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*Review*

# Glycolipid Biosurfactant Production from Waste Cooking Oils by Yeast: Review of Substrates, Producers and Products

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**Abstract:** Biosurfactants are a microbially synthesized alternative to synthetic surfactants, one of the most important bulk chemicals. Some yeast species are proven to be exceptional biosurfactant producers, while others are emerging producers. A set of factors affects the type, amount, and properties of the biosurfactant produced, as well as the environmental impact and costs of biosurfactant's production. Exploring waste cooking oil as a substrate for biosurfactants' production serves as an effective cost-cutting strategy, yet it has some limitations. This review explores the existing knowledge on utilizing waste cooking oil as a feedstock to produce glycolipid biosurfactants by yeast. The review focuses specifically on the differences created by using raw cooking oil or waste cooking oil as the substrate on the ability of various yeast species to synthesize sophorolipids, rhamnolipids, mannosylerythritol lipids, and other glycolipids and the substrate's impact on the composition, properties, and limitations in the application of biosurfactants.

## 1. Introduction

An increasing need for materials, energy, and water forces the shift from a fossil-based linear economy to a sustainable circular bioeconomy. With the bioeconomy concept high on the global agenda, search for biological feedstock that has the potential to generate a spectrum of bio-based products has been initiated in many areas of research and industry. In this context, biogenic waste is considered as a potential feedstock for developing a circular bioeconomy.

Surfactants are among the most important bulk chemicals that are used in almost every product and activity of human daily life, such as cleaning products, cosmetics, food, textiles, pharmaceuticals, mining, agriculture, paper production, etc. Biosurfactants (BSs) are an alternative to synthetic surfactants synthesized from fossil resources [1]. BSs are structurally diverse amphiphilic molecules produced by a variety of microorganisms—fungi (e.g., yeasts) and bacteria, especially actinomycetes [2]. Microorganisms synthesize BSs as secondary metabolites that can either remain attached to the cell surface or be secreted outside the cell [3]. Some yeast species, such as *Starmerella bombicola* and *Pseudozyma antarctica*, are proven to be exceptional BS producers [4]. In many aspects, yeasts are superior to bacterial producers. Yeasts do not produce toxic byproducts; many yeast species are used for making traditional food and beverages. Pathogenic strains are seldom (if any) among the yeast species used for biotechnological processes [5]. Yeasts can produce BS from lipid-rich substrates: vegetable oils, fats, and their products.

BSs have several advantages over their synthetic equivalents: (1) BSs can be produced from renewable feedstocks by fermentation; (2) BSs have greater environmental compatibility, as they are readily biodegradable and display low toxicity; and (3) BSs show better foaming properties and stable activity at a wide range of conditions (pH, salinity,

and temperature) [6]. Although BSs have many advantages over their synthetic analog, BSs are not yet competitive with synthetic surfactants due to very high production costs resulting from significantly low BS yields in the fermentative processes [7]. Still, in 2025, the global BSs' market is expected to be around 4.8 billion euro at a compound annual growth rate of 5.5% in the forecast period 2020–2025 [8]. Glycolipids, a type of BS, compose the greatest market share of BSs [9]. Glycolipid BSs are low-molecular-weight amphiphiles that consist of a hydrophilic polysaccharide headgroup and one or many hydrophobic fatty acid tails [3]. Glycolipid BSs have potential applications in the pharmaceutical, agricultural, and environmental sectors [9].

A set of factors affects the type, amount, and properties of the BSs produced: the used substrate (water-soluble/insoluble carbon source, nitrogen source, carbon-to-nitrogen ratio, carbon-to-phosphorus ratio, and metal ion concentration) and the fermentation conditions (pH, temperature, aeration, agitation, oxygen availability, and incubation time), as well as the selected microorganism, downstream processing of the BS, and application of genetic engineering of the producing organism [2,3]. The same factors also affect the cost and environmental impact of the BS production. If BSs are produced from raw cooking oils, then the substrate contributes up to 89% of the total production cost [10] and 47% of the total life cycle environmental impact [11]. For bioproducts to gain their market share, the quality and price must be comparable to their synthetic analogs [12–14]. BSs can replace synthetic surfactants only if the cost of the raw material and the process is reduced to the minimum [15]. Exploring alternative substrates for BS production can serve as an effective cost-cutting strategy. The use of low-cost substrate or cheap waste material may decrease the total BS production costs by 10% to 30% [16]. Waste cooking oil presents one such alternative material, as it is two-to-three times cheaper than raw cooking oils [17]. BS production based on industrial byproducts and waste effluents presents a huge potential for its application at the industrial scale in a more economic and environmentally friendly process, boosting the necessary transition towards a circular economy [1]. Meanwhile, the changes oils and fats undergo during frying or other thermal treatments may adversely impact the amount and properties of the BS produced or change the overall microbial metabolisms of the oils and fats when used as a carbon source in fermentation [18].

This paper aims to review the state-of-art knowledge of utilizing waste cooking oil as a feedstock to produce glycolipid biosurfactants by yeast. This article focuses on an extensive literature review on synthesizing, physicochemical characterization, and advanced applications of sophorolipids, rhamnolipids, mannosylerythritol lipids, and other glycolipids produced from waste cooking oils by yeasts. An outline of the recent progress (studies published up to May 2021) in WCO-derived BS research is presented in the corresponding sections covering the properties and pretreatment of waste cooking oils (Section 2) and yeasts as BS producers (Section 3), as well as the properties (Section 4) and applications (Section 5) of WCO-derived glycolipid BSs.

## 2. Waste Cooking Oils as a Substrate

Waste lipids such as fats, oils, and grease from catering service providers and households have become a major stream of biogenic waste in urban areas [19]. Waste cooking oil (WCO) from deep fat fryers is the most common form of waste lipids. Yet, waste lipid sources differ across the globe, from plant-based lipids (e.g., sunflower oil, rapeseed oil, olive oil, corn oil, margarine, coconut oil, palm oil, soybean oil, grape seed oil, canola oil, etc.) to animal-based lipids (e.g., animal fat, butter, cheese, ghee, fish oil, etc.) [20]. The estimated per capita consumption of products generating waste lipids ranges from less than 20 kg per annum in less-developed countries to over 50 kg per annum in developed countries [21]. The amount of waste lipids generated worldwide by the industry and households is increasing rapidly due to the growth in the human population and the incremental food consumption patterns. The worldwide generation of WCO is about 29 million tons [22]. WCO is one of the most-generated waste lipid streams in the European Union (EU), amounting to an estimated 4–10 million tons per annum [20,22]. Taking the

total EU population as around 500 million, the annual generated WCO amount is around 8 L per capita [23].

Meanwhile, the disposal and utilization of waste fats, oils, and grease is a significant challenge by itself. The European Waste Catalogue classifies waste lipids as municipal wastes under the codes 20 01 25 (edible oils and fats) and 19 08 09 (grease and oil mixture from oil/water separation containing edible oil and fats). Disposal of this waste to landfills is not permitted in many jurisdictions [19]. In addition, since 2002, the EU has banned the use of WCO in fodder production [24].

Currently, the collection and utilization of waste lipids are not at their full potential. Of the estimated potential collectable WCO amount in the EU, only 45% is collected from restaurants and only 16% from private households [25]. Following the annual increase of cooking oil usage, the amount of collectable WCO increases around 2% per year [23]. It has been determined that, in the EU, 60% of WCO is improperly disposed of, ending up in sewage systems. It is an illegal practice resulting in increased wastewater treatment costs and energy consumption, as well as greenhouse gas (GHG) emissions associated with waste fats, oils, and grease biodegradation [25]. Additionally, WCO disposal in municipal solid waste landfills is not an option, as it is associated with a loss of valuable resources and increase in GHG emissions. Meanwhile, the recycling rate of waste lipids to produce bioproducts is low. Currently, due to waste lipids' high energy content, it is mainly utilized for bioenergy, i.e., used as a secondary raw material in biodiesel production or as an additive to substrates in biogas generation [19,21]. Due to its high energy capacity, low cost, and wide availability, the use of WCO as a feedstock for microbial production is a promising alternative for the production of various value-added products. Finding sustainable WCO utilization alternatives that can be implemented at a full industrial scale would enhance their separate collection, especially if ambitious political goals are set with respect to the production and consumption of bio-based products.

The amount of WCO can be efficiently used as feedstock in waste biorefinery [26]. The application of various biotechnology processes (such as fermentation, transesterification, methanation, etc.) can result in value-added products, and biodiesel and biogas production has been implemented on an industrial scale. Yet, there are other, more valuable products that can potentially be produced, hence enabling a more carbon-efficient and sustainable circular bioeconomy. One such product is BSs for production, of which WCO utilization would also serve as a low-cost alternative to the raw or refined cooking oils that are currently used to produce BSs.

### 2.1. Composition of Waste Cooking Oils

In the literature, most studies on BS production by yeast have been conducted by using raw cooking oil as a substrate, i.e., oil that has not been exposed to high heat in the frying process (referred also as virgin vegetable oil or refined cooking oil). There are relatively few studies where WCO has been used and even less with waste fats and grease. The major physical and chemical changes oil undergoes in the cooking process alter its physicochemical properties and composition. Hence, the results of BS production obtained by using raw cooking oil may not be directly attributed to the BS production from WCO.

The composition of WCO is influenced by various factors like the origin of the oil; the number of frying cycles, frying time, and conditions; used equipment; and processed food [27]. Oil frying occurs for a prolonged period in the presence of air and light at high temperatures between 160 °C and 200 °C [28]. During the frying process, oils undergo chemical degradation reactions, i.e., oxidation, peroxidation, hydrolysis, and polymerization [28–30], leading to a change in the unsaturated and saturated fatty acid compositions [31] and the formation of various chemical compounds, like peroxides; hydroperoxides; hydrocarbons (i.e., alkanes, alkenes, and terpenes); aldehydes; and ketones, many of which are volatile. Degradation byproducts might inhibit microbial growth, the metabolism of microorganisms, and metabolite production [18,28,29]. Depending on the frying time, the formation of certain chemical compounds can increase or decrease. More-

over, during the frying process, oils can get contaminated with metal traces from the frying equipment, as well as spices, e.g., salt, water, and other organic molecules, from the fried food [28]. The sensory properties of oil, like color, appearance, off-taste, and odor, mainly due to the presence of volatile organic compounds [30], also change during the frying process [32,33]. Several studies have reported the composition and properties of WCO (see Table 1).

The physical and chemical properties of oils are mainly determined by the composition of unsaturated fatty acids [34]. The percentage of unsaturated fatty acids varies largely among different types of cooking oils and their respective WCOs. Table 1 shows that the major components of oil are long-chain fatty acids (>C12), mainly palmitic, oleic, and linoleic acids. The unsaturated fatty acids—oleic, linoleic, and linolenic acids—predominate in rapeseed, sunflower, soybean, olive and linseed oil, while palm, cottonseed, and rice bran oil are largely composed of palmitic acid (saturated fatty acid).

Thermal degradation leads to the vaporization of steam (water present in oil) and the formation of primary volatile compounds. Hydrolysis in the presence of water steam contributes to breaking ester bonds and the formation of free fatty acids, mono- and diglycerides, and glycerol. An increase in free fatty acids results in a lower pH value and higher acidity of WCO [35]. Oxidation leads to the formation of hydroperoxides (products of primary oxidation) and, thus, increase in the peroxide value of WCO. However, due to prolonged frying, other reactions occur: new compounds (e.g., dienes, trienes, and alcohols) are formed, and hydroperoxides break down rapidly [36,37]. As a result, the peroxide value can decrease over time. The Standard for Edible Fats and Oils [38] shows peroxide values of virgin (raw) oils and cold-pressed fats and oils ($<15$ mEq$\cdot$kg$^{-1}$) and other fats and oils ($<10$ mEq$\cdot$kg$^{-1}$). As shown in Table 1, the peroxide value of WCO is higher than that of raw cooking oils. However, the peroxide value is not above the range defined in the Standard for Edible Fats and Oils in all cases. This could be related to the oil processing (cooking or frying) conditions and, therefore, shows the heterogeneous nature of WCO. The absence of oxygen results in the further polymerization of oil, the formation of new carbon bonds, and other degradation products [39]. The acid value, total polar compounds, and changes in density and color are associated with the advanced degradation of WCO [37]. Meanwhile, the iodine value indicating the level of unsaturation and the number of double bonds decreases [17]. Therefore, highly saturated WCOs are characterized by a low iodine value [37]. In contrast, higher iodine values indicate a greater degree of unsaturation per triglyceride and higher susceptibility to functionalization that can be achieved by chemical modifications [34]. Moreover, the higher the percentage of unsaturation, the better the oil is in producing environmentally acceptable products [34].

**Table 1.** Composition and properties of the WCO and raw cooking oil.

| Oil | Fatty Acid Composition, wt% | | | | | | | Property | | Reference |
| | Myristic C14:0 | Palmitic C16:0 | Stearic C18:0 | Oleic C18:1 | Linoleic C18:2 | Linolenic C18:3 | Other | Acid Value, mg KOH·g$^{-1}$ of Oil | Peroxide Value, mEq·kg$^{-1}$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| WCO, n/s [a] | - | 6.70 | 1.60 | 19.30 | 72.40 | - | - | 4.1 | <5 | [22] |
| WCO, n/s | - | 8.50 | 3.10 | 21.20 | 55.20 | 5.90 | 4.20 | 3.6 | 23.1 | [40] |
| WCO, palm | 0.98 | 39.02 | 4.52 | 44.57 | 10.91 | - | - | 2 | 7.1 | [41] |
| WCO, palm olein | 1.93 | 45.68 | 4.25 | 40.19 | 7.95 | - | - | 36.81 | - | [42] |
| WCO, n/s | 0.14 | 11.73 | 2.10 | 27.75 | 53.29 | 0.83 | 2.44 | - | - | [43] |
| WCO, n/s | - | 18.16 | 8.12 | 40.72 | 25.81 | 7.19 | - | - | - | [44] |
| WCO, sunflower | - | 19.12 | 9.43 | 34.33 | 32.22 | - | 4.9 | 8 | 102 | [29] |
| WCO, rapeseed [b] | 0.14 | 7.17 | 2.83 | 63.62 | 17.39 | 5.81 | - | | | [45] |
| Sunflower | - | 8.92 | 3.36 | 26.27 | 60.24 | 0.38 | - | 0.29 | 0.36 | [46] |
| Sunflower | - | 7 | 5 | 20–25 | 63–68 | 0.2 | - | - | - | [34] |
| Palm | 1.00 | 37–41 | 3–6 | 40–45 | 8–10 | - | - | - | - | [34] |
| Rapeseed | 0.07 | 4.82 | 1.94 | 61.56 | 20.46 | 8.49 | - | 0.08 | 3.62 | [45] |
| Rapeseed | - | 4–5 | 1–2 | 56–64 | 20–26 | 8–10 | 9.1 | - | - | [34] |

n/s—WCO content not specified; [a] after pretreatment (filtration and washing); [b] rapeseed oil sample after 8 h of pan-frying.

### 2.2. Pretreatment of Waste Cooking Oils

Even though degradation products compose a minor part of the WCO, their presence might affect the direct valorization of WCO into personal care products or pharmaceutics [32,47]. Several authors have concluded that the yield of BS is higher when raw cooking oil is used as a substrate instead of WCO [18]. The literature suggests pretreatment methods for WCO before using it in the production of biodiesel, bio-lubricants [48], etc. However, little information exists on the pretreatment (as well as the necessity of a pretreatment) of WCOs used as a substrate in the production of BSs. Activated earth mainly consists of inorganic compounds, i.e., diatomaceous earth, clays, silicates, activated carbons, bentonite, etc. [48]. It is an adsorbent with a large specific surface area [33] and can remove oxidation products and pigments from WCO [49]. Wadekar et al. (2012) pretreated sunflower WCO with activated earth before the production of rhamnolipids by *P. aeruginosa* [29]. The pretreatment lowered the WCO's peroxide value while not changing the viscosity or fatty acid composition of the WCO [29,30] and concluded that filtration with activated earth has almost no effect on the fatty acid composition. Still, the pretreatment of WCO increased the titer of rhamnolipids from 2.8 $g \cdot L^{-1}$ without a pretreatment to 7.5 $g \cdot L^{-1}$ with pretreatment [29]. In this case, the pretreatment increased the final surfactant titers by 10–20% compared to untreated WCO. Additionally, the authors noted that acidic (not lactonic) sophorolipids were produced from WCO independently of the pretreatment. The authors concluded that some degradation products have not been filtered out from the WCO and might have inhibited the formation of acetylated lactonic sophorolipids [46].

Maddikeri et al. (2015) used the pretreatment of WCO with activated earth before the production of sophorolipids, aiming at the removal of suspended solids from WCO [22]. Salt impurities were separated using the washing method with water [22]. In the study, the treatment with activated earth led to the reduction of peroxide value as well [22]. Rincón et al. (2021) studied the activated earth treatment using three different adsorbents under different purification conditions [47]. In the study, removal of the acid value, total polar compounds, and color was analyzed by using two clay adsorbents and one synthetic amorphous silica adsorbent. Even though the use of clay adsorbents contributed to the reduction of the acid value and removal of the total polar compounds, this was reached at high adsorbent loadings of 15 wt% and 10 wt%, respectively. The authors pointed out that purification with adsorbents is not economically feasible and that complete adsorbent regeneration is not possible [47]. Therefore, when purifying WCO with activated earth, it is very important to find the optimal loading, as higher amounts of activated earth contribute to higher losses and the generation of unwanted waste during the WCO pretreatment stage.

Other studies, not directly focusing on the production of BSs, have described the existing pretreatment methods (including the treatment with activated earth) of WCOs. An extensive review of physical pretreatment methods: separation based on solubility, separation through filtration (or clarification) with specific materials, and separation based on the boiling point (distillation) was provided by Mannu et al. (2020) [48].

## 3. Nonconventional Yeasts as Biosurfactant Producers

Yeasts are a heterogeneous group of single-celled fungi. Many industrially important yeasts were "tamed" thousands of years ago during the dawn of modern agriculture and the dairy industry. Thus, due to the long history of applications and no record of pathogenic strains or conditions, many yeasts are assumed to be generally recognized as safe (GRAS). The GRAS status allows for wide use of an organism or its metabolites in food and/or feed, facilitating valorization of the organism's biomass as a valuable byproduct and therefore lowering its environmental impact. Bakers' yeast *Saccharomyces cerevisiae* is a typical GRAS yeast acknowledged by the US Food and Drug Administration [50]. Meanwhile, there are several yeasts that have been coined as "emerging GRAS producers", including *S. bombicola* with a long history of applications in food technology and no known threats to human health or environment [51]. Nowadays it is assumed that *S. bombicola*, one of the best-known BS-producing yeasts, has all the properties to be considered as GRAS [52].

Meanwhile, many bacterial BS producers (*P. aeruginosa*, *Escherichia coli*, etc.) bear a potential threat to the environment and human health, since pathogenic strains of these organisms exist [53,54].

The most recognized type of BS is glycolipids. These are ubiquitous molecules synthesized in the cells of animals, plants, fungi, and bacteria. These molecules facilitate nutrient assimilation, promote motility, and often participate in microbial biofilm formation [55]. Depending on the structural carbohydrate, glycolipids are classified into subgroups: rhamnolipids, sophorolipids, mannosylerythritol lipids, xylolipids, and cellobiose lipids [9]. Depending on the producer organism, BSs are categorized into bacterial, yeast, or fungal origin [55]. Further, the focus is on the glycolipids synthesized by yeasts and their downstream processing.

### 3.1. Sophorolipids

Sophorolipids consist of a sophorose (β-1,2-linked glucose dimer) sugar moiety and a long-chain hydroxy fatty acid linked by a glycosidic bond [3,56]. The hydrophilic head may be acetylated with one or two acetyl groups, and the tail usually consists of 16 or 18 carbon atoms. Naturally, sophorolipids are produced as a mixture of lactonic (closed-ring) or acidic (open) forms that determines their physicochemical properties (see Figure 1) [57].
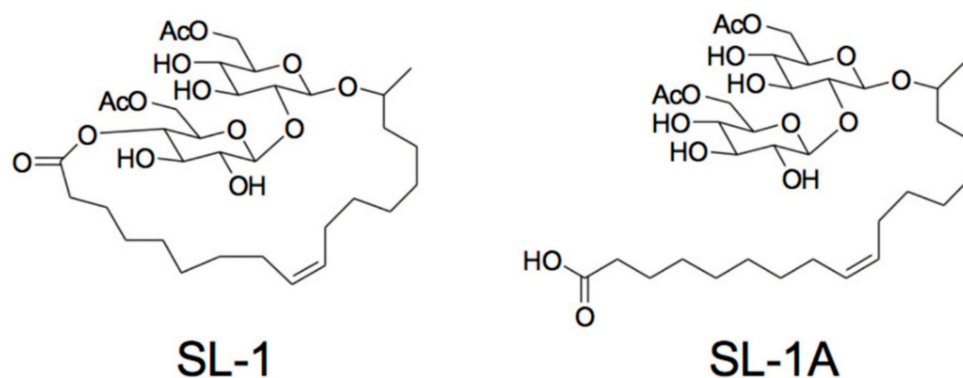


**Figure 1.** Structures of the sophorolipids predominantly produced by *S. bombicola*. The 17-L-[(2′-O-β-glucopyranosyl-β-D-glucopyranosyl)-oxy]-9-octadecenoic acid 1′,4″-lactone 6′,6″-diacetate (SL-1) and its free acid form (SL-1A) (Adapted from [58]).

Sophorolipids are produced by several nonpathogenic yeasts. The first study of sophorolipids produced by the yeast *Torulopsis magnoliae* (now *Candida apicola)* was reported in the early sixties. Shortly after, *Candida (Starmerella) bombicola* was isolated from the honey of a bumblebee [59], and its strain ATCC22214 is nowadays favored due to its high productivity rate [60]. The gene cluster responsible for sophorolipid production in *S. bombicola* has been identified [61]. The synthesis of the free, acidic form of sophorolipids from glucose and fatty acids is performed by four enzymes (cytochrome P450 monooxygenase, UDP-glucosyltransferase, UDP-glucosyltransferase, and acetyltransferase) and a specific sophorolipid transporter [61,62]. During exoproteomics studies, the extracellular enzyme lactone esterase was discovered to be responsible for catalyzing the formation of sophorolipid lactone [63].

Sophorolipid synthesis is affected by the culture conditions, operational modes [64], substrate type [65], and media content (with or without specific supplements). Typically, the sophorolipid production media contains hydrophobic and hydrophilic carbon sources. Considering the natural habitat of *Candida* spp., fermentation can be carried out in a media with a high glucose content—10% $w/v$ or more [60,66]. When a hydrophobic carbon source is added (vegetable oils, alkanes, or esters), *S. bombicola* starts to produce SLs in large excess.

Glucose provided in the medium is primarily directed towards glycolysis, and the sophorose molecule is synthesized de novo from trioses via gluconeogenesis [67]. Since the hydrophilic carbon source does not directly affect the formation of sophorose, glu-

cose can be substituted by less-expensive substrates, such as molasses and carbohydrate hydrolysates [12,68].

The physiological role of BSs in yeast is widely discussed, and several authors have declared different hypotheses. Considering that the production of sophorolipids in large quantities occurs during the stationary phase at a high C:N ratio (nitrogen as the limiting factor), Van Bogaert et al. (2011) indicated the similarities between intracellular and extracellular metabolite biosynthesis, i.e., the synthesis of intracellular storage lipids in oleaginous yeasts is also triggered by nitrogen starvation [66]. Thereafter, they assume that similar mechanisms activate the synthesis of sophorolipids. Hence, apart from an enhanced substrate uptake and improved motility [9], sophorolipids serve as an extracellular carbon reserve in a form that is "less available" to other microorganisms, in addition to displaying antimicrobial activity—factors, which, in combination, improve yeasts' survival chances in harsh environmental conditions. Hommel et al. (1994) proposed that sophorolipid synthesis in yeasts could be a response to high osmotic pressure in their natural environment [67]. However, it seems that sophorolipid biosynthesis is a result of an evolutionary adaptation to niche environments, and these various molecules possess more than a single function throughout the life cycle of yeast.

Since the molecular structure is linked to the functional properties of the glycolipids, microbial synthesis of sophorolipids with specific structure (lactonic or acidic with a short or long fatty acid chain) is desired at the industrial scale. This, however, has not been achieved yet. Partly, it is due to the fact that enzymes of the sophorolipid synthesis pathway have a higher affinity to C16–C18 fatty acids rather than C12 or C10 [69,70]. Additionally, the fermentation of raw cooking oils results in a viscous, oily end product containing a mixture of sophorolipids of various structures. Cavalero and Cooper (2003) succeeded in obtaining almost all the diacetylated lactones in the form of crystals when yeast was grown on certain alkanes (hexadecane or heptadecane) [65]. Additionally, growth on shorter-chain fatty acids is limited, imposing a toxic effect on yeast, possibly interfering with lipids in the cell membrane [69].

Sophorolipid synthesis is affected both by the chemical composition of the substrate and fermentation conditions. Table 2 summarizes the sophorolipid titers, yields, and volumetric productivity rates from different yeast fermentation studies of WCO and raw cooking oil. To avoid the effects elicited by the genetic background of the strain and demonstrate the impact of the cultivation mode and carbon and nitrogen source on the productivity parameters only, the fermentation results only from *S. bombicola* strain ATCC22214 were included.

Frying imposes complex effects on an oil's chemical composition; hence, it is difficult to compare the results of different studies. WCO fermentation generally results in lower production yields when compared to yields obtained from fermentation with raw cooking oils. Maddikeri et al. (2015) showed that sophorolipid titers from fermentations using WCOs are 56–77% of the titers from fermentations with raw cooking oils [22]. Similarly, Kim et al. (2021) reported sophorolipid titers up to 86% when compared to titers obtained from fermentation with raw cooking oil [18]. It seems that the cultivation mode is critical to reach higher sophorolipid titers. The fed-batch cultivation mode (up to 300 g·L$^{-1}$) clearly provides higher titers than the batch mode (20–80 g·L$^{-1}$), as shown in Table 2.

**Table 2.** Production of sophorolipids from WCO and raw vegetable oil by yeast *Starmerella bombicola* ATCC22214.

| Substrate | | Hydrophobic and Hydrophilic Substrate Ratio | Nitrogen Source | Titer, $g \cdot L^{-1}$ | Yield, $g \cdot g^{-1}$ | Volumetric Productivity, $g \cdot L^{-1} \cdot h^{-1}$ | Cultivation Method | Reference |
|---|---|---|---|---|---|---|---|---|
| Hydrophobic | Hydrophilic | | | | | | | |
| Restaurant oil waste | - | - | Yeast extract, urea | 34.0 | 0.23 | 0.14 | Batch in flask | [43] |
| WCO | Glucose | 0.27 | Yeast extract, urea | 50.0 | 0.24 | 0.25 | Fed-batch in a bioreactor | [71] |
| WCO | Glucose | 1.00 | Yeast extract, urea | 55.6 | 0.40 | 0.23 | Fed-batch with ultrasound in a fermenter | [22] |
| Raw sunflower oil | Glucose | 1.00 | Yeast extract, urea | 12.31 | 0.06 | 0.06 | Batch in flask | [46] |
| Waste frying (sunflower) oil | Glucose | 1.00 | Yeast extract, urea | 4.26 | 0.02 | 0.02 | Batch in flask | [46] |
| Yellow grease (soybean oil) | Sorghum bagasse hydrolysate | 0.18 [a] | - | 35.9 | 0.56 | 0.11 | Batch in flask | [72] |
| Yellow grease (soybean oil) | Corn stover hydrolysate | 2.13 [a] | - | 52.1 | 0.34 | 0.31 | Batch in a fermenter | [72] |
| WCO | Glucose | 1.00 | Yeast extract, peptone | 84.8 | 0.42 | 0.59 | Batch in flask | [18] |
| WCO | Glucose | 1.00 | Yeast extract, peptone | 315.6 | - | - | Fed-batch in a fermenter | [18] |
| Raw cooking oil | Glucose | 1.00 | Yeast extract, peptone | 365.8 | - | - | Fed-batch in a fermenter | [18] |

[a] Total monomeric sugars considered.

## 3.2. Rhamnolipids

Rhamnolipids are composed of one or two rhamnose sugars and two hydroxy fatty acids, with a typical chain length of 8 up to 22 carbons (see Figure 2). The OH- group of one of the fatty acids forms a glycosidic bond with the reducing end of rhamnose disaccharides, whereas the hydroxyl group of the second fatty acid is involved in ester formation [3]. Until now, *Pseudomonas* spp. (including *P. aeruginosa*, an opportunistic pathogen) are the main industrial producers of rhamnolipids [73].
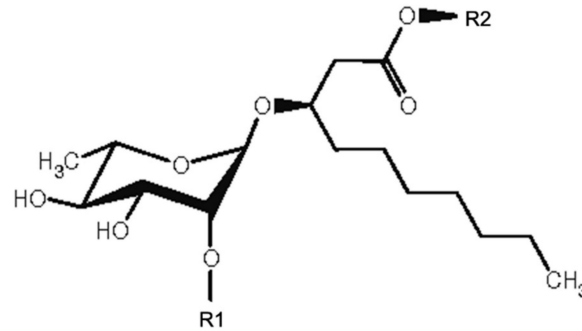


**Figure 2.** The rhamnolipid structure with all possible chemical modifications indicated. R1—position where the second rhamnose molecule can be attached and, thus, di-rhamnolipid is formed. R2—fatty acid of various lengths (Adapted from [73]).

Three enzymes are responsible for rhamnolipid synthesis in the *Pseudomonas* spp. First, two fatty acids are esterified together and linked to a carrier protein. Then, esterified fatty acids are linked to rhamnose by the glycosyltransferase—a glycosidic bond is formed, and a mono-rhamnose lipid is obtained. Rhamnosyl transferase II adds a second molecule of activated rhamnose to the mono-rhamnolipid; thus, di-rhamnose lipids are synthesized [74]. Precursor molecules for rhamnolipid synthesis are derived from the primary metabolism. Rhamnose is derived from glucose-6-phosphate. β-hydroxy fatty acids are synthesized either de novo or are taken up from the environment and shortened by β-oxidation [75].

Oil substrates and waste cooking oils can be used as a perspective substrate for microbial rhamnolipid production. There have been attempts to use WCO and similar substrates for rhamnolipid production [76,77]. Rhamnolipid yields from WCO substrates can be up to 0.62 g·g$^{-1}$ [78].

Although yeasts do not produce rhamnolipids, an engineering attempt for the heterologous expression of the rhamnolipid synthesis pathway in budding yeast *S. cerevisiae* is reported. The accumulation of intracellular lipid droplets was observed when sucrose was used as the carbon source in the budding yeast strain with the rhamnolipid pathway expressed [79]. In the future, rhamnolipid pathway expression in GRAS yeasts capable of fermenting oily substrates might lead to the sustainable and environmentally friendly valorization of WCOs into rhamnolipids without the use of a potentially pathogenic bacterial (*Pseudomonas* spp.) biomass.

## 3.3. Mannosylerythritol Lipids

Mannosylerythritol lipids (MELs) are BSs consisting of mannose, erythritol, and two fatty acid chains. The disaccharide mannosylerythritol is the "central" molecule of these glycolipids. In this molecule, erythritol is linked to mannose at the 1′ position. Two fatty acid chains are linked to mannose by ester bonds at the 2′ and 3′ positions. Depending on which chemical groups are attached to mannose at the 4′ and/or 6′ positions, the MELs are coined as MEL-A/B or C (see Figure 3).
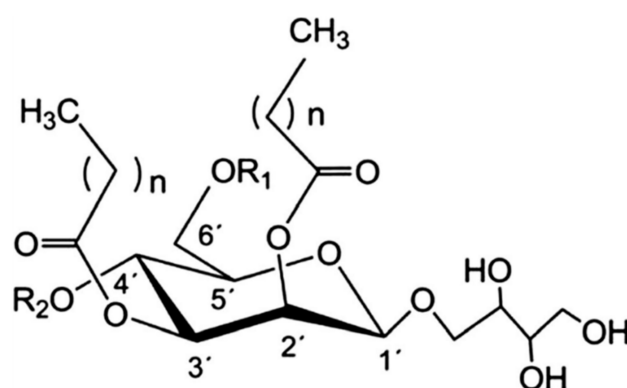
**Figure 3.** Chemical structure of mannosylerythritol lipids (Adapted from [80]).

MELs were first discovered to be produced by smut fungi *Ustilago maydis*, but later, more productive strains from Basidiomycota yeast *P. antarctica* (previously, *Candida antarctica*) were identified (reviewed in Morita et al. (2013) [81]).

The genes coding for MEL biosynthesis were first identified in the maize smut fungi *U. maydis*. Now, similar genes coding for the steps of this biosynthesis pathway have also been identified in *Pseudozyma* spp. yeasts [82]. The biochemical pathway of MEL synthesis consists of four steps. Mannosyltransferase catalyzes the mannosylation of erythritol, acetyltransferase catalyzes the double acetylation of mannosylerythritol (at positions 2' and 3'), and specific acyltransferase acylates mannosylerythritol at position 4' and 6'. In *U. maydis*, MELs acetylated in both (2' and 3') positions are exported out of the cell by a specific transporter, Mmf1 [81,83].

Most MELs produced by yeasts consist of medium-chain (8–10 C) fatty acids. However, vegetable oils typically contain fatty acids of 16–18 carbon atoms. Even if long-chain fatty acids are used as the sole carbon source, yeasts still produce MELs with medium- to short-chain fatty acids only [84]. MEL fatty acids are produced by cutting long-chain fatty acids in the β-oxidation pathway. When specifically inhibiting the yeast β-oxidation pathway, the production of MELs with medium- and short-chain fatty acids ceased [85].

*Pseudozyma* spp. can produce MELs from raw vegetable oils [86], raw soybean oil, and, also, WCO (waste soybean oil) [80]. Interestingly, the MEL yields from raw and WCO soybean oil did not differ significantly—in both cases, approximately 55–60% of the oil was converted into MELs. Besides, the maximum MEL production was achieved when more than half (55%) of the fermentation volume was filled with oil. No other supplementary carbon sources (sugar) were added to the WCO fermentation [80]. The composition of raw soybean oil and WCOs differ; hence, MELs produced from these substrates had different fatty acid compositions as well (see Table 3).

**Table 3.** MELs produced from raw or waste soybean oil. Adapted from [80].

| Fatty Acid [a], % | Caprylic C8:0 | Capric C10:0 | Decanoic C10:1 | Lauric C12:0 | Palmitic C16:0 | Stearic C18:0 | Oleic C18:1 | Linoleic C18:2 |
|---|---|---|---|---|---|---|---|---|
| Raw soybean oil | - | - | - | - | 13.08 | 4.36 | 33.6 | 48.11 |
| WCO | - | - | - | - | 18.59 | 5.13 | 43.57 | 30.63 |
| MELs from raw soybean oil | 23.19 | 47.49 | 0.7 | 5.94 | 2.13 | 1.6 | 7.37 | 9.57 |
| MELs from WCO | 1.5 | 6.66 | 3.22 | 0.31 | 7.92 | 3.79 | 59.78 | 13.78 |

[a] Each fatty acid composition expressed as % from the total fatty acids of the sample.

The fatty acid composition shown in Table 3 indicates that MELs produced from WCO contained long-chain fatty acids predominantly when compared to MELs produced from fresh soybean oil. Meanwhile, the activity of the BS extracts prepared from WCO or raw oil fermentations did not differ; their critical micelle concentrations and surface tension properties were identical [80].

### 3.4. Emerging Glycolipids Produced by Yeasts

Besides the "typical" BSs produced by yeasts—sophorolipids and MELs, other glycolipids are emerging as potential BSs. However, their industrial importance and synthesis from low-cost substrates, like WCO, are yet to be elucidated.

Xylolipids are glycolipids consisting of a xylose "head" and one or two fatty acid tails. Typically, xylolipids are produced by lactic acid bacteria [87]. There are reports that some yeasts are able to synthesize xylolipids. Joshi-Navare et al. (2014) reported that *Pichia caribbica* synthesizes xylolipids consisting of xylose and 9-heptadecanoic acid [88]. The maximum titer was 7.48 g·$L^{-1}$ of xylolipids when 4% raw olive oil was used as a substrate. The authors noted that the BS production depended on the nitrogen source—in a nitrogen-poor medium, the BS titer increased. Additionally, the value of the xylolipids' critical micelle concentration was approximately 100 mg·$L^{-1}$ [88]. However, there is no information on the mechanism of xylolipid synthesis within yeasts. Xylolipids are of special interest, since they could be produced from abundant and cheap substrates: waste lipids and xylose. Interestingly, the European Commission's funded Horizon 2020 project CARBOSURF aimed to improve the production of several industrially relevant BSs, including xylolipids. For this task, a bacterial producer was used.

Cellobiose lipids are another type of glycolipid produced by fungi. This BS has been found to be produced by the plant smut fungi *U. maydis*. A similar BS was also found to be produced by *Anthracocystis flocculosa* (previously known as *Pseudozyma flocculosa*), basidiomycete yeast, an antagonist of powdery mildew fungi (reviewed in reference [89]. Cellobiose lipids can be produced from sugars (sucrose), and their titers are increased if a medium-to-high C:N ratio is used [90].

### 3.5. Downstream Processing

Downstream processing (product extraction or separation) methods must be applied to separate the target product from the yeast biomass and fermentation broth. Downstream processing accounts for about 60% of the total cost of BS production [91], so the extraction methods should be selected carefully. There are at least seven different BS extraction or separation methods: solvent extraction, acid precipitation, salt precipitation, foam fractionation, gravity separation, ultrafiltration, and chromatography. To obtain BSs with a high degree of purity, a combination of different extraction and purification methods is used [92].

Several factors must be considered when choosing the most suitable method (or methods) for BS extraction. These include the physicochemical properties of the target BS, such as the critical micelle concentration (CMC), hydrophilic-lipophilic balance (HLB) [93], ionic charge of BS, solubility of the BSs in different solvents [92], and whether the BSs can form an insoluble separate phase within the fermentation broth [94]. Various extraction methods produce a BS with different degrees of purity; thus, the desired purity of the BS is also a criterion to choose an adequate extraction method [93]. The purity levels achieved using various extraction methods are shown in Table 4. Some of the methods have only been proven to be effective for use in lab-scale productions [95]; hence, the scale at which BSs are produced is also important in selecting the downstream processing method.

When using the gravity separation method, the amount and type of carbon source used for fermentation can also be a factor that affects the BS recovery process. When the glucose concentration in the fermentation medium is high, the BS phase can only be removed from the top (rather than the bottom) of the fermentation broth [94].

The purification method used can sometimes affect the ratio of different BS forms that are present in the product. If acid precipitation is used for sophorolipid extraction, the extract mostly contains lactonic sophorolipids, whereas the solvent extraction leads to roughly equal amounts of both lactonic and acidic sophorolipids in the crude extract product [101]. Liquid chromatography can be used if the separation of different BS congeners with high purity is desired [66].

**Table 4.** The glycolipid purity levels and recovery rates obtained using different BS extractions and purification methods, and the methods used to quantify the glycolipid BSs.

| BS Extraction Method | BS Type | Purity, % | Recovery Rate, % | Method Used to Quantify BS | Reference |
|---|---|---|---|---|---|
| Foam fractionation + foam adsorption | Rhamnolipids | No data | 40 | HPLC + charged aerosol detector | [96] |
| Acid precipitation + solvent extraction (with n-hexane) | Rhamnolipids | 90 | 99 | Anthrone-sulfuric acid assay [a] | [97] |
| Integrated gravity separation + solvent extraction (with hexane and ethyl acetate) | Sophorolipids | No data | 86 | Gravimetry | [94] |
| Filtration + solvent extraction (with methanol/water (pH 2)/n-hexane) | MELs | 100 | 90 | Orcinol method [a] | [98] |
| Multi-step integrated gravity separation | Sophorolipids | 74 | No data | Anthrone method [a] (residual glucose deduced from total sugar content to get sophorolipid content) | [99] |
| Foam fractionation + ultrafiltration | MELs | No data | 80 | Reverse phase HPLC | [100] |

[a] These BS quantification methods may not be precise [54].

Acid precipitation followed by solvent extraction is the most common combination of methods used for the extraction of low-molecular-weight BSs [95]. These methods are inexpensive and simple to perform [102]. The BS product obtained from solvent extraction and/or acid precipitation is often described as being completely pure, but this is not the case, since the product usually still contains up to 60% water and a number of other contaminants [55]. Ethyl acetate is the most common extracting solvent that is used either alone or in combination with other solvents, such as methanol and chloroform [76,80,97,102–107]. The use of ethyl acetate and chloroform in the production of environmentally sustainable products is not advisable, since these solvents are toxic and must be used in large amounts for these extraction procedures [98]. Zhou et al. (2020) suggest that these unfavorable solvents could be replaced by n-hexane, a less-harmful solvent [97].

Certain BS recovery methods, which are integrated with fermentation, could potentially solve problems that occur during the fermentation phase of some BS productions. For example, excessive amounts of foam can accumulate during fermentation, which causes fermentation inhibition [91] and makes it necessary to use a larger bioreactor [108]. The presence of BSs in the fermentation medium increases the viscosity, which decreases the amount of dissolved oxygen available for BS-producing yeasts [99]. These aspects further increase the costs of BS production, because more power is required to maintain the optimal agitation and aeration rates in the viscous fermentation medium [109]. Additionally, the foam formation must be controlled by supplementing antifoaming agents or mechanical systems for foam disruption [96]. The excess foam can be removed by using an integrated foam fractionation method while simultaneously separating the BSs that are in the foam [95]. The viscosity of the fermentation medium can be reduced by separating the BS-rich phase from the medium with an integrated gravity separation method [94]. The use of integrated BS recovery methods is also reported to increase the BS titer that is obtained at the end of production by enabling an extended fermentation process [91].

## 4. Properties of WCO-Derived Glycolipid Biosurfactants

The previous sections explained that BSs have both polar (hydrophilic) and nonpolar (hydrophobic) moieties that mostly define their role in producer organisms and the properties of surfactants. During the frying process, oil undergoes major physical and chemical changes altering its physiochemical properties and composition. Therefore, it is assumed that the type of substrate might have an influence on the molecular characteristics and

BS properties, including surface and interfacial tension, solubilization, and emulsification. The following sections discuss these issues, emphasizing the differences that might occur if WCO is used as a substrate for BS production.

### 4.1. Surface Tension and Interfacial Activity

Surface tension, interfacial tension, and surface rheology are aspects that play an important role in sanitizer, cosmetics, and cleaning product formulations. Understanding the surface activity of BSs produced by yeasts using WCO as a substrate is important to predict and ensure the stability and efficiency of the end product [110]. Other important properties of BSs, like the cleansing and foaming performances, are directly related to the surface activity [111].

The critical micelle concentration (CMC) provides a reference point to compare the efficiencies of different surfactants [112]. The CMC of BSs can even be 96% lower than in synthetic surfactants, meaning that a smaller amount of BS is necessary to achieve the minimum surface tension ($\gamma_{min}$) [3,111]. The presence of micelles is critical to evaluate the functional performance of the surfactant. The CMC and $\gamma_{min}$ values vary depending on the structural composition of the BSs that can differ not only between BS types but also between different forms of a specific BS [113]. For example, sophorolipids have various structural forms that determine their physiochemical properties. The presence of lactonic forms affects the BS hydrophilic/lipophilic balance, foam formation. capacity, and antibacterial activity. Lactonic sophorolipids express better surface tension-lowering properties and antimicrobial properties, while the acidic form has better foam production and water solubility [114].

The surface tension of distilled water is 72 mN·m$^{-1}$, and this value is often used as a reference point to compare the activity of a BS [115]. Rhamnolipids can lower the water surface tension to 26–29 mN·m$^{-1}$ and sophorolipids to 33–37 mN·m$^{-1}$, showing lower values than synthetic surfactants such as sodium dodecyl sulphate (37 mN·m$^{-1}$) or dodecylbenzene (47 mN·m$^{-1}$) [93,116]. Sophorolipids lower the surface and interfacial tension with a CMC of 40–100 mg·L$^{-1}$, though they are not effective emulsifying agents [117]. Meanwhile, rhamnolipids from *Pseudomonas* spp. demonstrate the ability to lower the interfacial tension and act as emulsifying agents [118].

Using WCO as a feedstock for BS production by yeast provides similar quality and value BSs as those obtained from raw cooking oil. Maddikeri et al. (2015) used a pretreated WCO as a substrate to produce sophorolipids using *S. bombicola* [22]. The application of sophorolipids at a loading of 500 mg·L$^{-1}$ reduced the surface tension of water from 72 mN·m$^{-1}$ to 32.6 mN·m$^{-1}$. The sophorolipids attained the maximum surface activity at a CMC value of 10 mg·L$^{-1}$. Similar research has been presented by other researchers, who obtained unspecified BSs produced by three different *Candida* species. Almeida et al. (2018) used canola WCO as a feedstock and fermented it with *Candida tropicalis* [119]. They obtained 4.11 g·L$^{-1}$ of BS. The CMC value of the BS was 60 mg·L$^{-1}$, and the surface tension of the water was reduced to 25.6 mN·m$^{-1}$. Similar CMC values (70 mg·L$^{-1}$ and 80 mg·L$^{-1}$) and surface reduction values (28.6 mN·m$^{-1}$ and 26.3 mN·m$^{-1}$) were obtained by Sobrinho et al. (2008) and Lira et al. (2020) [15,120]. Hence, the reported results showed that the CMC of the BSs obtained from WCO were similar to the CMC of the BSs obtained from raw cooking oil. The same applies to the ability to reduce the water surface tension.

### 4.2. Emulsification and De-Emulsification

BSs have a unique ability to emulsify hydrocarbons, thus enhancing their solubility and reducing the surface tension and increasing the biodegradation of oil in aquatic and land environments [121]. The emulsifying activity is determined by the molecular weight of the BSs. While BSs with lower molecular weights are more effective in reducing the surface tension at the air–water interface and interfacial tension of oil-in-water solutions, BSs with higher molecular weights effectively stabilize oil-in-water emulsions [122]. Among glycolipid BSs, rhamnolipids are found to be the most effective emulsifiers due to their

smaller droplet sizes, and as the rhamnolipid concentration increases, the emulsion particle size decreases. Furthermore, the stability of the emulsions is also evaluated as good; they are stable against coalescence, keep their consistency at temperatures from 30 to 90 °C, and have a high ionic strength sensitivity [111,123]. It has been reported that a BS produced by *Candida sphaerica* using ground nut oil residue with added corn steep liquor can achieve 65% emulsifying activity [15]. Even as high as 80% emulsification activity can be achieved with BS produced by *Candida guillermondii* and *Candida lipolytica* in emulsifying motor oil and petroleum [124]. This is comparable to the emulsifying activity of BS obtained from raw cooking oil.

The hydrophile–lipophile balance (HLB) value is an important characteristic that indicates the type of emulsion formed by BS in reaction with immiscible substances and the potential application of BSs (see Figure 4).
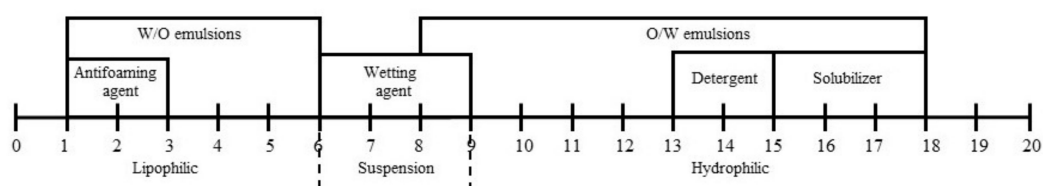


**Figure 4.** The application of surfactants according to their hydrophile–lipophile balance values [93].

The HLB values of the lactonic or acidic sophorolipds differ significantly. The lactonic form has lower HLB (4–5) values than acidic. In the literature, sophorolipids with low HLB values can be applied as wetting agents and as stabilizers for water–oil emulsions (W/O). The HLB value for the sophorolipid acidic form or its alkaline salts can vary from 8 to 15, suggesting its application as detergents or as stabilizers for oil–water (O/W) emulsions [11,125]. Structural modifications of sophorolipids facilitate achieving the required HLB value to broaden the application of sophorolipids to hair and skin care, detergents, and agriculture products [93].

### 4.3. Temperature and pH Tolerance

BSs have a high resilience to pH and temperature, and they exhibit unchanged surface tension-reducing activity up to 90 °C. It allows us to apply BSs in extreme conditions where synthetic surfactants lose their efficiency [112]. Sobrinho et al. (2008) tested the stability of a BS produced by *C. sphaerica* by incubating it for 1 h at temperatures ranging from 4 °C to 120 °C and concluded that the BS was stable at these temperatures and preserved its tension-reducing and emulsifying activity [15]. The industrial application of surfactants often involves extreme conditions, including a high temperature and pressure, alkaline and acidic conditions, and ionic concentrations that do not affect the activity of the BS [125]. In fact, higher pH values in the range of 4–11 can decrease the surface tension-lowering ability and increase the emulsion stability of BSs. Environmental variables such as the temperature, pH, and ionic strength can be applied to modulate the BS properties [3].

### 4.4. Biodegradation and Toxicity

BSs can be easily degraded and, therefore, can be used for natural applications, such as bioremediation and biosorption [56,118]. Rhamnolipids can be degraded under aerobic and anaerobic conditions, and in both cases, they show a soluble chemical oxygen demand removal efficiency by 74% after 10 days and by 47.2% after 6 days [126,127]. Moreover, the presence of BSs in the environment enhances the biodegradation of other pollutants [111]. This property does not differ depending on the substrate (raw cooking oil vs. WCO) that is used in BS production. BSs show low or no toxicity. Recent research with a BS produced by *C. guilliermondii* from WCO as a substrate showed no inhibitory effect on the terrestrial plant growth or negative impact on brine shrimp at concentrations of $1/2\times$ CMC, $1\times$ CMC, and $2\times$ CMC [120].

## 5. Applications of WCO-Derived Glycolipid Biosurfactants

The molecular and physical properties and activity of glycolipid BSs provide multiple application opportunities. Most popular applications include cleaning products, i.e., detergents and soil and water remediation from hydrocarbon pollution; yet, it extends to wide uses in agriculture, food, pharmacy, and cosmetics [113]. A low CMC of BSs allows us to effectively reduce the surface tension; therefore, a smaller amount of surfactant is necessary to remove the oils and fats [6]. The spectrum of BS properties represents a wide functional potential for their industrial application.

### 5.1. Agriculture and Bioremediation

The low toxicity and physiochemical properties of BSs allow us to apply BSs as environmental bioremediation agents to clean up oil spills, remove heavy metal contamination, and treat wastewater [3]. BSs increase the microbial cell surface hydrophobicity, allowing microbes to access environmental pollutant molecules and break down hydrocarbon chains transforming molecules into $CO_2$, water, and minerals [128]. BSs could replace the toxic surfactants used in pesticides and improve the quality of the soil by increasing the bioremediation [111]. If a pollutant is adsorbed into the porous surface of soil, BSs have the ability to transport and solubilize it in water [129].

WCO-derived BSs can solubilize hydrocarbons by partitioning them into the surfactant micelles above the CMC [118]. BS produced by *C. tropicalis* cultivated with canola WCO can be used as an oil spill remediation agent in a marine environment [31,128]. Luna et al. (2016) demonstrated the ability to remove 70% of motor oil adsorbed to a porous surface by using glycolipid BS produced when using WCO [128]. By encouraging the growth of indigenous microorganisms, BS stimulated the biodegradation of oil by 60% at a concentration of $1\times$ CMC and above 70% biodegradation at $3\times$ CMC, while $5\times$ CMC led to an oil biodegradation maximum of 73.8% [119].

Sophorolipids can be applied as an adjuvant in the formulation of herbicides and for phytopathogen control. They show antifungal activity and can prevent root diseases caused by phytopathogenic fungi [130].

### 5.2. Detergents, Personal Care, and Cosmetics

Surfactants are an important ingredient of cosmetic products like soaps, shampoos, toothpaste, deodorants, creams, makeup, laundry, and home care products. BSs have relevant surface-active properties and biological activity to act as wetting agents, solubilizers, dispersants, foaming agents, cleansers, detergents, and emulsion-forming agents [130]. Glycolipid BSs with HLB values close to 10 and CMC values ranging from 5 to 150 mg·L$^{-1}$ have great detergent, emulsifying, foaming and dispersing properties [118]. Sophorolipids have attractive properties, such as foaming and detergency, which are not affected by water hardness [131].

BSs have a wide range of properties; hence, depending on the application of the end product, various BSs are used [115]. The application of a BS on skin can improve the dermal fibroblast metabolism, and their hygroscopic properties help to moisturize the skin [55]. Due to their nonionic nature, BSs produced by yeast have antibacterial properties, while not harming skin cells or irritating the eyes [93,132]. Sophorolipids are detergents capable of dissolving biological membranes and, hence, pose some antimicrobial activity. They can be used against bacteria, algae, fungi, viruses, and some intracellular parasites (mycoplasma) [133].

In order to be used in cosmetics and body care products, BSs must be of high purity; however, purification significantly increases production costs [93]. The cosmetics industry is one where the high production costs of BSs would be acceptable, as consumers aim for a more sustainable and environmentally friendly lifestyle [111]. Additionally, consumers are interested in nonirritating cosmetics with low cytotoxic effects [132].

MELs are highly hydrophobic and can therefore be used as emulsifiers, dispersants, and detergents [134]. MELs also have the ability to regenerate skin cells after damage

caused by sodium dodecyl sulphate (sodium lauryl sulphate) and are able to activate skin fibroblasts and wart cells and restore damaged hair [82,135]. By replacing chemically synthesized silicones, MELs can be used as an ingredient in foundations, as MELs are able to encapsulate particles of metal oxides (titanium oxide, and iron oxides) [135]. MELs produced by *Pseudozyma tsukubensis* (fatty acid chain lengths (8–14 C) from raw olive oil are already commercialized for use in cosmetics. This product is called SurfMellow® and is produced by the Japanese company Toyobo Co. Ltd. (Osaka, Japan) [136].

### 5.3. Oil Recovery

Oil recovering technologies have their limitations, and BS-mediated oil recovery has the perspective to recover large amounts of crude oil that would otherwise stay entrapped in oil reservoirs [118]. The conventional methods that are used for oil recovery allow us to obtain only 30% of the oil from reservoirs [93].

The BS ability to reduce interfacial tension increases the rock-wetting ability and entrapped oil to be mobilized [137]. Surfactants are applied to increase the amount of oil recovered during the water flooding or steam injection processes. BSs have the ability to partially or fully replace synthetic surfactants and can be applied in surfactant-enhanced oil recovery processes [93].

Even applied in low concentrations, BSs are effective and do not lose their activity over a wide range of pH values, temperatures, and salinities that occur in oil reservoirs. The effectivity of BSs in surfactant-mediated oil recovery has been proven in laboratory experiments and field studies. Research shows that the in situ production of the BSs using WCO as a substrate is possible and recovers additional oil in the process [44].

### 5.4. Medicine and Pharmaceuticals

Glycolipid BSs possess antibacterial, antifungal, anticancer, and antiadhesive properties; hence, they have great potential for applications in medicine [138]. Sophorolipids have antibacterial properties against Gram-positive and Gram-negative bacteria. In addition, anticancer, anti-HIV, spermicidal, and hemolytic properties have been described. These properties are promising opportunities for therapeutic applications in wound healing, oral cavity, and dermatological care [139]. Another application of sophorolipids is as a gelation agent of silk fibroin, a protein produced by the domestic silk moth *Bombyx mori*. Lactonic and acidic sophorolipids produced from oleic acid initiate fast gelation and the formation of a porous 3D protein matrix. This matrix proved to be suitable for mural cell proliferation. Thus, sophorolipids could be used for biomedical purposes [140]. It has been reported that MELs promote cell neuronal separation in human promyelocytic leukemia cells and improve the movement of acetylcholine esterase, indicating that MELs could be used as novel agents for disease treatment [56].

### 5.5. Food and Feed

In the food industry, BSs are used for their ability to improve the physical properties (volume, texture, viscosity, and stability) of vegetable oil-based, starch-based, and animal food products [141]. Glycolipids could be used as preservatives because of their antibacterial properties [14,111].

Food pathogens become resistant to the chemical preservatives used over time, so it is necessary to find new preservatives that act against these pathogens [142]. Combining rhamnolipids with other substances that also possess antimicrobial properties (e.g., essential oils and biopolymers) would allow an increase in the spectrum of antimicrobial exposure, thus controlling the spread of food pathogens both during food production and packaging [111].

Adding sophorolipids to flour promotes a better volume, appearance, and shelf life of bread [143]. Additionally, rhamnolipids could be used to improve the properties of confectionery dough. However, their use for this purpose is hampered by the fact that rhamnolipids are most often produced by pathogenic bacterium *P. aeruginosa* [79].

*5.6. Other Emerging Applications*

Several other emerging applications have been reported for BSs. It is known that sophorolipids can be used as reducing and capping agents in the process of synthesis and of silver nanoparticles, avoiding the need for external reducing agents. Sophorolipids are known to reduce silver ions to silver nanoparticles and cap them in the sophorose moiety, creating glyconanoparticles. They are researched for their cell-mimicking function that allow us to understand protein–carbohydrate interactions and can be applied in various biomedical applications [144].

Another alternative to BS application is to use them as a feedstock to produce other surfactants with enhanced performances. Saerens et al. (2009) studied how to optimize the production of glucolipids from sophorolipids by using enzymes from *Penicillium decumbens* [145]. Glucolipids are glycolipid analogs that can serve as unique building blocks for the (chemo)-enzymatic synthesis of novel glycolipids with unique forms of carbohydrate moieties. This unique carbohydrate moiety would allow us to achieve better biological activity and reduced toxicity.

Some more novel applications of sophorolipids include methyl hydroxy-branched fatty acid (MHBFAs) preparation, which is an important chemical produced by a biochemical hybrid approach. In the process of bio-based plastic production, MHBFA is used as comonomer to improve the mechanical properties, such as the ultimate tensile strength and toughness [18,138].

There has been research on sophorolipids' application in biodegradable film development. Sophorolipids produced by *S. bombicola* were added to increase the thermostability and reduce the tensile strength. In addition, it was proven that sophorolipids can still express their chemical effects and act as an antifungal agent by reducing the fungal growth by 100%. This feature can be especially valuable for considering sophorolipids as a promising ingredient in food packaging [146]. Additionally, other studies have been initiated for finding innovative biomaterials.

## 6. Discussion

BSs present outstanding functional properties that form the foundation for their various applications. With the concepts of bioeconomy and circular economy high on the global agenda, the search for renewable bioresources and cheap waste materials as substrates has been initiated in many areas of research and industry, including BS production. Currently, most studies are focusing on the synthesis of BSs from raw cooking oils, and there are relatively few results where the use of WCOs is explored and even less where waste fats and grease are studied as substrates. Little information exists on the pretreatment (as well as the necessity of a pretreatment) of the WCOs used as a substrate in the production of BSs. Most pretreatment studies are currently done with the aim of studying the effect on biodiesel production rather than BS synthesis; hence, robust knowledge has not yet been obtained on the process, nor its effect on the BS properties. Additionally, limited-to-no information is published on the type, availability, costs (prices), and environmental impact of substrates and the processes applied.

BS production from WCOs should be based on economic, environmental, technological, and social justifications to ensure that the BS production does not contribute to input (materials, energy, and water) and output (waste and emissions) flows too high to not jeopardize the circularity of the WCO used and sustainability of the BSs produced. This is especially important, because it has been reported that the BS production phase is responsible for most of the environmental impacts in every cradle-to-the-grave life cycle assessment category, indicating the importance of the systemic assessment of the bioproduction impact on the environment. Again, BS production by yeast using WCO as a substrate can be affected by factors such as the temperature, stirring speed, aeration, medium volume, incubation time, pH, salinity, inoculum ratio, and mineral elements. Hence, multiple factors still must be considered and studied to draw solid conclusions.

Most of the published studies have concentrated mainly on BS synthesis from WCOs rather than their further use in products. In addition, often, details on the WCO (source) have not been identified. Yet, it is important, as different types of vegetable oils have different fatty acid profiles. Moreover, different types of vegetable oils dominate in various regions, e.g., soybean and palm oils predominate in Asia, while, in Europe rapeseed, sunflower, and olive oils are more popular. To enable the valorization of WCO into BS, the costs of the substrate should be minimized, e.g., the source of the feedstock should be closer to the BS production to reduce the logistic costs and emissions.

The chemical composition of the WCOs differs from the composition of raw cooking oils; therefore, the BS spectrum produced from WCO differs from that produced from raw oil. Additionally, since WCO is often a poorly defined mixture of several different cooking oils, not a single, but possibly several, inhibitory substances affecting BS production are present in the WCO. Lower titers of BSs have been reported when WCOs were used as the substrate in comparison to the titers achieved with raw cooking oils [18,46]. Moreover, the specific inhibition of lactonic sophorolipid synthesis occurs. Part of the inhibition could be alleviated by shifting the co-substrate from glucose to glycerol and part by applying a pretreatment to the WCOs [46]. The shift to the production of MELs containing long-chain fatty acids occurs when using the WCO as a substrate in comparison to raw cooking oil [80]. This effect is similar to the β-oxidation inhibition described by Kitamoto et al. (1998) [85]. When specific β-oxidation-inhibiting substances were applied, yeasts produced MELs with long-chain fatty acids only [85]. Due to the complex chemistry of WCOs, the presence of substances capable of inhibiting yeast β-oxidation cannot be ruled out. To further develop a BS production process from WCOs, the mechanisms of the inhibitory effects must be clarified. The potential inhibitory mechanisms could be the direct inhibition of extracellular or intracellular enzymes (sophorolipid lactonic esterase) or inhibition of the β-oxidation process.

Detailed knowledge on the metabolic pathways that synthesize BSs in the emerging BS producers—*P. caribbica* and *A. flocculosa*—is not known yet. With the help of genetic engineering, it would be possible to improve BS production from the endogenous pathway or to start BS synthesis from the heterologous pathway expressed in yeasts. There are successful genetic engineering examples of shifting *S. bombicola's* BS production to acidic or bolaform sophorolipids only by knocking out acetyl transferase and lactonic esterase [147]. Additionally, rhamnolipid production can be performed via bakers' yeast by expressing the bacterial synthesis pathway [79].

Yeast biomass is a valuable protein- and vitamin-rich product. It is traditionally harvested from breweries and used for yeast extract (paste or powder) production. Spent brewing yeast is a valuable side product, and the brewing industry benefits from the valorization of it. It is foreseen that if GRAS yeasts would be used for the production of BSs from WCOs, then the yeast biomass might become a valuable side product for protein or vitamin production. Alternatively, spent yeast from BS production might be recycled to enhance yeast biomass growth for BS production.

Further, the WCO used for BS production may limit its application options. Even though degradation products compose a minor part of WCOs, it has been reported that WCOs' heterogeneous physicochemical properties and the trace impurities also present in a pretreated WCO can limit its future use in personal care products, cosmetics, food, and pharmaceuticals, where high-purity products with constant composition are needed. Hence, further research is also needed on the substrate treatment and downstream processing methods and their efficiency in ensuring the quality requirements and sustainability of the end product. Currently, the purity and recovery rates of BS obtained from raw or WCO have not been disclosed. Some authors have described their BS product as being of "high purity" without assigning a numerical value to the purity. The purity and recovery rates should be mentioned, because they are good indicators of how well an extraction method works. Moreover, such values would help to determine the total production costs and environmental impact. On the other hand, even though the chemical composition of

BSs differs depending on the substrate (raw oil or WCO), the physicochemical properties of BS produced from raw or WCO are highly similar [80]. This aspect would be helpful in adopting or developing WCO-based BS production processes for specific applications where the total BS activity is more important than a precise chemical composition. Soil improvement, bioremediation, and oil extraction might be the typical examples [137].

Overall, there is still a huge potential for further research on WCO-derived BSs and their substrates, production processes, and applications, as well as economic and environmental impacts. We suggest focusing comparative studies on the effects of WCO composition and the properties of BS composition. For example, experimentally compare the BS yield and properties from a raw cooking oil and corresponding WCO as a substrate. The results of this and similar experiments would suggest application options for BS obtained from WCO fermentations. Additionally, research on BS synthesis and the properties when different types of defined WCOs (i.e., rapeseed, palm, etc.) are used would be necessary, since the differences in their oil fatty acid profiles will alter the chemical compositions of the produced BSs. Knowledge on these issues would open up the opportunity for high-value circularity in the food industry, where more homogeneous WCOs are generated as compared to the catering and household sectors.

### 7. Conclusions

Alternative waste-origin substrates are sought to solve several modern environmental challenges, including the valorization of waste streams, the mitigation of greenhouse gas emissions, the efficient use of resources, and the production of bio-based, nonhazardous chemicals. Currently, there is a lack of knowledge and evidence on how large the positive impact is that can be achieved by switching from raw cooking oil to WCO when used as a substrate for BS production, as there are very few studies focused on the issue. Meanwhile, studies on the substrate quality, BS synthesis, product outcomes, extraction methods, and application options must be continued to establish a robust process at the commercial scale.

Considering the natural ability of living organisms to adapt to various conditions, including changes in the substrate, research testing the stability (or alterations) of the composition, quality, and properties of WCO-derived BS is suggested. This is a research question for circular economy in general, as there will always be a need to ensure stable processes and the robust quality of products.

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