palm oil refineries.

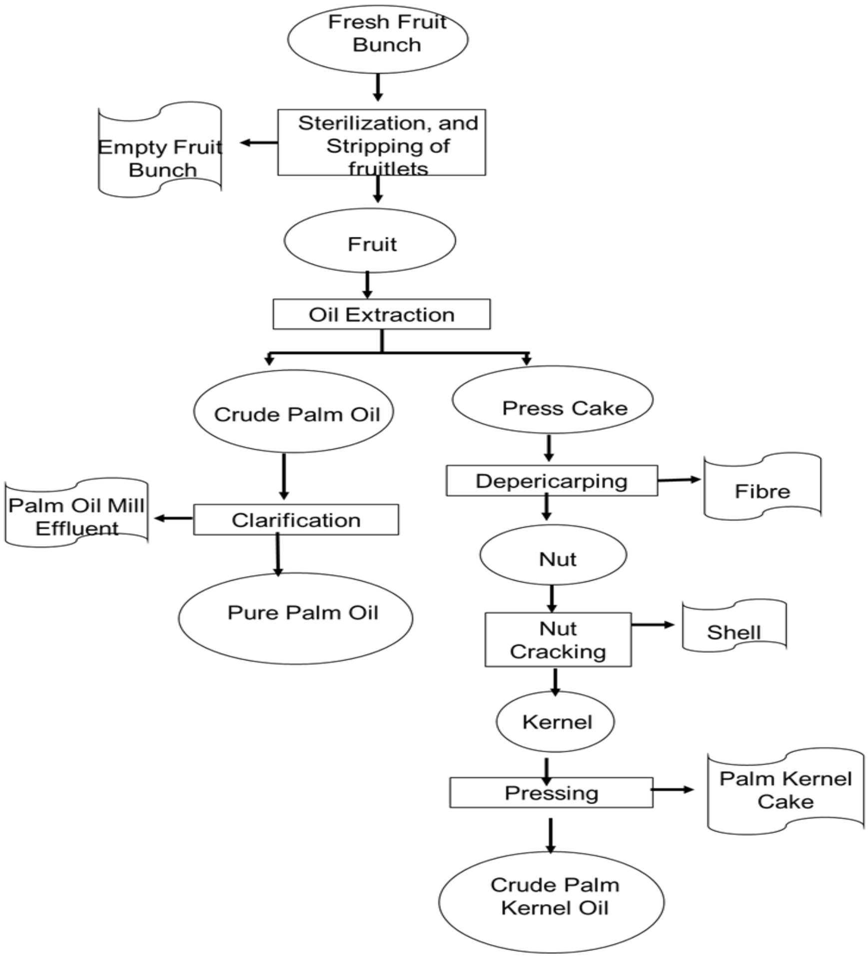

Fig. 1: Palm oil production process and by-products. Adapted from the study by Mahlia *et al.* (2019)

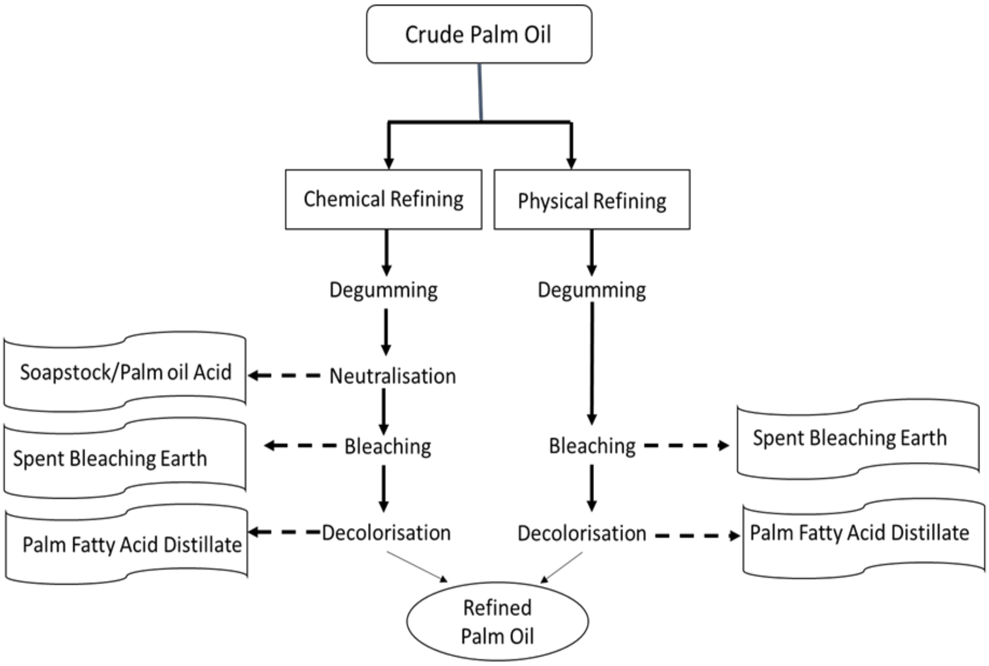

Fig. 2: Waste and products of Palm Oil Refineries.
Adapted from the study by Lee and Ofori-Boateng (2013).

According to Sundalian *et al.* (2021), only approximately 11% of the oil palm fruit is converted into high-value products such as CPO, RPO, and PKO; the remaining accounts for the different waste products mentioned above. This would imply that in a large palm oil-producing country like Nigeria, over 11 million metric tons of waste will be generated annually from the 1.4 million metric tons of palm oil produced (Sasu, 2023). The amount of waste generated from the palm oil industry poses an environmental risk because disposal remains a public health concern (Kee *et al.*, 2021). A Nigerian study by Achoja *et al.* (2019) identified four main waste disposal methods in palm oil mills: recycling, sales, burning, and dumping. Recycling implies that the waste is reused in palm oil plantations for different purposes, such as composting and fueling (Supriatna *et al.*, 2022). Some of these wastes are sold and used as animal feed, construction materials, and fuel (Mahlia *et al.*, 2019). This serves as a source of additional revenue for palm oil farmers (Achoja *et al.* 2019). Other methods of disposal, such as burning and dumping, however, pose a serious environmental challenge because of the environmental pollution they cause, and there is a need to channel the waste into more sustainable use (Ogunsina and Akintan, 2020).

Composition of Palm Oil Waste (POME)

The waste generated from palm oil mills or refineries can be categorized as liquid waste, which is mainly POME, and dry biomass, which includes EFB, PKC, palm kernel shells, and fronds (Kee *et al.*, 2022).

Palm Oil Mill Effluent

Palm Oil Mill Effluent is the liquid waste produced during palm oil processing. The processing of oil palm to produce CPO requires a large quantity of water, such that for every ton of CPO produced, approximately 5 to 7 tons of water is used (Ogunsina and Akintan, 2020), and about half of this ends up as POME in many palm oil mills (Kamyab *et al*., 2018). Typically, POME is produced at very high temperatures ranging between 80 oC and 90 oC (Kee *et al*., 2022). It is brownish, acidic, and viscous water that contains fruit debris and residual oil (Sundalian *et al*., 2020). However, the composition and characteristics of POME vary depending on the quality of the oil palm being processed and the process involved in palm oil milling (Kamyab *et al*., 2018). The carbon and hydrogen contents of POME are very high, although they have low sulfur and nitrogen contents (Saad *et al*., 2021). According to Hassan *et al*. (2013), POME may contain sublethal concentrations of heavy metals such as lead, which are introduced by pipes, plastics, paints, and glass that they come in contact with in the mill. Although POME is mainly organic, as no chemicals are used in the processing of palm oil, it is still hazardous to the environment due to its high chemical oxygen demand (COD) and biological oxygen demand (BOD) (Kee *et al*., 2022). The ranges of COD and BOD found in POME are 44,000–103,000 mg/L and 25,000–66,000 mg/L respectively (Wang *et al*., 2015). These values are over 100 times the BOD and COD of domestic waste, and should not be discharged into the environment without treatment (Chan and Chong, 2019). Hence, palm oil mills must implement effective waste treatment practices because the release of POME into the environment can result in both water and air pollution due to biomethane emissions (Ogunsina and Akintan, 2020). In Nigeria, some laws and regulations guide the discharge of industrial effluence, including POME, into the environment (Emodi, 2015). However, many palm oil mills do not comply with these regulations, hence, they discharge the untreated POME directly into the environment (Ogunsina and Akintan, 2020).

Empty Fruit Bunch

About 25-30% of the solid waste from the palm oil industry is EFB (Sundalian *et al*., 2020). Empty Fruit Bunches are typically used for composting; approximately 90% of the EFBs generated from CPO production still end up as waste (Achoja *et al*., 2019). The main components of EFB are cellulose, xylose, lignin, and ash (Suraya *et al*., 2017). This makes it an excellent substrate for the production of biofuels, such as bioethanol (Sundalian *et al*., 2020).

Palm Kernel Fibre and Shell

Palm kernel shell accounts for approximately 7% of the waste from palm oil production and is often used to prepare activated charcoal (Sundalian *et al*., 2020). On the other hand, palm kernel cake is rich in non-starchy polysaccharides, proteins, lignin, and mannose (Sundalian *et al*., 2020).

Biosynthesis of Bio-surfactants and the Suitability of Palm Oil and Palm Oil Wastes for Bio-surfactant Production

Bio-surfactants contain both hydrophobic and hydrophilic moieties (Santos *et al*. 2016). To produce bio-surfactants with these moieties, microorganisms must have both water-soluble and water-immiscible substrates for the biosynthesis of hydrophilic and hydrophobic moieties, respectively (Nurfarahin *et al*., 2018). Hydrophilic substrates are typically involved in metabolic processes and cell growth via the glycolytic pathway, whereas hydrophobic substrates are typically channelled to the production of hydrophobic moieties of bio-surfactants via the lipolytic and de novo fatty acid pathways (Nurfarahin *et al.*, 2018). Multiple metabolic pathways are involved in the biosynthesis of bio-surfactants, and the preferred pathway depends on the nature of the carbon source available to the microorganism (Santos *et al.*, 2016).

When a water-soluble carbon source, such as glucose, is used as the sole carbon source, glucose is phosphorylated to glucose-6-phosphate (G-6-P) via glycolysis. Figure 3 shows the metabolic pathways involved in bio-surfactant synthesis using water-soluble substrates. Glucose-6-phosphate is an important precursor for the synthesis of hydrophilic moieties of bio-surfactants (Kee *et al.*, 2022). In the presence of the AlgC gene, G-6-P is transformed to Glucose -1-phosphate and eventually, the production of Deoxythymidine diphosphate (dTDP)-l-sugar, the hydrophilic moiety including mannose, trehalose, rhamnose, sophorose, and polysaccharide (Pardhi *et al.*, 2022). For the formation of hydrophobic moiety, glucose forms pyruvate via the glycolytic pathway and then enters the tricarboxylic acid (TCA) cycle to form acetyl-CoA (Nurfarahin *et al.*, 2018). Acetyl-CoA combines with oxaloacetate to synthesise malonyl-CoA, which is then transformed into fatty acids that are important for the formation of the hydrophobic moiety (Santos *et al.*, 2016).

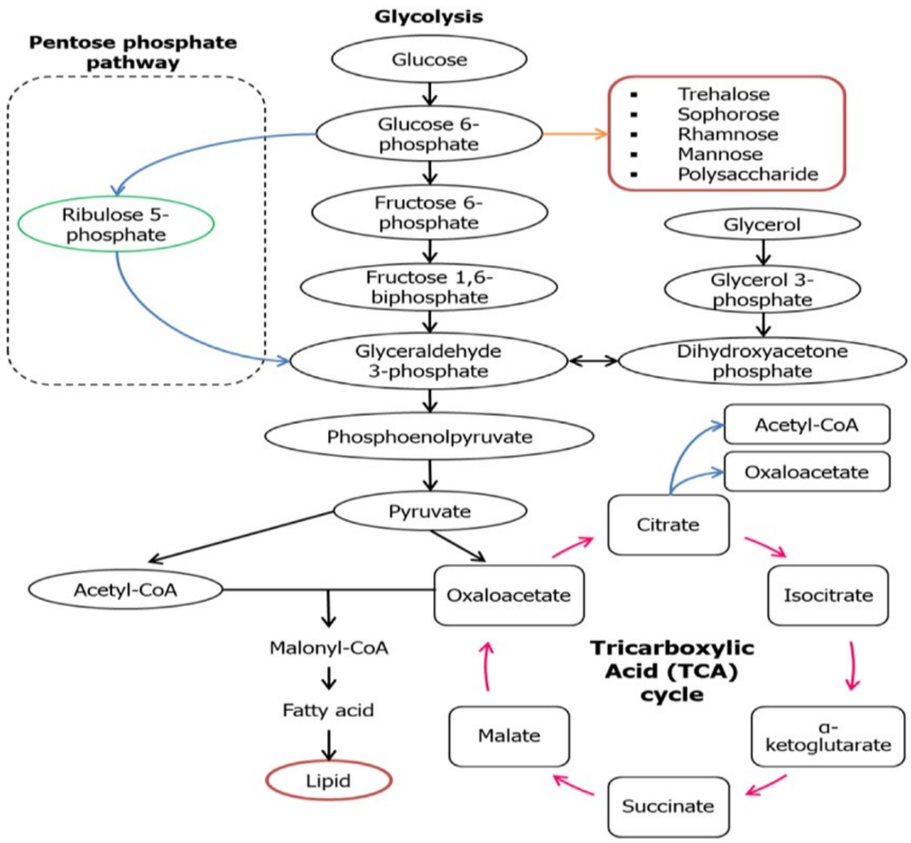

Fig. 3: The Metabolic Pathway Involved in the Synthesis of Bio-surfactants using Water Soluble Substrates.
Source: Nurfarahin *et al.* (2018)

In situations where hydrophobic substrates such as hydrocarbons or palm oil and its by-products are used as sole carbon sources, the metabolic mechanism of the microorganism self-regulates to trigger gluconeogenesis (GNG) and the lipolytic pathway. Figure 4. (Nurfarahin, 2018). The lipolytic pathway and GNG result in the synthesis of the hydrophobic moiety, whereas the hydrophilic moiety is produced from the de novo synthesis pathway through GNG. If the substrate is a hydrocarbon, it is metabolised through the n-alkane degradation pathway to form fatty acids and subsequently lipids and acetyl-CoA. Acetyl-CoA then enters the TCA cycle to form pyruvate. The pyruvate enters the GNG pathway to form G6P, thereby initiating the synthesis of the hydrophilic moiety. Palm oil and its by-products are not hydrocarbons, they contain three types of fatty acids: palmitic acid, oleic acid, stearic acid, and linoleic acid (Estiasih and Ahmadi, 2018). As such, these substrates are metabolised for bio-surfactant production via the GNG and lipolytic pathways.

The suitability of palm oil and the byproducts of its milling and refinery processes as substrates for bio-surfactant production is due to their chemical composition, low cost, and availability. As emphasised by (Kee *et al.*, 2022), palm oil and its by-products contain fatty acids, lignocellulose, and other organic matter, which makes them an excellent source of carbon for microbes. Table 1 shows the compositions of the different palm oil by-products. Palm oil by-products are agricultural wastes that cause significant environmental pollution (Ogunsina and Akintan, 2020). Considering the magnitude of waste generated by the palm oil industry, the need for more effective methods of disposing of these wastes, as well as the low cost of production of these wastes, makes them more suitable substrates for bio-surfactant production.

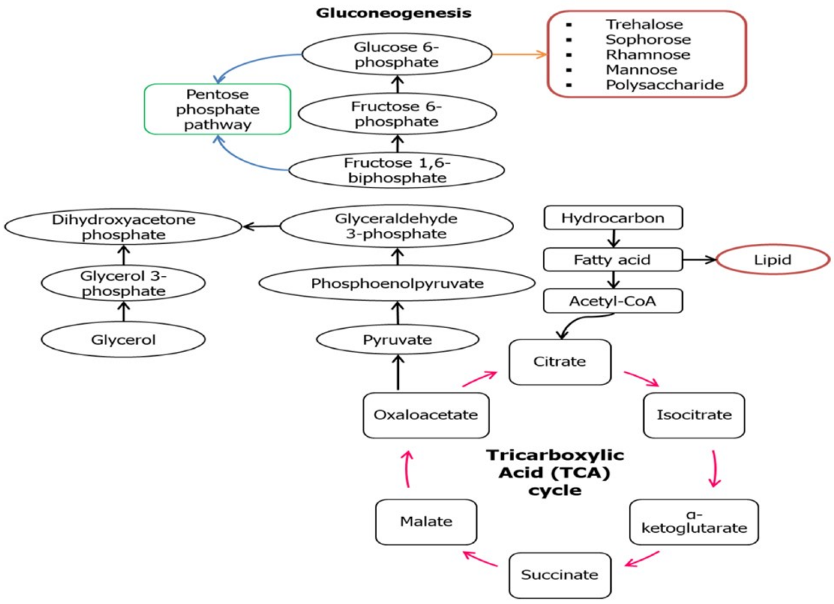

Fig. 4: The Metabolic Pathway Involved in the Synthesis of Bio-surfactants Using Water-immiscible Substrates.
Source: Nurfarahin *et al.* (2018)

Table 1: Composition of Palm Oil Waste/By-products

Palm Oil By-Product/ Waste	Composition	References
Empty Fruit Bunch, Palm Kernel Shell, fibre, fronds, Palm kernel cake	Lignin, moisture, hemicellulose, ash, cellulose, xylose, Fibre, oil	Sundalian *et al.* (2020)
Palm Oil Mill Effluent	Lipid, Protein, ash, carotenoids, moisture, carbohydrates	Hassan *et al.* (2013)
Palm Fatty Acid Distillate	Fatty acids, Moisture	Estiasih and Ahmadi (2018)

Bio-surfactant production by isolates from palm oil contaminated sites

Palm oil-contaminated sites and palm oil mill effluent are rich sources of bio-surfactant-producing microorganisms, including bacteria, moulds, and yeasts (Chooklin *et al.*, 2013). Table 2 shows the different bio-surfactant-producing organisms that have been isolated from palm oil-contaminated sites and the conditions under which they were used to produce bio-surfactants.

Marañón *et al.* (2015) isolated Bacillus tequilensis and Bacillus subtilis from palm oil mill effluent, and both bacteria produced bio-surfactants that have the surface tension of 29.2 and 29.0 mN/m. However, the authors neither characterised the bio-surfactants nor qualitatively assessed their ability to produce bio-surfactants. Saisa-Ard *et al.* (2014) also isolated an array of bio-surfactant-producing bacteria from palm oil -contaminated soil but found that Azorhizobium doebereinerae AS54 and Geminicoccus roseus AS73 were the highest bio-surfactant producers amongst all. They also utilised different waste products from the palm oil industry, including palm oil decanter cake, palm oil, crude palm oil, and palm oil mill effluent, as carbon sources for the production. They reported that with the aforementioned bacteria, combining both palm oil decanter cake and palm oil gave the highest yield of bio-surfactant, which was determined using the emulsification indices of the bio-surfactants produced. The bio-surfactants produced by A. doebereinerae AS54 and G. roseus AS73 had emulsification indexes of 63% and 69%, respectively. However, they did not determine the type or chemical structure of the bio-surfactant produced.

Ogbonna *et al.* (2023) isolated Bacillus thuringiensis c25 from palm oil-contaminated soil, and the isolate produced a lipopeptide using glucose, lactose and mannose as carbon sources. While these sugars are excellent carbon sources, they are neither water-insoluble nor inexpensive.

Jamal *et al.* (2014) also isolated Providencia alcalifaciens SM03 from degraded palm kernel cake (PKC), which is a solid residual obtained after the extraction of palm oil from the palm fruit. The PKC has a high concentration of fatty acids, protein and minerals, and this makes it a suitable source of bio-surfactant-producing microorganisms and an excellent substrate for bio-surfactant production. Using PKC as a carbon source, Jamal *et* *al.* (2014) reported that the bacterium, P. alcalifaciens SM03, produced a glycolipid with a surface tension of 33.69 mN/m.

Dikit *et al.* (2019) evaluated the microbial diversity of the bio-surfactant-producing bacteria which were isolated from palm oil-contaminated soils, as well as the low-cost substrates they utilise for bio-surfactant production. They isolated the bio-surfactant -producing bacterium Marinobacter hydrocarbonoclasticus ST1 from palm oil-contaminated soil, and the bio-surfactant produced was a glycolipid with an emulsification index of 81% and a yield of 1.8g/L. They used a wide range of carbon sources, including glucose, molasses, commercial sugar, rice bran oil, palm oil and soybean oil. The Bio-surfactant produced from molasses gave the highest emulsification index (40.40 ± 4.25%), followed by palm oil (34.21 ± 4.25%) and commercial sugar (20.35 ± 6.14%). Interestingly, commercial sugar, glucose and rice bran oil produced higher dry cell masses than palm oil, although palm oil resulted in the production of more bio-surfactants. This corroborates the notion that water-immiscible substrates are more effective for the production of bio -surfactants (Singh *et al.*, 2019)

Chooklin *et al.* (2013) also isolated Nevskia ramosa NA3 from palm oil-contaminated sites and used palm oil mill effluent as the substrate. The bio-surfactant produced has a surface tension reduction of 27.2 mN/m. Using the Plackett-Burman experimental design, they emphasised that using NaNO3 as a nitrogen source (25.25%) and FeCl2 as an important cofactor in the biosynthesis of many enzymes is important in the production of bio-surfactants. However, salts such as MgCl2 are only required in small quantities for a high yield of bio-surfactants. They also reported that adding glucose and/or commercial sugar in low concentration to the medium as an initial carbon source alongside POME resulted in a higher yield of bio-surfactant during production. Pansiripat *et al.* (2010) also reported similar findings with glucose and palm oil. In both studies, adding glucose and/or commercial sugar as co-substrates was done at the initial stage of the production to initiate cell growth and consequently, yield bio-surfactants. While the addition of either glucose or commercial sugar may increase the production cost, further studies may be required to determine the cost-effectiveness of the addition of a co -substrate. The report by Chooklin *et al.* (2013) gave an insight into the nutritional requirements for the production of bio-surfactants using palm oil mill effluent. It, however, accentuates the need for more studies on the use of palm oil wastes and other factors that contribute to their effectiveness.

Chiewpattanakul *et al.* (2010) isolated a yeast-like fungus, Exophiala dermatitidis SK80, from palm oil-contaminated soil. Using palm oil (5% v/v) as the carbon source, the fungus produced a bio-surfactant identified as monoolein with antiproliferative activity against cervical cancer (HeLa) and leukaemia (U937) cell lines (Chiewpattanakul *et al.*, 2010). Williams *et al.* (2021) also used Palm oil mill effluent as a substrate for the production of sporolipids by three yeast isolates from palm oil-contaminated soil samples. The isolates include Candida haemulonis, Pichia kudriavzevii, and Saccharomyces cerevisiae MW182017.

Table 2: Microorganisms used in the production of Bio-surfactants using Palm Oil waste/By-products

Microorganism	Source	Type of Bio-surfactant	Type of fermentation and fermentation conditions	Carbon Source	References
Pseudomonas alcaligenes	Crude Oil contaminated soil	Glycolipid	Submerged fermentation. 29 ºC, 150 rpm	Palm Oil	Oliveira *et al.* (2006)
Ochrobactrum anthropi 2/3	Mangrove Sediment	Rhamnolipid	Submerged fermentation. 30 ºC, 150 rpm	palm oil decanter cake	Noparat *et al.* (2014)
Streptomyces sp. PBD-410L	Mangrove Sediment	Lipopeptide	Batch and fed-batch fermentation in a 3-L stirred-tank reactor (STR), 200 rpm and an aeration rate of 0.5 vvm	Palm Oil	Zambry *et al.* (2021)
Recombinant *Escherichia coli*	-	Rhamnolipid		POME (20% v/v) Autoinduction (1% w/v)	Suhandono *et al.* (2021)
Bacillus subtilis	-	Surfactin	Submerged fermentation. 30 ºC, 150 rpm	POME (50% v/v)	Abas *et al.* (2013)
P. aeruginosa PAO1	-	Rhamnolipid	Submerged fermentation. 37 ºC, 150 rpm	palm fatty acid distillate	Radzuan *et al.* (2017)
Starmerella riodocensis GT-SL1R sp	Honeybee sample	Sporolipid	Submerged fermentation. 30 ºC, 150 rpm	Palm oil, glucose	Alfian *et al.* (2022)
Starmerella bombicola	Culture Collection				
S. bombicola	Culture Collection	Sporolipid	Submerged fermentation. 30 ºC, 180 rpm	Palm oil, glucose	Shah *et al.* (2017)
Pseudomonas aeruginosa SP4	Crude oil- Contaminated soil	Rhamnolipid	Submerged fermentation in a bioreactor.	3.5% v/v Palm oil supplemented with glucose at the ratio of 40:1	Pansiripat *et al.* (2010)

Exophiala dermatitidis SK80	Palm oil-contaminated soil	Monoolein	30 °C, 200 rpm	5% v/v Palm Oil	Chiewpattanakul *et al.* (2010)
Azorhizobium doebereinerae AS54 and *Geminicoccus roseus* AS73	Palm oil-contaminated soil		30 °C, 150 rpm	palm oil decanter cake (1% w/v) and used palm oil (1% v/v)	Saisa-Ard *et al.* (2014)
Marinobacter hydrocarbonoclasticus ST1	Palm oil-contaminated soil	Glycolipid	30 °C, 150 rpm	glucose, molasses, commercial sugar, rice bran oil, palm oil and soybean oil	Dikit *et al.* (2019)
Bacillus tequilensis, Bacillus subtilis	Palm oil mill effluent (POME)	-	-	POME	Marajan *et al.* (2015)
Bacillus thuringiensis c25	Palm oil-contaminated soil	Lipopeptide	30 °C, 150 rpm	Glucose and Starch	Ogbonna *et al.* (2023)
Nevskia ramosa NA3	Palm oil-contaminated soil	-	30 °C, 150 rpm	POME and glucose/commercial sugar	Chooklin *et al.* (2013)
Candida haemulonis Pichia kudriavzevii, Saccharomyces cerevisiae MW182017	Palm oil-contaminated soil	Sporolipid	30 °C, 150 rpm	POME and glucose/commercial sugar	Williams *et al.* (2021)
Providencia alcalifaciens SM03	Degraded palm kernel cake (PKC)	Glycolipid	37 °C, 180 rpm	PKC (2% w/v)	Jamal *et al.* (2014)

Key: v/v – Volume per volume, w/v – Weight per volume, vvm- volume of air per volume of liquid per minute, rpm - Revolutions per Minute

The Use of Palm Oil Industry Waste for Bio-surfactant Production

Several studies have described the use of palm oil and the waste products of palm oil refineries as substrates for the production of bio-surfactants by microorganisms isolated from different sources. (Table 2). For instance, Oliveira *et al.* (2006) reported that Pseudomonas alcaligenes isolated from crude oil-contaminated soil produced a bio-surfactant using palm oil (5% v/v). The surface tension of the glycolipid produced was 50 mN/m and the critical micelle dilution (CMD) was 39 ± 1 mN/m. Pansiripat *et al.* (2010) also isolated Pseudomonas aeruginosa SP4 from crude oil-contaminated soil and used Palm oil supplemented with glucose at a ratio of 40:1 for bioproduction of bio-surfactant. The production was done in a sequencing batch reactor. They reported that the bio-surfactant, a rhamnolipid, had a CMD of 28–30 mN/m. They also observed that the use of palm oil as a source of carbon increased the chemical oxygen demand of the medium, but that COD removal from the medium was highest at a 40:1 oil-to-glucose ratio. Pansiripat *et al.* (2010) maintained that the addition of glucose to the medium enhanced the performance of the reactor and initiated the proliferation of the microorganisms. However, glucose is required in a minimal quantity to prevent an increase in the chemical oxygen demand within the system.

Zambry *et al.* (2021) investigated the influence of different bioprocessing parameters on the production of lipopeptides by Streptomyces sp. PBD-410L using palm oil as the substrate in a stirred tank reactor (STR). Alfian *et al.* (2022) also used palm oil (100 g/L) and glucose (100 g/L) as both hydrophobic and hydrophilic carbon sources, respectively, for the production of sporolipid by the yeast Starmerella riodocensis GT-SL1R sp and Starmerella bombicola. They used the same production medium, having the same composition and quantity, as reported by Shah *et al.* (2017). While Shah *et al.* (2017) reported the highest bio-surfactant yield of 32 g/L by S. bombicola, Alfian *et al.* (2022) reported that the same organism gave the highest yield of 42.81 g/L. They also reported the highest yield of 45.70 g/L on Day 3 of cultivation by S. riodocensis. Shah *et al.* (2017) investigated the effect of different hydrophilic carbon sources, including Tapis oil, Melita oil, and Ratawi oil. They reported that compared to these three oils, palm oil had the highest yield of sporolipid, the highest glucose utilisation rate, and the lowest biomass production (11.4 g/L). The authors inferred that this inverse proportionality between biomass production and bio-surfactant yield could be because palm oil diverted more carbon sources to the bio-surfactant biosynthesis pathways rather than biomass production (Shah *et al.*, 2017). Both Alfian *et al.* (2022) and Shah *et al.* (2017) reported similar emulsification indices for the sporolipid produced by S. bombicola (60.2% and 69.0%, respectively).

Abas *et al.* (2013) demonstrated the production of surfactin by Bacillus subtilis using palm oil mill effluent as a nutrient source. An interesting finding of the study showed that when used at 50% v/v of the production medium, palm oil mill effluent as a carbon source produced the highest bio-surfactant yield compared to lower concentrations. In the study by Suhandono *et al.* (2021), the authors engineered Escherichia coli for the production of rhamnolipid and optimised the production process using autoinduction medium and palm oil mill effluent as carbon sources. They inserted two plasmids, pPM RHLAB and pPM RHLABC plasmids for the production of mono-rhamnolipid and di-rhamnolipid production respectively, into E. coli to give two separate recombinant strains, E. coli pPM RHLAB and E. coli pPM RHLABC. They reported that using autoinduction as a carbon source, the maximum yield of rhamnolipid was 1245.68 mg/L and 318.42 mg/L using 20% (v/v) of POME. Suhandono *et al.* (2021) used different concentrations of POME, including 10%, 15%, 20% and 25% v/v and reported that the highest yield was obtained at 20% v/v. These results contradict those by Abas *et al.* (2013), who reported the highest yield at 50% v/v of POME. Nonetheless, findings by Suhandono *et al.* (2021) suggest that the concentration of POME might be required in high degrees to facilitate increased yields.

Noparat *et al.* (2014) explored the viability of using palm oil decanter cake as a carbon source for the production of bio-surfactant by the bacterium Ochrobactrum anthropi 2/3, which was isolated from mangrove sediment. The surface tension of the bio-surfactant, rhamnolipid, was 25.0 mN/m and its critical micelle concentration was 8.0 mg/L. Radzuan *et al.* (2017) used palm fatty acid distillate (PFAD), a waste product from a palm oil refinery, as the carbon source in the production of rhamnolipids by the bacterium P. aeruginosa PAO1. The process resulted in the yield of 0.43 g/ L of rhamnolipid, which had a surface tension of 29 mN/m and a critical micelle concentration of 420 mg/ L. Although the study demonstrated the suitability of PFAD as a carbon source for rhamnolipid production, the yield reported was low, suggesting the need for further research to improve bio-surfactant yield from these substrates.

Environmental Benefits of Using Palm Oil Bio-surfactants

Owing to the multiple applicability of bio-surfactants, their low toxicity and environmental friendliness, there is a growing demand for bio-surfactants (Akbari *et al.*, 2019). Using cheap palm oil milling and refinery waste/by-products will no doubt reduces the cost of production and reduce the environmental burden of disposing of these agro wastes (Kee *et al.*, 2021). The uncontrolled disposal of wastes from the palm oil industry can result in environmental pollution and increased emissions of carbon dioxide (Ogunsina and Akintan, 2020). This is because when these palm oil industry wastes are inappropriately released into the environment, their decomposition results in significant greenhouse gas emissions, thereby contributing to climate change and an increased carbon economy (Gautam *et al.*, 2023). However, using waste products for bio-surfactant production aligns with the practice of circular and bio-economy, which targets resource efficiency, waste reduction and cost-effectiveness of production processes (Cheah *et al.*, 2023). From an economic standpoint, the use of these agro wastes for bio-surfactant production is lucrative and cost-effective because it ensures environmental sustainability and reduces carbon footprint (Mgbechidinma *et al.*, 2022). It is a double-edged sword that converts the waste to high-value commodities and tackles the problem of waste management.

Furthermore, there is an increase in the global demand for bio-surfactants. According to Fortune Business Insights (2022), the global demand for bio-surfactants is expected to rise from \$4.18 billion to \$6.04 billion between 2022 and 2029. The rise in demand for bio-surfactants is largely due to environmental concerns associated with the use of chemical surfactants, fluctuating oil prices and the introduction of more stringent environmental regulations (Gautam *et al*., 2023). Bio-surfactants are suitable alternatives to the toxic surfactants. They have several environmental applications that offer many environmental benefits. The use of bio-surfactants for the bioremediation of hydrocarbon-contaminated sites is well documented (Patel *et al*., 2019). They have also been used for heavy metal removal (Luna *et al*., 2016), antifouling activities (Alemán-Vega *et al*., 2020), biofilm inhibition (Sriram *et al*., 2011) and microbial oil recovery (Alvarez *et al*., 2020).

Challenges and Future Perspectives of Bio-surfactants in Industry

The waste products of palm oil mills and refineries are valuable for the production of bio-surfactants and other bioproducts (Kee *et al*., 2022). However, since these waste products can be reused or recycled, they are more appropriately termed by-products if they are directed for further processing (Amasuomo and Baird, 2016). Having the mindset that the wastes from the palm oil industry are by-products is very important if these by-products are to be used for bio-surfactant production. Currently, the improper disposal of palm oil by-products into the environment is a common practice in Nigeria, although palm oil farmers complain of the problem of waste disposal (Ogunsina and Akintan, 2020). Selling palm oil wastes can be a source of additional money for the farmers and palm oil factory owners, however, they do not recognise the value in the by-products (Achoja *et al*., 2019). This suggests that there is a gap between research and practice, such that findings from research on the suitability of these by-products for bio-surfactant production have not yet been adopted in practice. Hence, there is a need for increased environmental awareness of the dangers of uncontrolled waste disposal and the economic value of the by-products of the palm oil industry. This would require collaborations between academia, government as well and the industry.

Furthermore, there is a need for further research to increase the yield of bio-surfactants when using palm oil and its by-products because of the low yield (Zanotto *et al*., 2019; Kee *et al*., 2022). For instance, Suhandono *et al*. (2021) reported a maximum yield of 12.5g/L using 20% (v/v) of POME in the production medium. Alfian *et al*. (2022) also reported a maximum yield of 42.81 g/L with palm oil (100 g/L) as substrate. This suggests that a large amount of palm oil-based substrate is required to produce a smaller proportion of bio-surfactants. The downstream processing of bio-surfactants is expensive, and using such a process for low-yielding bio-surfactant production might not be cost-effective, thereby rendering the use of low-cost by-products inefficient. There is, therefore, a need for further research to increase the efficiency of the bio-production process using low-cost by-products from the palm oil industry, thereby increasing the yield of bio-surfactants from palm oil-based substrates. Additionally, although Nigeria is the world's fifth-largest producer of palm oil, the number of Nigerian studies on palm oil-derived bio-surfactants is minimal compared to those from other larger oil producers. It is, therefore, no surprise that the uncontrolled disposal of palm oil by-products persists in the country. Nigerian researchers must therefore consider exploring this uncharted world of palm oil bioconversions and the circular economy.

CONCLUSION

This review critically evaluates the application of palm oil and its associated industrial by-products as alternative substrates for microbial bio-surfactant synthesis. The palm oil sector produces considerable volumes of residual materials, such as palm fatty acid distillate (PFAD), palm oil mill effluent (POME), palm kernel shells, fibres, and empty fruit bunches. These by-products are characterised by their richness in diverse nutrients, positioning them as promising feedstocks for bio-surfactant production. In particular, lipid-rich residues have been extensively explored for this purpose. The valorisation of palm oil derivatives as fermentation substrates offers a strategic advantage by significantly lowering bio-surfactant production costs, thereby addressing a major economic barrier in the industry. Additionally, the redirection of these waste streams towards value-added bioproduct synthesis provides a sustainable solution to the pressing environmental challenges associated with their disposal, while concurrently presenting an opportunity to enhance the profitability of palm oil producers. Despite these advantages, the bio-surfactant yields obtained from palm oil-based substrates are often suboptimal, which may compromise the economic feasibility of downstream processing due to elevated recovery costs. Consequently, further research is imperative to optimize both bio-surfactant yield and the efficiency of downstream purification processes.

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