

Review

Recent Advances in Biosurfactant Production in Solid-State Fermentation

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Abstract

Biosurfactants are amphiphilic molecules synthesized by some microorganisms. Biosurfactants have a wide range of applications in fields such as the bioremediation, petroleum, and pharmaceutical industries. Currently, biosurfactant production is carried out mainly by submerged fermentation (SmF). Biosurfactant production by SmF requires the use of antifoams, which hinder biosurfactant recovery and have a high energy requirement. Biosurfactant production by solid-state fermentation (SSF) has been little explored, but it has some advantages over SmF: it allows the utilization of cheap agro-industrial by-products that function as a support-substrate, does not present foam formation, and allows for improved oxygen and mass exchange. Several research groups have explored different strategies to improve the yields in biosurfactant production by SSF and have demonstrated that it is a viable technology for obtaining these products. Some of the parameters studied are temperature, moisture, substrates, supports, aeration, and, in some cases, agitation. These studies have shown advantages of SSF over SmF for biosurfactant production, such as higher product-substrate yields and higher product concentrations. However, further study of the causes of these results is necessary to implement SSF technology for commercial biosurfactant production.

Keywords: solid state fermentation; biosurfactants; glycolipids; rhamnolipids; sophorolipids; mannosylerythritol lipids; lipopeptides; surfactin



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1. Introduction

Surfactants, also known as surface-active agents, are amphipathic organic molecules composed of a nonpolar, hydrophobic portion and a polar, hydrophilic portion. The nonpolar portion is often a hydrocarbon chain, and the polar portion can be highly ionic, anionic, or amphoteric. Due to the presence of these hydrophobic and hydrophilic groups, surfactants are usually located at interfaces, forming a film and reducing the surface tension of the medium.

Many of these surfactant substances, called biosurfactants, are synthesized by microorganisms, which have a natural origin compared to synthetic surfactants obtained by

chemical methods. Most biosurfactants have common properties, being more effective than conventional surfactants. Among the advantages of biosurfactants obtained naturally are: (i) a lower concentration of biosurfactant is required to reduce surface tension, (ii) they tolerate changes in pH, temperature and ionic strength better, allowing them to be used in a wider range of conditions, (iii) they are biodegradable so they do not cause environmental problems, and (iv) they have very low toxicity so they can be used safely in the pharmaceutical, cosmetic and food industries [1–4].

2. Classification of Biosurfactants

Surfactants are classified according to their origin into petroleum-derived surfactants, bio-based surfactants, and partially bio-based surfactants. Bio-based surfactants, or biosurfactants, can be extracted directly from plant material, produced by chemical synthesis from renewable materials, or obtained through biological processes such as microbial culture [5]. The production and use of biosurfactants represents a sustainable option compared to chemical surfactants.

Biosurfactants are classified into two main groups: high molecular weight and low molecular weight biosurfactants [6,7]. Table 1 shows the classification of biosurfactants according to their chemical structure.

Table 1. Classification of biosurfactants.

Group	Sub-Group	Class
Low Molecular weight	Glycolipids	Rhamnolipids
		Sophorolipids
		Trehalolipids
	Lipopeptides	Mannosylerythritol lipids
		<i>Bacillus</i> -related LP
		<i>Pseudomonas</i> -related LP
		Other bacteria-related LP
		Fungi-related LP
	Fatty acids, neutral lipids, and phospholipids	Fatty acids
		Neutral lipids
Phospholipids		
High molecular weight	Polymeric biosurfactants	Emulsan
		Biodispersan
		Alasan
	Particulate biosurfactants	Liposan
		Vesicles and fimbriae
		Whole cells

Each of these groups of biosurfactants has different physicochemical properties due to their chemical structure. These properties determine the biological function and commercial applications of a biosurfactant [8].

3. Solid State Fermentation (SSF)

In recent years, significant progress has been made in the production of biosurfactants through solid-state fermentation (SSF), utilizing solid materials and agro-industrial waste as a promising alternative to valorize these wastes, thereby reducing production costs and promoting environmental sustainability [9,10]. Likewise, strategies have been developed to optimize processes through control of fermentation conditions, such as the specific selection of microorganisms and the improvement of parameters (temperature, moisture, etc.), which allows increasing the efficiency of the process [11,12].

Solid-state fermentation is a technique frequently used to obtain fungal biosurfactants from oilseeds or agricultural waste and is considered an excellent fermentation option [13]. The selection of alternative substrates utilizing industrial and agro-industrial waste as sources of carbon and nitrogen has proven effective in improving sustainability and reducing costs in the production of biosurfactants [9,10]. A variety of lignocellulosic byproducts have been used as a carbon source for biosurfactant production in submerged fermentation. However, to utilize the nutrients in these byproducts, they must undergo processes such as thermal pretreatment, chemical or enzymatic hydrolysis, and detoxification [14]. These steps increase the energy requirements and overall cost of the process. SSF allows the use of solid lignocellulosic by-products with minimal pretreatment. These by-products can be used as inert support or as both a support and substrate.

Karmakar et al. [15] mention that the design of microbial consortia through the combination of microorganisms with complementary functions helps to improve the efficiency of the process and production of desired compounds, as well as the optimization of nutrients to adjust the proportions of carbon and nitrogen in the substrate through statistical methodologies such as the design of experiments, which has allowed the maximization of the production of specific metabolites [10]. Jiménez-Peñalver et al. [16–18], focus their research on the production of sophorolipids as biodegradable glycolipid-type compounds using the yeast *Starmerella bombicola*, which requires a hydrophilic and a hydrophobic carbon source (glucose and oleic acid) reaching a viable production by obtaining high yields and a substrate conversion of up to 70%, in this research, they developed two types of fermentation, submerged fermentation (SmF) and SSF, demonstrating that SSF has the advantage of being able to use hydrophobic solid substrates. In addition, SSF does not present problems of viscosity increase or foam formation, improving oxygen transfer and thus requiring less water [7,16,18].

SSF is a biotechnological process in which microorganisms such as bacteria, filamentous fungi, and yeasts grow in the absence of free water, generating different products of interest in the market [19]. Unlike submerged fermentation, microorganisms grow on solid materials that act as a support and source of nutrients. This process is widely used in the production of foods, enzymes, biofertilizers, biofuels, organic acids, and bioactive compounds [20,21]. Its popularity stems from advantages such as lower water consumption, lower liquid waste production, and lower operating costs compared to submerged fermentation systems. Furthermore, SSF typically mimics the natural growth conditions of many microorganisms, which favors its efficiency and adaptability. For example, this method is common in the production of products such as miso and tempeh in the food industry.

4. Industrial and Environmental Applications

Biosurfactants produced using SSF have proven effective in applications such as bioremediation of contaminants, soil quality improvement in agriculture, and food processing [10,12]. In this sense, Martins et al. [22], compared a biosurfactant produced by SSF and a chemical remediation by in situ tests of a diesel spill, achieving results of 99% hydrocarbon removal with the biosurfactant, while with the chemical dispersant, the maximum reduction was 90% in 180 days. Recently, advanced technologies have been implemented to improve the extraction and purification of biosurfactants, which has allowed obtaining higher quality products with specific properties [15].

5. Production of Biosurfactants by SSF

Biosurfactants can be produced by the well-known submerged fermentation (SmF) system or by solid-state fermentation (SSF). Most of the published articles correspond to studies performed by SmF at the laboratory level (0.1–1.2 L); only a few articles report

production at the bench level (around 3 L) or at the pilot level [23]. SSF can be an interesting alternative for large-scale production of biosurfactants since it offers some advantages over SmF, such as: smaller bioreactor size, reduced recovery process, lower sterilization costs, and the possibility of using cheap agro-industrial byproducts as support and substrate [24].

Despite the potential advantages of SSF over SmF, there are only a few reports on biosurfactant production by SSF. Table 2 shows some examples of biosurfactant production by SSF, highlighting the production of glycolipids such as rhamnolipids, sophorolipids, and mannosylerythritol lipids, as well as lipopeptides such as surfactin, iturin A, and fengycin.

Table 2. Examples of biosurfactants produced by SSF.

Biosurfactant Type	Microorganism	Solid Support	Yield (g/kg Solid Support)	Reference
Rhamnolipids	<i>Pseudomonas aeruginosa</i>	Soybean meal	19.68	[25]
Rhamnolipids	<i>Pseudomonas aeruginosa</i>	Soybean meal	17.05	[26]
Rhamnolipids	<i>Pseudomonas aeruginosa</i>	Soybean meal	12.6	[27]
Rhamnolipids	<i>Pseudomonas aeruginosa</i>	Polyurethane foam	39.8 *	[28]
Rhamnolipids	<i>Pseudomonas aeruginosa</i>	Corn bran and corn germ	19.5	[29]
Rhamnolipids	<i>Pseudomonas aeruginosa</i>	Sugarcane bagasse and sunflower seed meal	46.85 *	[30]
Rhamnolipids	<i>Pseudomonas aeruginosa</i>	Rapeseed meal and wheat bran	18.7 *	[31]
Rhamnolipids	<i>Serratia rubidaea</i>	Mahua oil cake	N.D.	[32]
Rhamnolipids	<i>Pseudomonas aeruginosa</i>	Sugarcane bagasse and corn bran	45 *	[33]
Rhamnolipids	<i>Pseudomonas aeruginosa</i>	Sugarcane bagasse and sunflower seed meal	172	[34]
Sophorolipids	<i>Starmerella bombicola</i>	Wheat bran and winterization oil cake	190	[35]
Sophorolipids	<i>Starmerella bombicola</i>	Winterization oil cake	141	[10]
Sophorolipids	<i>Starmerella bombicola</i>	Sunflower oil cake	N.D.	[18]
Sophorolipids	<i>Starmerella bombicola</i>	Polyurethane foam	211	[17]
Sophorolipids	<i>Starmerella bombicola</i>	Winterization oil cake and wheat straw	179	[16]
Sophorolipids	<i>Starmerella bombicola</i>	Sunflower oil cake	495	[36]
Mannosyl-erythritol lipids	<i>Moesziomyces bullatus</i>	Wheat bran and winterization oil cake	98.0	[37]
Mannosyl-erythritol lipids	<i>Ustilago maydis</i>	Wheat bran and winterization oil cake	12.2	[37]
Lipopeptides	<i>Bacillus subtilis</i>	Aleppo pine waste	27.59	[38]
Lipopeptides	<i>Serratia marcescens</i>	Wheat bran	52	[39]
Lipopeptides	<i>Bacillus subtilis</i>	Date molasses	N.D.	[40]
Lipopeptides	<i>Bacillus subtilis</i>	Tuna fish flour and potato waste flour	116	[41]
Lipopeptides	<i>Bacillus subtilis</i>	Millet	20.8	[42]
Lipopeptides	<i>Bacillus subtilis</i>	Potato peels	67	[43]
Lipopeptides (surfactin and iturin)	<i>Bacillus natto</i>	Wheat bran and bean pulp	4.40	[44]
Lipopeptides (surfactin and fengycin)	<i>Bacillus subtilis</i>	Polyurethane foam	3.1 *	[45]
Lipopeptides	<i>Trametes versicolor</i>	Two-phase olive mill waste	3.7	[46]
Surfactin	<i>Bacillus amyloliquefaciens</i>	Rice straw and soybean flour	15.03	[47]
Surfactin	<i>Bacillus subtilis</i>	Okara	2	[48]
Iturin	<i>Bacillus velezensis</i>	Soybean meal powder	12.46	[49]
Carbohydrate-peptide-lipid complex	<i>Pleurotus ostreatus</i>	Sunflower seed shell	4.69 *	[50]
Protein-polysaccharide-lipid complex	<i>Pleurotus djamor</i>	Sunflower seed shell	10.2	[51]

N.D. = Not determined, * g/L.

5.1. Rhamnolipids

Rhamnolipids (RL) are glycolipids consisting of a hydrophilic fraction with one or two rhamnose molecules linked together by an α -1,2-glycosidic bond, and a hydrophobic

fraction that may have one or two hydroxylated fatty acid molecules of between 8 and 14 carbons. They are produced mainly by species of the genus *Pseudomonas* and are synthesized as secondary metabolites [52], although there are also strains of the genus *Burkholderia* and some fungi that can synthesize RL. Figure 1 exemplifies the diversity of RL congeners that are synthesized since Mono-RL and Di-RL can be present with one or two lipid chains of different lengths.

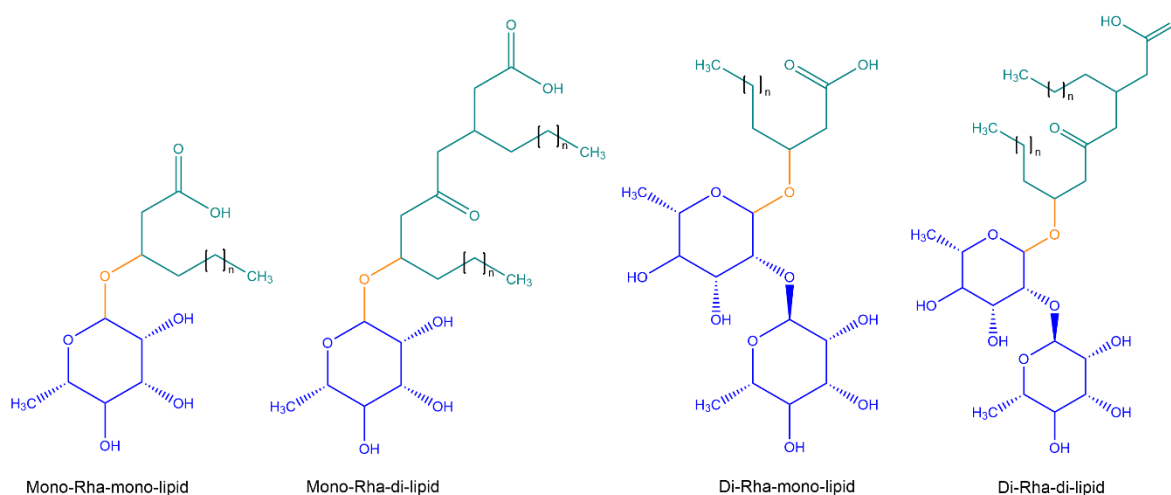


Figure 1. General structure of RL, the hydrophilic fraction is indicated in blue, the ester bond to join the fatty acid fraction (green) in orange, n refers to the carbon units in the hydrophobic fraction.

RLs produced by *Pseudomonas aeruginosa* are capable of reducing the surface tension of water to 25–30 mN/m and have shown critical micellar concentration (CMC) values ranging from 10 to 230 mg/L [53]. RL is already being produced commercially, although its costs are higher than those of synthetic surfactants. The commercial price of RL with a purity of 85 to 90% is USD 5000/kg (AGAE TechnologiesCorvallis, OR, USA).

Some researchers have employed SSF to produce RL. The use of sugarcane bagasse in combination with sunflower seed meal impregnated with a glycerol solution has been reported [33]. Subsequently, Camilios Neto et al. [33] explored the mixture of sugarcane bagasse and corn bran supplemented with glycerol and soybean oil for the production of RL in SSF. In this study, 17 rhamnolipid congeners were identified.

Research conducted by Dabaghi et al. [25–27] explored RL production by *P. aeruginosa* PTCC 1074 using soybean meal and different types of bioreactors, from Erlenmeyer flasks to a rotating drum bioreactor.

Gong et al. [28] used polyurethane foam as an inert support in which the nutrient solution was impregnated and coconut oil as an inducer for the synthesis of RL. They also used air pulses; through a Plackett–Burman design, they found that coconut oil and NaNO_3 have an inducing effect on the production of RL, subsequently the afore mentioned factors were optimized through a Box–Behnken experimental design.

Ranjbar and Hejazi [29] evaluated the effect of temperature and initial moisture content on growth and RL by *Pseudomonas aeruginosa* using a mixture of corn bran and corn germ as substrate packed-bed bioreactors. The highest growth and RL production were obtained at 35 °C and an initial bed moisture of 70%.

Biosurfactant yields are, in some cases, limited or determined by the synthesis of the hydrophilic fraction. For example, in rhamnolipids, rhamnose synthesis is strain-limited; the formation of L-rhamnose from glucose-1-phosphate in *P. aeruginosa* is highly induced, whereas in *Escherichia coli* K12 or *P. putida* KT2440, which contain the *rml* gene, this activity is attenuated, thereby reducing RL formation [54]. Regarding the carbon source, it influences the synthesis of the hydrophobic fraction. In the study by Nicolò et al. [55],

when evaluating hydrophilic and hydrophobic carbon sources, they found that the carbon flow when using glycerol or glucose led them to obtain a greater quantity of Di-RL, while when using myristic acid, the activity of *rlhC* expression (responsible for the formation of Di-RL) decreased, and, as a consequence, a greater quantity of Mono-RL was obtained.

Some of the alternatives that have been used to improve yields include the overexpression of genes involved in biosurfactant synthesis or the deletion of biosynthetic pathways for other metabolites that compete for the substrate. Funston et al. [56] improved RL synthesis using *Burkholderia thailandensis* by knocking out the synthesis of polyhydroxyalkanoates that use hydroxyalkanoic acids as a precursor, which form the hydrophobic fraction of RL.

5.2. Sophorolipids

Sophorolipids consist of a hydrophobic hydroxylated fatty acid chain and a hydrophilic head formed by a disaccharide (sophorose). The fatty acid chain can be attached at one or two points to the sophorose molecule, giving rise to acidic and lactonic sophorolipids [57]. Figure 2 shows the structure of typical acidic and lactonic sophorolipids.

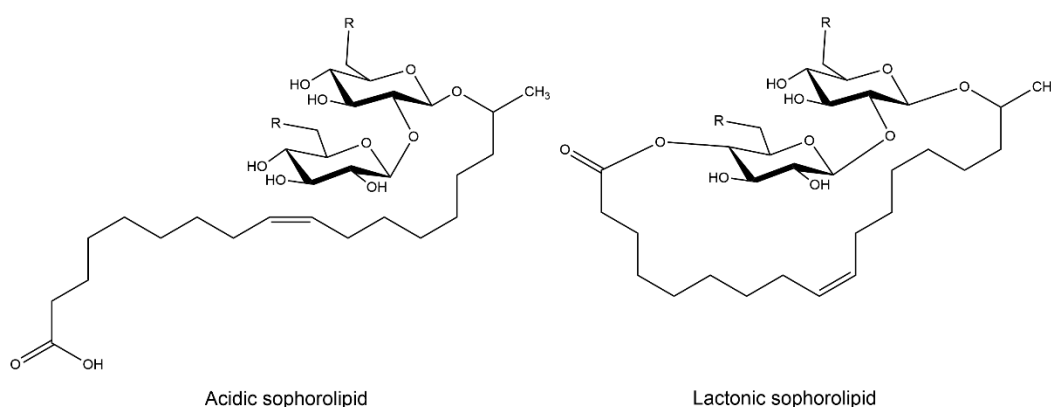


Figure 2. Chemical structure of typical acidic and lactonic sophorolipids.

Sophorolipids have excellent surface and interfacial tension properties. They also have antimicrobial, anti-inflammatory, spermicidal, anti-HIV, and even anticancer effects. Thus, these molecules could be used in the cosmetic and pharmaceutical industries, as well as for bioremediation purposes [7].

Sophorolipids are produced by some yeasts of the genera *Starmerella*, *Candida*, *Rhodotorula*, *Pseudohyphozyma*, *Cryptococcus*, *Cyberlindnera*, *Lachancea*, and *Wickerhamiella* [58]. *Starmerella bombicola* (formerly known as *Candida bombicola*) is the most efficient microorganism for sophorolipid production. *S. bombicola* produces trace amounts of biosurfactants when grown with glucose as the sole carbon source. However, when a hydrophobic carbon source is added to the culture medium, sophorolipid production increases significantly [59]. For example, Hirata et al. [60] obtained a sophorolipid concentration of 142.8 g/L and a product-substrate (Y_{PS}) yield of 72% with *S. bombicola* JCM 9596 in a 5 L stirred-tank bioreactor using waste frying oil and oleic acid as hydrophobic carbon sources and glucose as a hydrophilic carbon source. The sophorolipid concentration can be further increased by the addition of substrate during fermentation. For example, Pekin et al. [61] developed a process for obtaining sophorolipids with *S. bombicola* ATCC 22214 using corn oil and honey as substrates, obtaining a sophorolipid concentration greater than 400 g/L in a fed-batch system.

Recently, several researchers have studied the production of sophorolipids in SSF. One of the main advantages of this type of fermentation is the use of solid, fat-rich agroindustrial byproducts as an economical support and substrate. These investigations have been conducted primarily using winterization oil cake (WOC), a byproduct of vegetable oil

refining. For example, Jiménez-Peñalver et al. [16] used WOC and sugar beet molasses as substrates and wheat bran as an inert support to produce *S. bombicola* sophorolipids in SSF. They obtained a yield of 0.179 g of sophorolipids per g of dry matter after 10 days of fermentation in a laboratory-scale static bioreactor. Intermittent agitation led to a 31% increase in sophorolipid production. Subsequently, Rodríguez et al. [35] scaled up this process to 22 and 100 L mixed bioreactors, filled with 3 and 10.5 kg of wet matter, respectively. Under the conditions studied, they obtained yields of up to 0.195 g per g of dry matter.

The addition of hydrophobic substrates to a solid support can help increase yields. For example, Rashad et al. [36] used sunflower oil cake waste as a support, added with soybean oil as a hydrophobic carbon source, to produce sophorolipids from *S. bombicola*, obtaining a yield of 49.5 g of sophorolipids per 100 g of substrates. On the other hand, Jiménez Peñalver et al. [17] studied the production of sophorolipids using polyurethane foam as a solid support, stearic acid as a hydrophobic carbon source, and sugar beet molasses as a hydrophilic carbon source. Under the conditions studied, they obtained a yield of 0.211 g per g of substrate after 13 days of fermentation.

Sophorolipid production is related to the assimilation of hydrophobic substrates and tolerance to osmotic stress. Therefore, sophorolipids are produced primarily by osmophilic yeasts [62]. The wild strain *S. bombicola* ATCC 22214, isolated from bumblebee honey, has been the workhorse for sophorolipid production for decades. However, despite the high yields and productivity obtained, new sources and strain improvement are still being sought to increase the production of these biosurfactants [63]. For example, Liu et al. [64] studied the effect of a Rim9-like protein (Rlp) and three transcription factors (*ztf1*, *leu3*, *gcl*) on the production of sophorolipids by a wild-type *S. bombicola* strain. They used genetic engineering to construct a modified strain with three genes knocked out ($\Delta rlp\Delta leu3\Delta ztf1$) and observed an increase in the flow of glucose to SLs synthesis pathways, and a reduction in the synthesis of branched-chain amino acids. As a result, the sophorolipid production of $\Delta rlp\Delta leu3\Delta ztf1$ increased by 50.51% to that of the wild-type strain.

Regarding carbon sources, efficient sophorolipid production requires both a hydrophilic and a hydrophobic carbon source. The typical culture medium for sophorolipid production by *S. bombicola* consists of glucose, vegetable oil, and yeast extract or urea [63]. The carbon sources used affect not only the yield of sophorolipids, but also their chemical structure. Studies conducted in submerged culture show that sophorolipids produced from vegetable oils have a higher content of diacetylated lactones than those produced from their corresponding esters. Substrates rich in polyunsaturated acids, such as sunflower or linseed oil, lead to the formation of higher amounts of acidic sophorolipids [62]. However, information on the effect of the carbon source on sophorolipid synthesis by SSF is limited.

5.3. Mannosyl-Erythritol Lipids (MELs)

Mannosyl-erythritol lipids (MELs) are glycolipids containing 4-O- β -D-mannopyranosyl-erythritol or 1-O- β -D-mannopyranosyl-erythritol as the hydrophilic group, linked to a variety of fatty acids as a hydrophobic chain (Figure 3) [65]. MELs can be used in products such as detergents and dispersants due to their high hydrophilic properties and low critical micellar concentration. Their low toxicity and skin compatibility make them potential candidates for future use in the cosmetics industry [66].

MELs are mainly produced by yeasts of the genus *Pseudozyma*, such as: *P. tsukubaensis*, *P. churashimaensis*, *P. antarctica*, *P. aphidis*, *P. hubeiensis*, *P. crassa* and *P. siamensis*, and some fungi of the genus *Ustilago* [67]. The biotechnological production of these biosurfactants has been reported primarily as secondary metabolites in SmF using vegetable oils or other hydrophobic substrates as the sole carbon source. Under these conditions, biosurfactant

concentrations in the range of 30–40 g/L are achieved [68]. Furthermore, the addition of hydrophilic substrates significantly increases the production of MELs by these microorganisms. Using soybean oil and mannose or erythritol as a carbon source, MEL concentrations of up to 75 g/L per SmF have been obtained in shaken flasks [68]. The cultivation strategy also has a great impact on the yield obtained. For example, Rau et al. [69] They obtained up to 165 g/L of MELs in a stirred tank bioreactor using soybean oil and glucose as carbon sources, in a fed-batch culture [69].

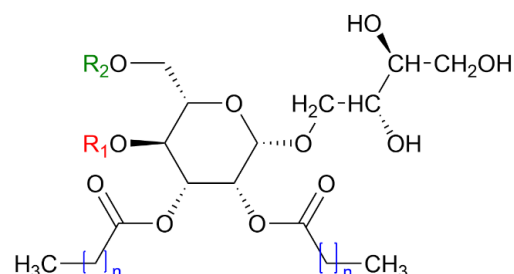


Figure 3. General chemical structure of MELs.

Recently, Bueno-Mancebo et al. [37] evaluated for the first time the production of MELs by *Moesziomyces bullatus* and *Ustilago maydis* in SSF. They used wheat bran as a support, WOC as a hydrophobic carbon source, and glucose as a hydrophilic carbon source. Under these conditions, they obtained up to 98 and 5.9 g of crude MELs per kg of WOC for *M. bullatus* and *U. maydis*, respectively.

5.4. Lipopeptides

These biosurfactants consist of a peptide linked to a lipid chain (Figure 4). The length, structure, and composition of the lipopeptide (LP) can vary, determining its physicochemical properties and biological activities. One of the most notable LPs is surfactin, produced by *Bacillus subtilis*, although some strains of the genus *Pseudomonas* also produce it. LPs are also classified based on their synthesis pathway. For example, iturin, surfactin, fengycin, and kurstakin are the most representative of the LPs synthesized by non-ribosomal synthases (NR-LPs) [70].

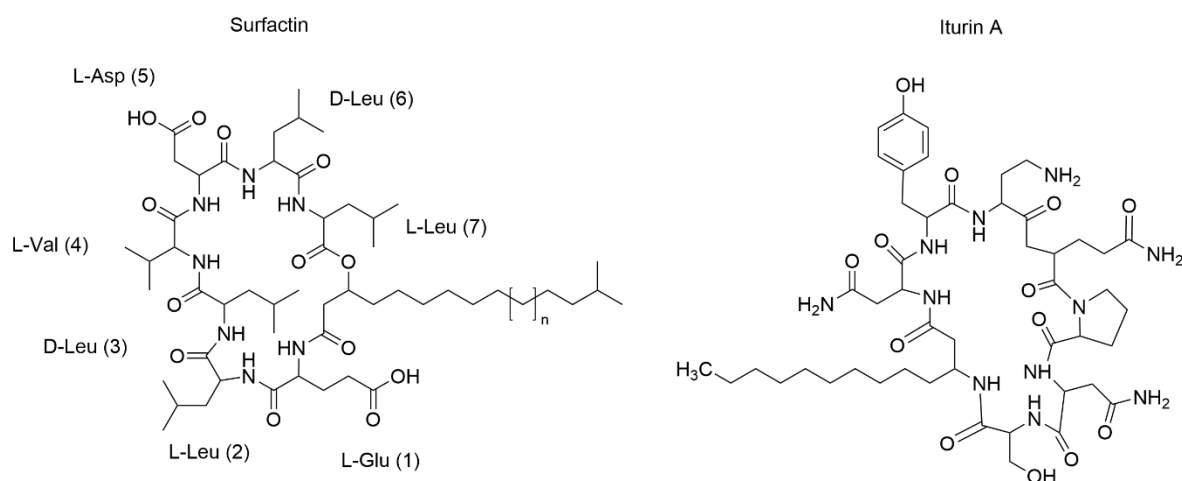


Figure 4. Structure of the lipopeptides surfactin and iturin A, n refers to the number of carbons in the chain.

Surfactin reduces the surface tension of water from 72 to 27 mN/m and has a CMC of around 10 mg/L. Among the applications and biological activities reported for surfactin are antibacterial activities and antitumor properties [71].

LPs have various applications, for example, for the elaboration of biodegradants, in the petroleum industry for the enhanced recovery of oil, and in bioremediation to remove heavy metals. Among the biological activities that have been reported are antioxidant activity, hypocholesterolemic activity, and immuno-modulatory activity, in addition to having an antifungal activity against several phytopathogens [72]. LPs have also been used in personal hygiene products such as shampoo, shower gels, and makeup removers. Furthermore, this LP has been documented to have antifungal activity and is used as a biocontrol agent.

Several authors have studied LP production in SSF using different supports, substrates, and microorganisms. Ohno et al. [48] studied surfactin production by a recombinant strain of *Bacillus subtilis*. They evaluated the effect of temperature and humidity on surfactin growth and production in SSF using *okara* as a solid support and compared it with the production in SmF. Under the best conditions (37 °C and 82% humidity), they obtained about 2 g per kg of wet matter. Surfactin production was 4 to 5 times more efficient in SSF than in SmF.

Zhu et al. [47] optimized surfactin production by *Bacillus amyloliquefaciens* in SSF using rice straw and soybean meal as supports and substrates. Under the selected conditions, they obtained a yield of 15.03 mg of surfactin per g of dry substrates in a 50 L bioreactor with 9 kg of dry support.

More recently, Valdés-Velasco et al. [45] evaluated surfactin and fengycin production by four wild-type *Bacillus* sp. strains and one mutant strain unable to develop biofilm on SSF and SmF. They used a defined medium and polyurethane foam as an inert support for SSF. The highest LP production was obtained with *B. subtilis* ATCC 21332 on SSF at 72 h of culture. Maximum lipopeptide production was 3.5 times higher in SSF than in SmF for this strain.

6. Techniques Used to Optimize Solid-State Fermentation

There are key techniques used to optimize SSF, maximizing process efficiency and improving the quality of the products obtained. Some of the most relevant are:

Microorganism selection: Through the identification and use of microorganisms that are highly productive and adaptable to the specific solid substrate used. For example, the use of spores, which serve as biocatalysts, facilitates reactions due to their practicality and adaptability, allowing them to have a longer shelf life, although a larger amount of inoculum is required during fermentation [73].

Substrate modification: By improving the physical and chemical properties of the solid material, such as its porosity, moisture, and nutrient content, to facilitate the growth of the microorganism used. Such as agro-industrial waste, due to its high nutrient content, such as sugarcane bagasse, rice straw, coffee waste, or cassava [74].

Moisture control: Maintaining adequate moisture levels in the system is essential to promote microbial growth and the production of desired compounds. If the substrate moisture content is too low, the solubility of the substrate and nutrients is reduced, and swelling can be observed. Conversely, if the moisture content is too high, oxygen transfer is hindered due to particle agglomeration [75,76].

Environmental conditions: Adjusting temperature, pH, and oxygen parameters to create an optimal environment for fermentation.

Temperature is an important factor, as it regulates microbial growth during the process of producing enzymes and other secondary metabolites [75,77]. Furthermore, pH is a very important parameter during solid-state fermentation, as it affects the growth of microorganisms by forming organic acids, which lowers the pH. Therefore, it must be constantly monitored [76,78].

On the other hand, agitation and aeration influence oxygen transfer, heat exchange, and mass transport during the process. In this sense, agitation is vitally important to maintain homogeneity and improve the process [76,79].

Use of specialized bioreactors: With the implementation of equipment specifically designed for spongy foam fermentation (SSF), better control of fermentation conditions is achieved, and process monitoring is facilitated [80].

Use of additives or pretreatments: Incorporation of necessary agents, such as additional nutrients, enzymes, or pretreatments, which promote the bioavailability of substrate components and improve process efficiency. Zhu et al. [81], used a highly effective method: incorporating three strains that control nutritional components, antioxidant activity, and antinutritional factors, achieving excellent results after 60 h, with significant changes after this time of fermentation.

Modeling and optimization using artificial intelligence: Emerging research focuses on the implementation of Artificial Intelligence (AI) techniques in solid-state fermentation to produce biofactants, although these techniques are not yet widely used. Studies using advanced statistical techniques, numerical optimization, predictive modeling, and some hybrid methods are an innovative strategy that allows for the optimization of different complex biotechnological processes. These methods can be considered within the AI spectrum, using artificial neural networks and genetic algorithms to predict and adjust optimal process conditions, such as temperature, humidity, and fermentation time [21].

The use of AI systems applied to the production of fermented food and beverage products has revolutionized the world of fermentation as a strategic tool for transformation. By integrating these intelligent systems, industries gain real-time control through sensors, automatically monitoring and reproducing data. Likewise, through algorithms and flowcharts, production reaches an optimal point, thereby increasing process efficiency and reducing production costs [82,83].

AI is being applied to technological innovation projects such as wine and kombucha production by Spanish companies, using AI designs and digital microscopy based on deep learning; the main objective is the detection of microorganisms during fermentation [84]. Jimenez-Peralta and Pizango-Linares [85] used an intelligent system, also based on deep learning, to optimize temperature and pH in cocoa fermentation, achieving greater efficiency compared to traditional methods and improving the sensory quality of the product.

Bezerra et al. [86], carried out an SSF using peach-palm residues for the production and optimization of cellulase through an artificial neural network and the ANN-AG genetic algorithm, which allowed determining the influence of optimal conditions on fermentation, reaching an algorithm efficiency in the process of 98% of the endoglucanase activity. These results indicate that the biotechnological use of residues is a process with great applications in the production of biofuels and food through AI algorithms and neural networks.

The application of AI with innovative techniques such as neural networks in the fermented beverage industry allows for greater efficiency in the quality of the final product with the identification of patterns through the application of algorithms that predict and identify parameters in fermentation, improving the results of the process, for example, in the aromatic profile of wine or beers, taking into account their organoleptic characteristics [87–89].

Rodera Martínez [90] developed a case study developed with sustainable biomaterials through fast and accurate simulations for the optimization of bacterial cellulose as a natural polymer produced by fermentation of bacteria of the genus *Gluconacetobacter*. The experiment was based on published data using AI tools such as real-time monitoring systems that include sensors and algorithms to predict optimal process conditions and

cellulose obtaining parameters, improving the quality and increasing the production of the biomaterial.

Research continues, and according to Beatriz-Cuellar et al. [21], AI techniques have a promising future that will allow them to be applied in more complex biotechnological processes, which will translate into more sustainable, profitable, and more precise growth in fermentation industries.

7. Challenges for the Production of Biosurfactants in SSF

Biosurfactant production has been primarily studied through submerged fermentation. Recent studies have shown the advantages of SSF for obtaining biosurfactants in SSF. Moreover, SSF presents significant challenges for commercial biosurfactant production, such as difficulties in monitoring and controlling process variables, heat dissipation, and SSF scale-up.

7.1. Monitoring and Controlling Process Parameters

SSF is a process that involves the growth of microorganisms on the surface of wet solids. It is a heterogeneous process, which makes monitoring and controlling operating parameters difficult.

pH is a critical parameter that affects microbial growth, enzyme production, and overall microbial activities. However, SSF presents unique challenges for real-time monitoring of pH [91]. Jiang et al. [92] developed a non-invasive method based on Fourier-transform near-infrared (FT-NIR) spectroscopy for the determination of pH in an SSF system. They used a genetic algorithm and synergy interval partial least-squares to calibrate the method. More recently, Kabir et al. [91] successfully used a conventional pH probe to monitor the pH of an SSF for the production of hydrolytic enzymes by *Aspergillus niger*. However, due to the heterogeneous distribution of moisture, biomass, and metabolic activity, the measurement from an inserted probe may not fully represent conditions across the substrate. Additionally, the low water activity of SSF matrices may lead to poor probe-substrate contact.

Temperature is a key parameter for microbial growth. Real-time monitoring is necessary to ensure proper operation of SSF systems. This is a relatively easy parameter to measure. However, due to the heterogeneity of the solid substrate, multiple probes must be inserted into the reactor to obtain a reliable temperature measurement. Thermocouples are the most widely used temperature sensors in industrial environments due to their low cost, wide measurement range, and fast, linear response. On the other hand, Resistance Temperature Detectors (RTDs) are more suitable for monitoring SSF systems due to their fast, stable response and the lack of periodic calibration [93].

Although SSF has low water content, the moisture of the substrate is a key parameter of the process. Therefore, it is necessary to measure the moisture during the fermentation process to ensure that the ideal operation is maintained. The traditional dry weight method is time-consuming and cannot achieve the purpose of real-time monitoring and control. To achieve rapid and non-destructive online monitoring of moisture, several methods, including nuclear magnetic resonance, near-infrared spectroscopy, and hyperspectral imaging, have been studied [94]. These methods provide reliable real-time information on moisture. However, they require expensive equipment and have not been widely adopted.

Biomass is the most important variable for assessing the state of the culture. However, no reliable method is available for online measurement of biomass in SSF bioreactors [93]. Online gas analysis in the inlet and outlet gas stream is very useful for indirect measurement of microbial growth in SSF [95]. Exhaust gases can be monitored by gas chromatography or using specific sensors for CO₂ or O₂ [93]. The analysis of exhaust gases can be used to determine oxygen transfer rate, CO₂ production rate, and respiratory quotient, and to

determine physiological stages in aerobic SSF. This data could be useful for the quantitative comparison of cultures carried out under different conditions [95].

7.2. Heat Transfer

Due to the nature of the SSF, the dissipation of metabolic heat is one of the factors that could interfere with the production of metabolites of interest. Depending on the type of bioreactor (tray, packed bed, mixed, or stirred bioreactors), the temperature within the bed can be monitored and thus maintained at adequate conditions so that the temperature is homogeneous and does not interfere with the metabolic activity of microorganisms. One of the actions that helps reduce heat accumulation is the use of forced aeration in column and packed bed bioreactors, while in tray reactors, aeration is by natural diffusion [96]. Another strategy to reduce heat accumulation is the use of cooling jackets, the height and porosity of the bed for tray bioreactors [97]. Lopez-Ramirez et al. [98] used a stirred bioreactor to reduce heat build-up, agglomerate formation, maintain homogeneity of the system, and increase mass transfer.

7.3. Scale up of SSF

One of the main challenges to producing biosurfactants in SSF is the scaling up of the process. Most research on biosurfactant production in SSF has been conducted at the laboratory level; only a few studies in large-scale bioreactors have been published [35,47]. Scale-up practices are process-specific, and the information on bioreactor design and the effect of fermentation conditions on microbial growth and metabolite production is scarce [99]. The scaling up of the SSF process is considered a bottleneck because the accumulation of heat and the apparition of water and gas gradients inside large-scale bioreactors [24].

Zhu et al. [47] studied surfactin production in SSF by a *Bacillus amyloliquefaciens* strain using soybean flour and rice straw as substrates. Under optimal conditions, they estimated a production of 15.17 mg of surfactin per g of dry substrate. They scaled up the process by a factor of 1000 using a 50 L mixed bioreactor (50 rpm) with forced aeration (0.4 vvm) and observed no differences due to the production scale. However, the authors do not mention the criteria they used for scale-up.

Rodríguez et al. [35] scaled up the production of sophorolipids by *S. bombicola* in SSF using winterization oil cake and molasses as substrates, and wheat straw as solid support. They used the aeration rate from a lab-scale bioreactor (0.5 L) to pilot-scale a bioreactor (22 and 100 L). They initially used the aeration rate determined in a laboratory-scale bioreactor (0.5 L) to scale up the process to a 22 and 100 L bioreactor. However, during fermentation in the pilot-scale bioreactors, temperatures rose to over 40 °C. Increased aeration and intermittent mixing were necessary to dissipate heat and control temperature. Despite the difficulties in controlling bioreactor temperature, sophorolipid yields were similar to those obtained at lab-scale under controlled temperatures.

8. Conclusions

SSF is a promising technique for large-scale biosurfactant production. It solves some technical problems, such as foaming, and allows the use of agroindustrial byproducts as support and substrate without the need for costly pretreatment such as hydrolysis or saccharification. If the substrates and growth conditions are appropriate, higher product-to-substrate yields and higher volumetric product concentrations can be obtained compared to SmF, leading to a reduction in the reactor volume required to obtain biosurfactants.

Studies on biosurfactant production in SSF are limited, but significant advantages over SmF have been observed. However, the exact causes of this behavior are unknown.

Transport phenomena have been hypothesized to play a crucial role in explaining the advantages of SSF; therefore, further study of mass and heat transfer is necessary to better understand this system and achieve commercial-scale biosurfactant production in SSF.

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Abbreviations

The following abbreviations are used in this manuscript:

AI	Artificial intelligence
CMC	Critical micellar concentration
LP	Lipopeptide
MELs	Mannosyl-erythritol lipids
RL	Rhamnolipid
RTDs	Resistance Temperature Detectors
SmF	Submerged fermentation
SSF	Solid State Fermentation
Y _{PS}	Product-substrate yield
WOC	Winterization oil cake

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