

Biosurfactants: Sustainable and Versatile Molecules

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Biosurfactants are amphipathic molecules produced by plants, animals, and microorganisms, that present emulsifying properties and may act reducing surface and interfacial tensions. When compared to synthetic surfactants, these biological analogues have high biodegradability potential, and may be produced from renewable raw materials within overall biotechnological processes involving low generation of residues. The production and application of microbial surfactants have been recently considered in several industrial sectors, as these low toxicity versatile compounds find applications in food, pharmaceutical, cosmetic, and petrochemical products, in nanotechnology and agriculture, and in the bioremediation of xenobiotic-contaminated areas. Herein, the main conceptual aspects and physicochemical properties, as well as the classifications of biosurfactants according to their origin and their chemical structures, are addressed. The production of microbial biosurfactants through sustainable processes are also described, with particular focus on new applications and on the increasing relevance of such bioproducts for the sustainable development of modern society.

Keywords: renewable resources, biotechnology, biorefineries, sustainable products, biosurfactants

1. Introduction

The current environmental concerns related to the burning of fossil fuels—especially petrol and coal, have led to an increased social awareness that has motivated the search for sustainable processes. In fact, practically various countries have adopted specific measures to follow the concept of sustainable development, aiming at minimizing the climate and environmental crisis that puts the whole planet in danger. One of the strategies used in this context involves strong investments in the development of sustainable bio-based products to replace, at least partially, petroleum-based synthetic commodity products.¹

Surface active agents, also known as surfactants, are broadly used in different industrial, domestic, and biological processes, performing functions as emulsifiers, wetting, suspension and phase dispersion agents, lubricants, among others.² They are, therefore, of significant importance in people's daily lives, representing a market in full growth, with

a compound annual growth rate (CAGR) of 5.6% between 2017 and 2022.³⁻⁵ However, surfactants have contributed to massive pollutions of water sources and groundwater, which may be exemplified by the foams of Tietê's river stretches in Brazil, especially between the cities of Pirapora do Bom Jesus (SP) and Salto (SP) during the dry months, when the rivers' flow is smaller, and the concentration of wastes increases. The search for benign analogues that present similar physicochemical properties, increased biodegradability, and low toxicity is, therefore, mandatory.^{2,6}

In recent decades, the concept of biosurfactants has become widespread, which reflected in the number of related scientific publications. Between years 2011 and 2021, publications with the term "biosurfactant" increased by 223.6%, and several companies have sought to include these bioproducts in their portfolios.⁷ Such natural surfactants, whose chemical structures differ widely depending on their origins, possess excellent physicochemical and biological properties, and potential biodegradability, biocompatibility, and low toxicity, with the additional advantage of being produced from renewable raw materials, such as agro-industrial by-products.^{8,9} Therefore, biosurfactants are

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considered sustainable biobased products that may be easily inserted in the context of biorefineries and applied in the most varied industrial segments.¹⁰

Herein, the conceptual aspects, physicochemical properties, and classifications of biosurfactants according to their origin and chemical structures will be addressed, considering their technological, economic, and environmental relevance. The production of microbial biosurfactants through sustainable processes (fermentation processes and via enzymatic syntheses) will be additionally discussed, together with potential new applications and their role on the sustainable development of modern society.

2. Biosurfactants: Main Definitions and Current Interest

Biosurfactants are metabolites produced by animals, plants, or microorganisms, that bear a pronounced amphipathic character that is responsible for typical surface-active and/or emulsifying properties. Such properties are related with the critical micelle concentration (CMC) and the hydrophilic-lipophilic balance (HLB). By definition, the CMC is the concentration from which the micelle formation process begins; it is an intrinsic and characteristic property of surfactant substances at a given temperature and a given electrolyte concentration. In general, micellar structures are classified as normal or reverse, depending on the specific medium.¹¹ Normal micelles, on the one hand, are aggregates of surfactants built in a polar (mostly aqueous) media, forming a polar surface of hydrophilic portions directly in contact with the media and a nonpolar central nucleus. In reverse micelles, on the other hand, aggregates are usually formed in nonpolar media, and the hydrophilic groups are concentrated within the micellar aggregate, therefore forming a hydrophilic central core.¹²

The combination of the hydrophilic and lipophilic groups of a given surfactant molecule possess, as a result, a balance related to the corresponding size and strength of these two groups, which is the hydrophilic-lipophilic balance (HLB). According to the precise HLB value, surfactants are classified in water/oil emulsifier, oil/water emulsifier, wetting agent, detergents and solubilizers^{13,14} (Table 1).

Table 1. General applications of surfactants with respect to the HLB value

HLB range	Utilization
4-6	water/oil emulsifier
7-9	wetting agent
8-18	oil/water emulsifier
13-15	detergents
15-18	solubilizers

HLB: hydrophilic-lipophilic balance.

Although the common sense tends to agree that bioemulsifiers and biosurfactants belong to the same group in terms of chemical structures, physicochemical properties, and physiological functions, a recent and pertinent contribution of Uzoigwe *et al.*,¹⁵ correctly stated otherwise. In recent years, in addition to surface-active and emulsifying properties, several studies have also highlighted some biological functions of biosurfactants, such as larvicidal and mosquitocidal activity,¹⁶⁻¹⁸ as well as antimicrobial,¹⁹ antitumor,²⁰⁻²² anti-inflammatory,^{23,24} and immunomodulatory²⁵⁻²⁷ properties, which puts them into the spotlight for various applications in industrial segments.

As it is the case for synthetic surfactants, biosurfactants may be classified with respect to their origin, chemical composition, and to the charge of the hydrophilic portion. A more recent classification separates biosurfactants into first and second generation compounds.¹⁸ First-generation biosurfactants, on the one hand, include alkylpolyglycosides and sugar esters, and despite being produced from natural raw materials (sugars and vegetable oil), other organic synthesis techniques are needed to achieve the precise target chemical structure. On the other hand, second-generation biosurfactants are biosynthesized by animals, plants or microorganisms by fermentation and enzymatic processes using agro-industry by-products as raw materials.²⁸ This current classification is less complex than other existing ones, allowing for an easy and systematic understanding.

In addition to their outstanding physicochemical and biological properties, biosurfactants have also been highlighted as ecofriendly, biobased sustainable products. With respect to synthetic petroleum-derived surfactants, biosurfactants have equal (and, sometimes, superior) physical-chemical properties, are potentially biodegradable, have low toxicity, and may be produced from industrial wastes. However, the production of biosurfactants on industrial scale and their further commercialization still present some bottlenecks,²⁹⁻³¹ such as:

- (i) Overall costs: most biosurfactants are produced by fermentation processes, enzyme-based routes, or from extractives, which are still expensive methods that may increase the price of the final products up to 50 times when compared to synthetic surfactants;
- (ii) Low productivity: biosurfactants are mostly produced in low concentrations and the processes often require long time intervals;
- (iii) Downstream step: the purification of the target biosurfactant from the cultivation medium often requires several steps, reducing product recovery and generating large amounts of waste.

Despite the abovementioned disadvantages, the current appeal for sustainable development have propelled the industrial production of biosurfactants by several companies around the world, and they are now considered real potential substitutes for synthetic surfactants.

2.1. Classification of biosurfactants according to their origin

As previously mentioned, biosurfactants may be produced by animals, plants, and microorganisms, or may be synthesized via enzymatic routes. In each case, specific structural characteristics and physicochemical properties are obtained.

2.1.1. Animal biosurfactants

Bile salts are natural phospholipids and monoacylglycerols surfactants produced in the liver of most vertebrates as greenish-brown or yellowish fluids, which are stored in the gallbladder and released into the duodenum to help emulsifying lipids for further digestion and intestinal absorption.^{32,33} After emulsification, the lipases dissolved in the aqueous medium of the intestinal tract catalyze lipid digestion. The lipid dissolution in the presence of bile salts occurs because micelles do not coalesce, as their negatively charged surfaces repel each other electrostatically,

remaining dispersed in the aqueous medium. Also, the hydrophilic polar character of the surface allows the dispersion of micelles in the aqueous environment of the intestine, enabling the action of lipases.³³⁻³⁵ Therefore, the action of bile salts is crucial for the digestion and absorption of most of the lipids present in food. Commercial animal biosurfactants are usually extracted from the gallbladder of animals such as bears, swine, cattle, and poultry such as chicken, ducks, or goose, or produced by synthetic methods.^{36,37} Xu *et al.*³⁸ reported the possibility of industrially producing tauroursodeoxycholic acid and its derivatives via fermentation using an engineered *Escherichia coli* strain. The production of bile salts using fermentation or biocatalysis represents itself a great advance over the direct animal extractive processes, which is strongly criticized by several protective institutions.

In general, bile salts have an anionic steroidal core bearing a 24C-cyclic structure of cyclopentanoperhydrophenanthrene, derived from cholesterol (Figure 1). The main human bile salts are cholate, chenodeoxycholate, deoxycholate, glycocholate, lithocholate, and taurocholate. Other less common bile salts such as ursodeoxycholate and obeticholate are also produced by other vertebrates. According to their production in the human body, bile salts are classified as primary and secondary, with the former

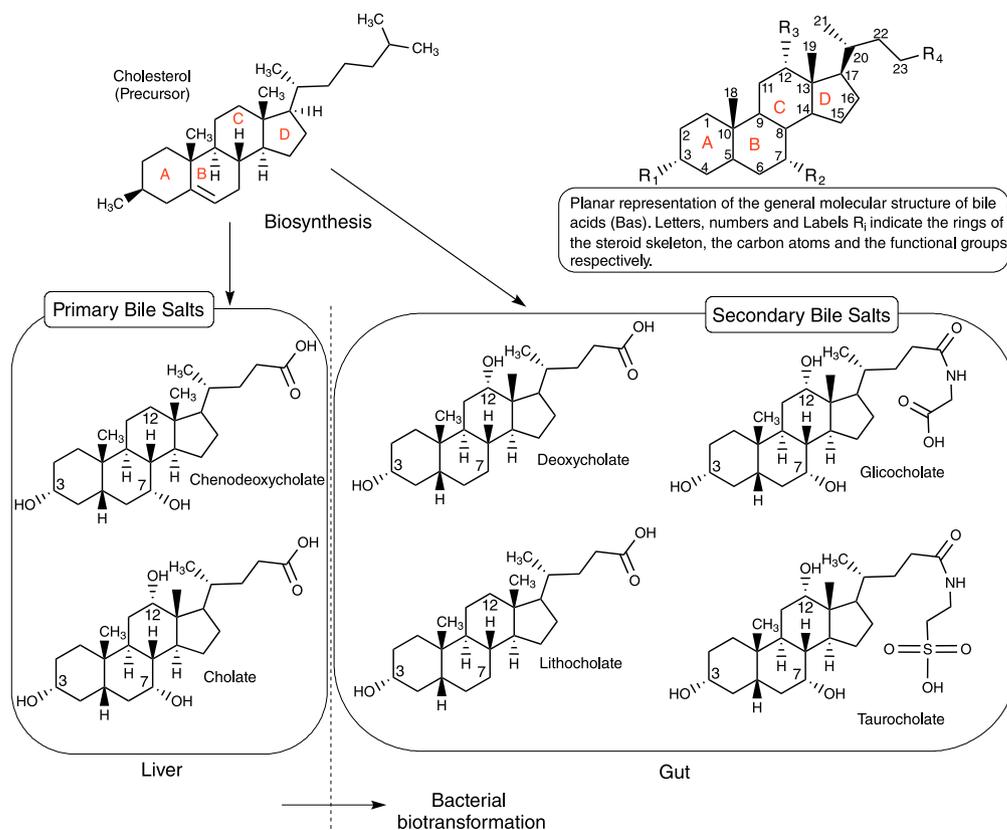


Figure 1. Types of animal biosurfactants.

being produced in the human liver, and the latter as a result of biotransformations of primary bile salts by the action of the intestinal microbiota (Figure 1).^{33,39}

Concerning their physicochemical properties, bile salts produce, in general, micelles with smaller dimensions when compared to other biosurfactants, which is of course dependent on the precise chemical structure (hydrophobicity, hydrophilicity, presence of charges, and steric effects). The critical micellar concentrations (CMC) range from 2 to 19 mmol L⁻¹ for natural bile salts and from 20 to 135 mmol L⁻¹ for semi-synthetic analogues. The presence of the molecular rigid cyclic core, on the one hand, allows the formation of bile salts-aggregates with specific geometries, and on the other, hinders the possibility of adopting the same rules used to estimate the head-tail self-assembly geometry model. Furthermore, bile salts form micellar systems in which the polar and non-polar domains are not completely separated, and the corresponding interactions are driven by hydrophobic forces and hydrogen bonds of functional groups.³⁶⁻⁴⁰

In addition to emulsification, bile salts possess other fundamental biological roles, such as antimicrobial and anti-inflammatory ingredients,⁴⁰ and the pharmaceutical industry often apply them as therapeutic agents, pro-drug formulations, and in drug delivery systems. Many of these applications are enhanced after chemical modification, which mainly occur through the C24 carboxylic function and hydroxyl groups to form derivatized or semi-synthetic bile salts. In this context, avicholate (avicholic acid sodium salt and 6 α -ethyl-avicholic acid sodium salt), synthesized from 16-epi-avicholate (3 α ,7 α ,16 β -trihydroxy-5 β -cholan-24-oic-acid sodium salt and 3 α ,7 α ,16 β -trihydroxy-6 α -ethyl-5 β -cholan-24-oic-acid),³² may be highlighted.

Mikov *et al.*⁴¹ and Sarenac and Mikov³⁶ reported the antimicrobial effect of taurocholate-3-sulfate against *Herpes simplex* 1 and 2 viruses, human immunodeficiency virus (HIV), *Neisseria gonorrhoea*, and *Chlamydia trachomatis*, with low or no toxicity to human cells. Ursodeoxycholate and chenodeoxycholate also exhibited anti-inflammatory effects against primary biliary cirrhosis (PBC), cholangitis, choledocholithiasis, and hepatolithiasis, often associated with Caroli syndrome. Other interesting results of the *in vitro* hypoglycemic effect of a 3 α bile salt derived from cholate, 7 α -dihydroxy-12-keto-5 β -cholate, in association with glicazide or stevioside, were also reported.⁴¹ Due to their ability to form liposomes, particularly known as bilosomes, bile salts can also be applied in the formulation of drug delivery systems. Bilosomes help to stabilize administered drugs, facilitate transmembrane transport, and increase absorption efficiency. Bilosomes of sodium glycocholate, sodium

taurocholate, and deoxycholate were simultaneously used in the development of drug delivery systems with insulin.^{40,42} Bilosomes of deoxycholate with cefotaxime showed outstanding physicochemical properties that allowed the drug to be administered orally.⁴³ Chilkawar *et al.*⁴⁴ reported the use of bilosomes in the development of vaccines for hepatitis B, tetanus, and influenza A.

2.1.2. Plant biosurfactants

Saponins, or saponosides, are well-known biosurfactants produced by plants as secondary metabolites that regulate the plant growth and act as a natural defense against insects and pathogens.⁴⁵ With respect to the chemical structures, saponins may appear as steroid glycosides (steroidal saponins) or polycyclic terpenes (triterpenic saponins), with molecular masses ranging from 600 to 2000 g mol⁻¹.⁴⁶ Saponins may be obtained by various plant species via extractive methods, such as autoclave, microwave, and thermostatic bath, using water or common organic solvents, like methanol and ethanol.⁴⁷

Saponins bear a dual chemical structure, with lipophilic (triterpene or steroid) and hydrophilic (sugars) sites, which are determinant to their surface-active and emulsifying properties and the reason why they are said to be natural plant detergents or plant biosurfactants.^{48,49} The lipophilic region of the molecules is commonly referred as aglycone, and the hydrophilic one as a glycoside (Figure 2).

Steroid saponins bear 27 carbon atoms and present the aglyconic group derived from cyclopentanoperhydrophenanthrene (steroid nucleus), as it is the case for hecogenin; triterpene saponins have 30 carbon atoms and a triterpene nucleus as an aglyconic portion, as it is the case for hederagenin and oleanolic acid.⁴⁹ In both saponins, the aglyconic portion is biosynthesized in the isoprenoid pathway, which is widely studied in secondary plant metabolism. The aglyconic portions can undergo rearrangement reactions, cyclizations, oxidations (formation of hydroxyl, carbonyl, and carboxyl groups), glycosylations, unsaturations, and degradations. In addition, the presence of groups such as DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) and angeloyl as hydroxyl substitutes leads to subdivisions in the steroidal and triterpenic groups of saponins.⁵⁰⁻⁵²

The glycoside portion of saponins can be formed by monosaccharides, such as D-glucose, D-galactose, D-glucuronic acid, D-galacturonic acid, L-rhamnose, L-arabinose, D-xylose, D-fucose, and glucosamine, and by oligosaccharides.⁴⁵ Saponin glycosylation is generally associated with the modulation of metabolite stability, biological activity, solubility, and signaling for cell accumulation or transport.⁵³

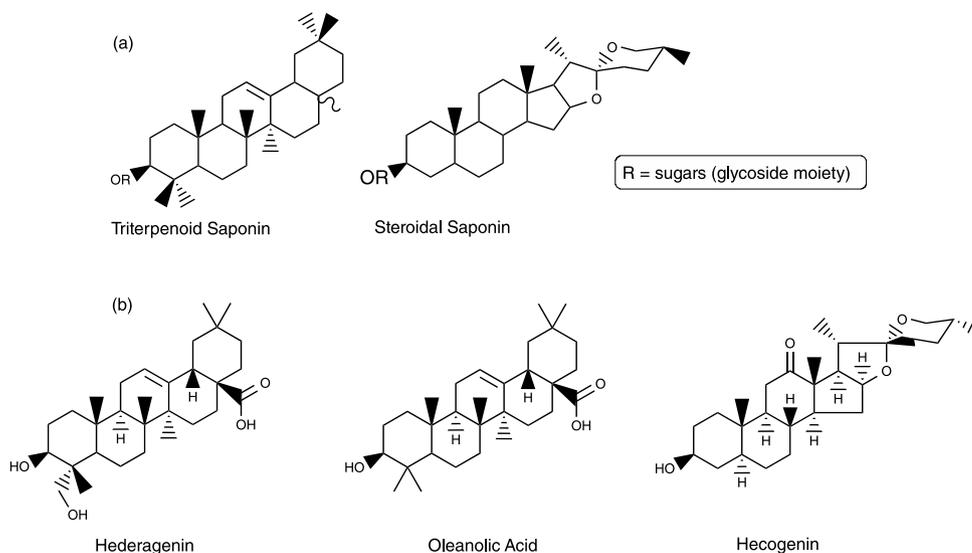


Figure 2. Types of plant biosurfactants.

In addition to the classification regarding the aglyconic group, saponins can also be classified as mono-, di-, or tridesmosidic saponins, according to the number of glycosylations, and with respect to their acid-base character. Acid saponins have carboxylic groups in the aglyconic or glycoside part; basic saponins have nitrogen atoms in the glycoside portion, usually in the form of secondary or tertiary amines.⁵⁴

The broad structural diversity of natural saponins results in a variety of corresponding physicochemical properties, such as foaming, emulsification, solubilization, sweetening and bitterness, and biological properties, such as membranotropic, antimicrobial, and antitumor effects.^{45,52,55,56}

The micellar properties of saponins, such as CMC, maximum surface density, and aggregation number, are influenced by physicochemical variables such as temperature, salt concentration, pH, concentration, and chemical nature of the solvent in which they are dissolved. Furthermore, micelles formed in aqueous solutions can vary in size and shape depending on the type of saponin.⁵⁷ These facts were described in the studies of Mitra and Dungan⁵⁷ and Pekdemir *et al.*,⁵⁸ who demonstrated that the CMC values of saponins from *Quillaja saponaria* and Indian nut saponins varied significantly with pH, temperature, and ionic strength of the medium. Furthermore, the maximum surface densities of saponins reached values over 80 A² (higher than those of synthetic surfactants), and a probable lay-on orientation (all hydrophilic groups immersed in water) of the molecules at the water-air interface.^{57,59}

As multifunctional molecules with amphipathic structures, saponins easily interact with other biomolecules,

which is of fundamental importance for their various applications. Studies regarding the interaction of saponins with sterols and proteins are very useful to explain their biological properties, such as (i) membranotropic and antimicrobial effects; (ii) hypocholesterolemic effect in animals and humans; (iii) the induction of anti-inflammatory, immunomodulatory, hepatic and neuroprotective actions; (iv) apoptosis and adipogenesis; (v) inhibition of deoxyribonucleic acid (DNA) and metalloprotease synthesis in tumor cells, which may contribute to an anti-tumor and anti-metastatic action; and (vi) inhibition of collagenase, elastase and hyaluronidase enzymes found in the skin, which may be associated with an anti-aging action and with the inhibition of the renin-angiotensin system, therefore preventing hypertension problems.^{45,52,60-62}

Saponins can also be applied as larvicides to combat vectors that cause neglected diseases, as it is the case of their demonstrated interaction with lipid molecules present in the cuticle of *Aedes aegypti*, for example.⁶³ This is a burgeoning field of research, with many studies still being carried out to verify new potential applications. The pharmaceutical industry has been testing saponins as precursors for steroidal compounds such as hormones, contraceptives, and diuretics, and as adjuvants to increase the immune response in vaccines.⁶⁴⁻⁶⁷ Recently, saponins obtained from *Quillaja saponaria*, a tree of the Andes region (South America), have been used in the formulation of soaps, shampoos, bath salts, among other cosmetic products.

Chemically modified (or semi-synthetic) saponins find innumerable applications in the pharmaceutical industry, with molecular derivatizations often carried out in the sugar chain or in the aglycone portion by means

of organic synthesis techniques or by biotransformations using microorganisms or enzymes. As examples of the most common structural changes in saponins, one can mention glycosylations, which aim to increase the glycidic content and solubility of saponins, changing the structure-activity relationship and enhancing their pharmaceutical applications. Structural modifications made at the aglyconic portion can also improve biological activities and potential pharmaceutical uses due to their limiting hemolytic and cytostatic properties.⁴⁸

In addition to saponins, other surfactant compound that is worth of mention is betaine, a glycine derivative bearing three methyl groups attached to the nitrogen atom that possess a direct effect on the osmotic balance of plants. Betaine and their derivatives (mainly cocamidopropyl betaine) are widely applied in cosmetic formulations.⁶⁸

2.1.3. Biosurfactants produced by enzymatic routes

Sugar esters or glycan-based surfactants are synthesized from renewable substrates, such as sugars and fatty acids, with great chemical diversity, enabling specific applications for each case (Figure 3). Their particular structural characteristics and physicochemical properties make them comparable or even superior, to synthetic surfactants in terms of efficiency.⁶⁹ The large-scale production of biosurfactants based on fats, oils, and sugars is relatively recent when compared to conventional synthetic surfactants. At the present moment, the most important sugar-based surfactants are alkyl polyglycosides, sorbitan esters, and sucrose esters.⁷⁰

The syntheses of glycan-based surfactants can be carried out by chemical or enzymatic processes. Chemical methods have a high cost because, for the esterification reactions to occur, high temperatures, pressures and toxic solvents are needed, and the overall reaction is often limited to low selectivity, resulting in heterogeneous mixtures of products with different degrees of esterification, which can affect the quality of the final product.^{71,72} The enzymatic process, on the other hand, uses lipases as catalysts.⁷³

The lipases (EC 3.1.1.3) considered for the synthesis of sugar esters are typical hydrolases, used in the hydrolysis of lipids in aqueous media. In the presence of moderate water contents, however, the reaction equilibrium shifts to

the synthesis of the ester bonds. Fungal-based extracellular lipase enzymes, such as those produced by *Candida rugosa*, *Candida antarctica* and *Geotrichum candidum*, are the most promising for industrial applications, as they exhibit selectivity, stability, and substrate specificity, and the large-scale production is viable.^{73,74} A relevant drawback of the enzymatic synthesis of biosurfactants is the limited production related to the low solubility of the sugars in a media containing organic solvents of reduced polarity. Also, the concentration of water in the reaction medium must be minimized to avoid hydrolysis of the ester bonds formed. Research initiatives aim at designing strategies to overcome this limitation by employing, for example, ionic liquids, supercritical carbon dioxide, and deep eutectic solvents.⁷⁵⁻⁷⁷ Also worth of mention are the high enzyme costs, and further studies are being conducted all over the world to optimize the production and to make enzymes cheaper for large-scale applications.

Sugar esters have emulsifying, foaming and stabilizing properties, and present therefore potential application as food additives. Sucrose monolaurate, maltose monolaurate, lactose monolaurate, sucrose monodecanoate, maltose monodecanoate, lactose monodecanoate, sucrose monoctanoate, maltose monoctanoate and lactose monoctanoate were tested and presented CMC values ranging from 0.31 to 0.78 g L⁻¹, and hydrophilic-lipophilic balance (HLB) between 12.4 and 14.5 g L⁻¹.⁷⁸ Although several sugar esters have outstanding physicochemical properties for the food industry, only the sucrose ester has been applied in food products so far.⁷⁹⁻⁸² It should be noted that due to the raw materials needed for the production of biosurfactants from enzymatic routes, lignocellulosic and oleaginous biorefineries can be highlighted as potential biofactories for such compounds.

In opposition to the extractive methods previously mentioned, that inevitably cause direct and indirect socio-environmental problems, such as the reduction of biodiversity and the extinction of animal and plant species, alternative technologies are now being considered. In fact, several animal protection organizations have issued warnings against traditional medical practices that extract and use bear bile to treat diseases, such as coronavirus disease 2019 (Covid-19).⁸³ The concern is mainly because

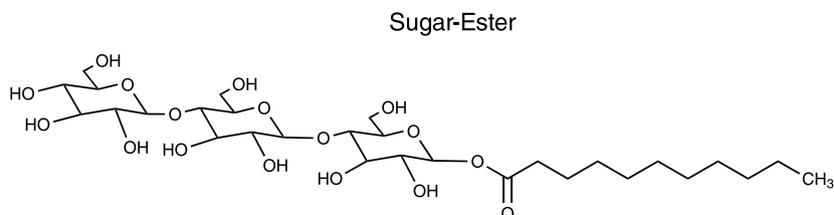


Figure 3. Structure of enzymatic biosurfactant.

during the processes of biosurfactants extraction from animals, direct contact between a man and wild animals can lead to the adaptation of many zoo pathogens, in a process known as spillover that can lead to epi- and pandemics. Plant biosurfactants, on the other hand, require large land areas for cultivation, and the structure-property relationship of the isolated products depends on plant species and edaphoclimatic conditions, which directly interfere in the corresponding productivity. Enzyme-based and chemically modified biosurfactants are safer processes that, however, involve expensive substrates and still demand optimization steps to reach economic viability. The whole scenario points out, therefore, to biosurfactants of microbial origin, that exhibit extraordinary overall cost-benefits and are promising biomolecules that can be easily and advantageously inserted to the biorefinery context.

2.1.4. Microbial biosurfactants

Microbial biosurfactants are produced by bacteria, yeasts, and filamentous fungi via different bioprocesses.^{6,84} Studies have demonstrated that biosurfactant and bioemulsifier-producing microorganisms are found in terrestrial habitats, such as soils and rocks, and in aquatic ecosystems,⁸⁵⁻⁸⁷ and several recent contributions highlighted the scientific and technological importance of biosurfactant-producing microorganisms that may be isolated from extremophiles environments, such as marine environments of high salinity, regions of extreme temperatures, such as Antarctica, and desert and volcanic regions.⁸⁸⁻⁹⁰

Despite their unquestionable importance for many industrial sectors and for daily life, the biological function of biosurfactants in microorganisms is still not fully understood. It is known, however, that many physiological functions that support the development of microorganisms and their survival in their natural habitats can be performed by surface-active molecules^{91,92} that provide emulsification, solubilization, transport of insoluble compounds in aqueous media, cell release in biofilms, and antimicrobial activity.^{69,87}

3. Why do Microorganisms Produce Biosurfactants?

During microbial growth, the ecological processes are carried out by microbial interactions with different environments.⁹³⁻⁹⁵ Some recent observations indicate that biosurfactants have different biological roles, that may range from enabling the assimilation of water-insoluble nutrients to serving as nutrient reserves. Also, they promote motility behaviors, assist in biofilm development, and act as antimicrobial and antiviral agents.^{96,97}

Under certain environmental conditions, microorganisms need to emulsify, solubilize, and transport water-insoluble compounds that may be used as substrates for energy generation. Biosurfactants are key molecules in this case, as they act as mediators for establishing the proper contact with hydrophobic substrates.^{93,98,99} This characteristic is often observed with microorganisms that are found in sites contaminated with hydrophobic organic compounds, and biosurfactants have, then, a clear role in hydrocarbons assimilation and biodegradation by microorganisms.¹⁰⁰⁻¹⁰³

The antimicrobial activity of biosurfactants can additionally favor the microbial survive,^{104,105} as they can provide a potential defense mechanism when the microorganism is in an environment of competition for resources in different niches.^{106,107} The property is a result of the interaction of biosurfactants with the lipids of the plasma membrane, changing its permeability, and then causing solubilization, rupture, and disruption.¹⁰⁵

Among all biosurfactants, lipopeptides and glycolipids possess higher antimicrobial action.^{108,109} The lipopeptides produced by *Bacillus* sp., such as surfactin, are widely known for such biological property, as it is also the case for other lipopeptides such as fengycin, iturine, bacilomycins, and mycosubtilins.¹¹⁰⁻¹¹² Kourmentza *et al.*¹⁰⁵ recently reported that a mixture of lipopeptides can favor antimicrobial action, which was observed by the action of mycosubtilin/surfactin mixtures against *Paecilomyces variotii*, *Byssoschlamys fulva*, and *Candida krusei* filamentous fungi.

In general, many environmental factors induce the synthesis of biosurfactants by microorganisms; also, the microbial strains are varied and may lead to a diversity of molecules with different chemical structures.⁸⁷

3.1. Microbial biosurfactants: molecular structures and physicochemical properties

The various biosurfactant-producing microorganisms, their different habitats, and environmental conditions (such as pH, temperature, oxygen), as well as nutritional conditions (source of carbon, nitrogen), are responsible for the broad molecular diversity of biosurfactants, and for the ensuing biological and physicochemical properties.^{86,113,114} In general, biosurfactants are classified according to their chemical skeleton and molecular weight.^{69,115,116} With respect to the molecular structure, biosurfactants are classified as the biochemical components that build up the molecule, i.e., glycolipids (rhamnolipids, sophorolipids, mannosylerythritol lipids, and trehalose lipids), lipopeptides/lipoproteins (surfactin, fengycin), and polymers (lipopolysaccharides, heteropolysaccharides, and proteins) (Table 2).¹¹⁷⁻¹¹⁹

High molecular weight biosurfactants, also known as bioemulsifiers, are produced by many bacteria and yeasts, and act as stabilizers for water/oil emulsions. Polymeric biosurfactants are constituted by polysaccharides, proteins, lipopolysaccharides, and lipoproteins (Figure 4).^{86,134,135} Bioemulsifiers such as emulsan, lipomannan, alasan, liposan, and other polysaccharide-protein complexes are the subject of many research investigations, and deserve attention in the field.^{80,123} Emulsan, for instance, is an extracellular heteropolysaccharide bearing 80% of lipopolysaccharide and 20% of high molecular weight exopolysaccharide.¹³⁶

Low molecular weight biosurfactants such as free fatty acids, phospholipids, lipopeptides, and glycolipids (Figure 4) generally act to reduce surface and interfacial tension.^{86,132} The lipopeptide produced by *Brevibacillus* sp. reduced the surface tension of water up to 29 mN m⁻¹ at its CMC (80 mg L⁻¹).¹³⁷

Microbial biosurfactants can be chemically modified to produce derivatives with targeted properties. Some contributions dealt with the functionalization of glycolipids,

especially rhamnolipids and sophorolipids, by the insertion of amino acids aiming at increasing the molecular functionality for application in gene therapy (such as nucleic acid encapsulation), drug delivery systems for pharmaceuticals, antimicrobials against resistant microorganisms, and as physicochemical and biological modifiers of biomaterials used in medicine.¹³⁸⁻¹⁴²

Azim *et al.*¹⁴³ modified an acid sophorolipid by conjugation with serine, leucine, glycine, phenylalanine, glutamic acid, and aspartic acid through the following steps: (i) hydrolysis of a natural sophorolipid mixture produced by *Candida bombicola* with aqueous alkali to give sophorolipid free acids, (ii) coupling of free acids with protected amino acids using dicarbodiimide, and (iii) removal of the amino acid carboxyl protecting groups. The sophorolipid-amino acid conjugates obtained showed antibacterial, anti-HIV, and spermicidal activities. Similar results were obtained by Zerkowski *et al.*,¹⁴⁴ who conjugated an acidic sophorolipid with *N*- ϵ -benzyloxycarbonyl lysine [Lys(Cbz)], leading to a conjugate with CMC ranging from 10⁻⁶ to low 10⁻⁵ mol L⁻¹ and minimum surface tension

Table 2. Classification, chemical, and biological characteristics of biosurfactants

Biosurfactant	Chemical characteristics	Biological characteristics	Example
Glycolipids	composed of carbohydrates (monosaccharides-glucose, xylose, rhamnose; oligosaccharides-sophorose) and lipids (fatty acids: β -hydroxydecanoic acid (C10), hydroxy-hexadecanoic acids (C16:0, C16:1 and C16:2), hydroxy-octadecanoic acids (C18:0, C18:1 and C18:2)); ¹²⁰⁻¹²² the glycidic and lipidic portions are connected by ether or ester bonds; CMC between 20 and 366 mg L ⁻¹ ; ^{123,124} reduce water surface tension from 72 to 20 mN m ⁻¹ ; HLB: 6-24 (mannosylerythritol lipids 6-12; sophorolipids 12-15; rhamnolipids 22-24) ¹²⁵⁻¹²⁷	antimicrobial action; pesticide action predominantly produced by <i>Pseudomonas aeruginosa</i>	rhamnolipids, sophorolipids, cellobiose lipids, mannosylerythritol lipids, trehalose lipids, and liamocins (polyol lipids)
Lipopeptides/lipoproteins	composed of peptides or proteins (y L-glutamine, L-leucine, D-leucine, L-valine, L-asparagine, D-leucine, and L-leucine residues linked by peptide bonds) and lipids (fatty acid); exhibit higher molecular mass (\geq 1000 Da); CMC around 10 μ mol L ⁻¹ or 23 mg L ⁻¹ ; water surface tension reduction from 72 to 20 mN m ⁻¹ ; ²¹ HLB: 10-12 (surfactin); ¹²⁸	antimicrobial action; produced main by bacteria <i>Bacillus subtilis</i>	iturin, surfactin, fengycin, viscosin, and amphisin
Polymeric	composed by complex mixture with varied structures (heteropolysaccharides); exhibit higher molecular mass (\geq 1000 Da); reduction of water surface tension from 72 to 30 mN m ⁻¹ ; ^{129,130}	produced by yeast, for example, <i>Yarrowia lipolytic</i> ¹³³	alasan, liposan, lipomannan, emulsan, and other polysaccharide-protein complexes
Particulates	vesicles of proteins, phospholipids, and lipopolysaccharides with size between 20 and 50 nm ^{131,132}		extracellular membrane vesicles

Adapted from Marcelino *et al.*¹¹⁹

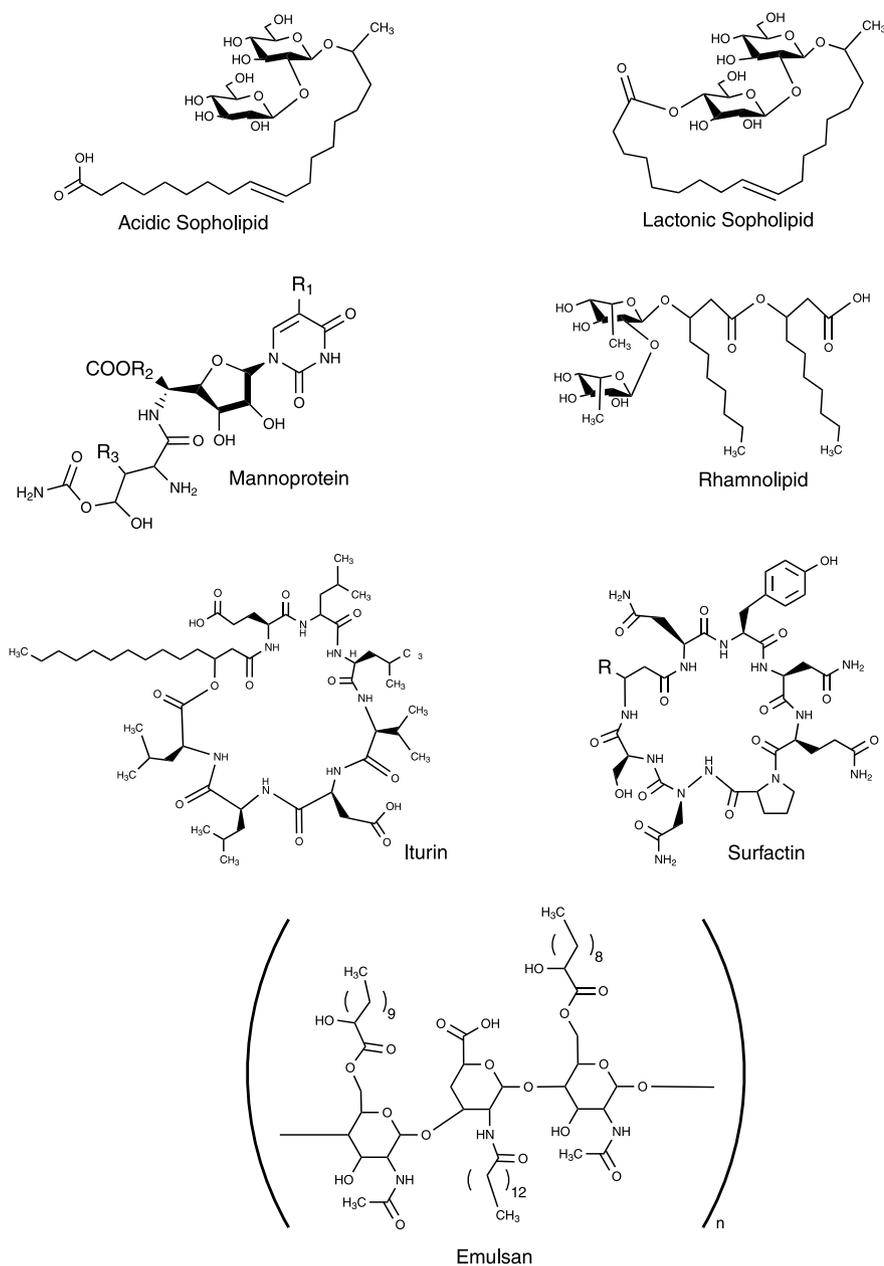


Figure 4. Types and classification of microbial biosurfactants.

values below 40 mN m^{-1} . The conjugation of mono- and dirhamnolipids with arginine and lysine was also reported (Figure 5), and conjugates with CMC ranging from 8 to 15 mg L^{-1} and minimum surface tension values between 28 and 31 mN m^{-1} were obtained.¹⁴² The biosurfactant-amino acid conjugates are considered important tools for the design of new drugs, since they can be used as “trojan-horses” in the treatment of some infections and tumors. Regio- and non-selective reactions at the sugar head group, modifications at the end of the lipid tail, polymerizations, modifications towards short-chained, and degradation in smaller building blocks have also been reported with sophorolipids.¹⁴⁵ The structural modifications of microbial

biosurfactants are of extreme high relevance, as they may enhance some physicochemical and/or biological properties of this class of bioproducts and thus further expand their applications.

3.2. Sustainable production of microbial biosurfactants in the context of biorefineries

Biosurfactants are naturally produced by many microorganisms via fermentation.^{6,84} Among the most relevant producing bacteria genera are *Pseudomonas*, *Acinetobacter*, *Bacillus*, *Brevibacterium*, *Clostridium*, *Rhodococcus*, *Thiobacillus*, *Citrobacter*, *Corynebacterium*,

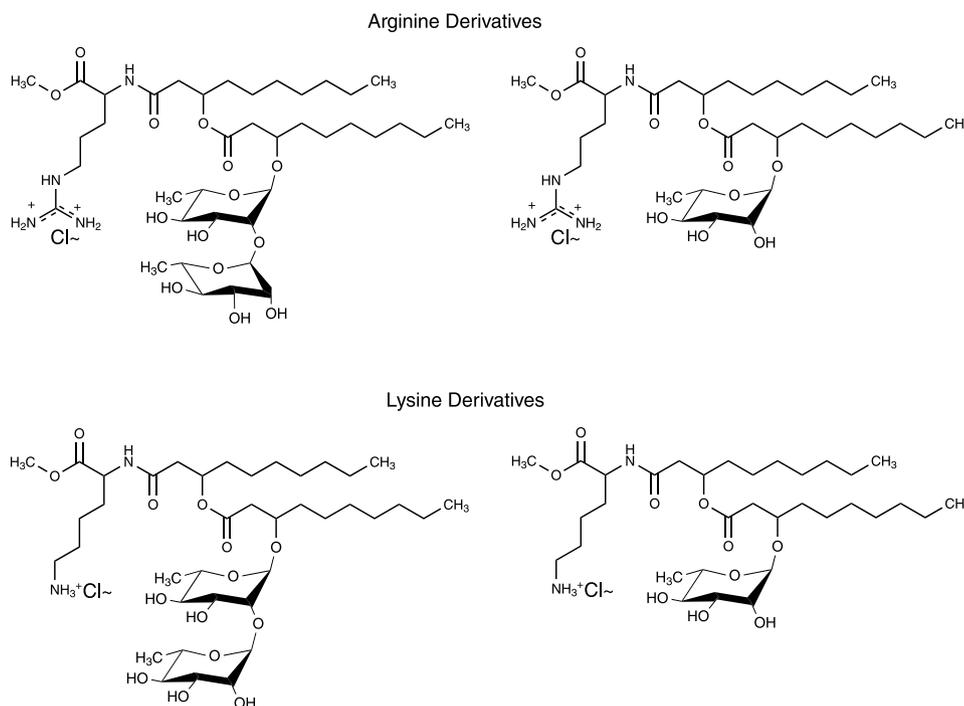


Figure 5. Types of modified microbial biosurfactants.

Enterobacter, and *Lactobacillus*,^{130,146} which can be isolated from oil contaminated soils, Antarctic and marine environments.^{91,147}

Marine microorganisms produce biosurfactants with varying chemical structures, including lipoaminoacids and lipopeptides: the lipoaminoacid known as proline lipid is synthesized by *Alcanivorax dieselolei* and *Brevibacterium luteolum*,^{148,149} lipopeptides such as rhodofactin are obtained by *Rhodococcus* sp.; didemnin B is produced by *Tristrella* sp. The production of glycolipids, such as a glucose lipid formed through a glycosidic bond between glucose and four 3-hydroxy fatty acids, was observed in *Alcanivorax borkumensis*, an alkane-degrading marine bacteria,¹⁵⁰ and a glucosyl palmitate structure, in which glucose molecules are esterified with medium and long-chain fatty acids (C14 to C18), were found in *Serratia marcescens* bacterium.¹⁵¹

Biosurfactant-producing bacteria were found in places with extreme temperatures, as it was the case for *Oceanobacillus* sp.,¹⁵² *Halomonas* sp.,¹⁵³ *Rhodococcus* sp.,¹⁵⁴ *Streptomyces luridus*;¹⁵⁵ *Paenibacillus antarcticus*, *Janthinobacterium svalbardensis*, *Psychrobacter arcticus*, and *Serratia* sp.^{156,157} Biosurfactants produced by microorganisms isolated from such environments have typical structures, such as the glucotriose lipids produced by *Rhodococcus* sp.¹⁵⁸ and glucose lipids of *Alcanivorax borkumensis*.¹⁵⁹

The main producers of biosurfactants are *Pseudomonas* sp. and *Bacillus* sp. bacteria. Due to the

pathogenic nature of some species, such as *P. aeruginosa* and *B. cereus*, their products have limited applications in food, pharmaceutical, and personal care industries.^{160,161} For these specific purposes, it is important to select a microorganism that has GRAS status (generally recognized as safe), that does not present risks of pathogenicity and toxicity.^{162,163} In this case, some yeast species are often considered,¹⁶⁴⁻¹⁶⁶ such as *Candida* sp. and *Starmerella* sp.^{133,167} Biosurfactant-producing yeasts derived from Antarctica are worth of mention, and a related contribution demonstrated the production of biosurfactants in a medium containing a hemicellulose hydrolysate from sugarcane straw. In the literature there are reports of the emulsion indexes (IE24h) above 50% for the yeasts *Rhodotorula mucilaginosa* L65, L07, L67; *Metschnikowia australis* L02; *Tausonia pullulans* L109, *Naganishia albidosimilis* L94, L95; *Papiliotrema laurentii* L59, *Leuconeurospora* sp. L107 and *Candida guilliermondii* FTI 20037.¹⁶⁸

After determining the producing microorganism, it is necessary to select the carbon and nitrogen sources involved in microbial growth and biosurfactant production. Some carbon sources that can be considered for fermentation processes are water-insoluble sources, such as animal fat, vegetable oils (soybean oil, corn oil), oil residues, hydrocarbons (*n*-hexadecane, *n*-dodecane, *n*-tetradecane), and hydrophobic mixtures (engine oil, diesel, crude oil, paraffin, kerosene). Industrial and agro-industrial by-products can also serve as carbon and lipid sources, and some very promising contributions indicate

the use of wheat straw, rice straw, corn, rice, molasses, bagasse, and sugarcane straw, rice and soybean, dairy cheese whey, and whey residues as raw materials for microbial fermentation.^{119,169-173} The strategy could be easily inserted in a given biorefinery, leading to high value-added products and therefore contributing to its economic viability.

In addition to the choice of raw material, the production capacity and the final yield of the bioprocess are dependent on the type of fermentation performed, viz. submerged fermentation (SmF) and solid-state fermentation (SSF).^{119,174,175}

At SSF, solid biomass residues, such as agro-industrial residues, may be exploited. This fermentative process involves the microbial development occurring on the surface of a solid biomass that can provide nutrients and absorb water.¹⁷⁵⁻¹⁷⁷ SSF is a bioprocess in which substrates are converted into chemical substances by microorganisms that grow and develop in environments with low free water content.^{174,178} SmF, on the other hand, is the consolidated industrial method to produce various bioproducts and is based on the cultivation of microorganisms in culture media with high free water content.¹⁷⁹ In this process, it is possible to control parameters like pH, dissolved oxygen, temperature, substrate concentration, agitation, biomass separation, and recovery of the products of interest.¹⁸⁰

Some limitations of SmF, mainly related to foam formation, make the scale up of biosurfactant production very challenging,³⁰ and this is particularly difficult in systems with intense agitation and aeration.^{175,181} Due to the excess of foam, the medium may leak out, causing contamination and loss of product. In addition, microorganisms can be dragged to the top layer of the foam, reducing their concentration in the culture medium, and therefore decreasing the conversion of the substrate into biosurfactants.^{175,182} Anti-foaming agents are often used in many bioprocesses, which increases the overall production cost. Another possible strategy is to add a hydrophobic substance to the system, such as vegetable oils, which decreases foam formation.^{133,183}

SSF are interesting methods that avoid foam formation, at the same time as favor the transfer of O₂ and reduce the risk of contamination.^{175,184,185} However, the large-scale production of biosurfactants by SSF is limited due to the reduced transfer of mass and energy, which generates the accumulation of heat and water residues inside the bioreactor.¹⁷⁶

Due to the high overall cost of production, biosurfactants still cannot compete with synthetic surfactants in the industrial market. The use of low-cost renewable raw materials is, therefore, mandatory to reach economic viability.^{135,170} That goes in the same direction of the current

global sustainability trend, and benign processes, capable of reducing negative effects on the environment, are in the spotlight. The use of industrial and agro-industrial wastes for the development of sustainable biorefineries is fully inserted in this context.¹⁸⁶

Different raw materials may be used in bioprocesses, such as lignocellulosic biomass, starch, and oleaginous. The selection of industrial residues must ensure the balance of nutrients that favors microbial growth and the production of the desired products.^{132,187} During biosurfactant production, oleaginous (hydrophobic) by-products from the food industries and glycerin from the biodiesel industry are generally used.¹⁸⁸ In addition, carbohydrate rich by-products such as lignocellulosic biomass may also be considered.^{17,119,132}

Lignocellulosic biomass is present in the form of agricultural, forestry, and urban waste, representing the most abundant resource of low-cost renewable raw materials, and one of the richest sources of organic carbon. It is mainly constituted of polysaccharides and lignin, which can be converted into high value-added products.^{189,190}

The specific chemical constitution of lignocellulosic biomass includes 35 to 50% cellulose, 20 to 35% hemicellulose, and 15 to 20% lignin.¹⁹¹ Cellulose, one of the most abundant natural linear homopolymer, is formed by D-glucose chains linked by β -(1,4) glycosidic bonds, and intra- and intermolecular hydrogen bonds, which contributes to cellulose rigidity, as well as to its strong, crystalline, and hydrolysis resistant structure. Cellulose fibers are arranged in the form of microfibrils via van der Waals interactions and hydrogen bonds, which are covered up with hemicellulose chains.¹⁹² Hemicelluloses, in their turn, are heterogeneous polymers consisting of different monosaccharides, such as β -D-xylose, α -L-arabinose, β -D-mannose, β -D-glucose, α -D-galactose.^{193,194} Last but not least, lignin is a polyphenolic macromolecule, which provides recalcitrance and structural support to biomass and acts as a natural barrier to microbial attacks.¹⁹⁵

The use of lignocellulosic biomass in fermentation processes is somehow limited to their chemical structure and composition. Prior to the biological process, the raw material often needs to undergo pre-treatment processes, where the cellulose and hemicellulose fractions will be depolymerized, releasing fermentable sugars that will be used by microorganisms.^{196,197} The pretreatments commonly used are chemical (diluted acid hydrolysis), physical and biological.^{198,199}

Among the agro-industrial by-products used to produce biosurfactants, one can mention barley bran, corn cob,²⁰⁰ distilled grape pomace,²⁰¹ sweet sorghum bagasse

hydrolysate,²⁰² orange peel,²⁰³ sugarcane bagasse,^{17,204} and xylose-rich corn cob hydrolysate.²⁰⁵

In addition to the high cost of biosurfactant production, the downstream stage accounts for about 70-80% of the overall production price.¹³² Biosurfactants may be recovered from the fermentation medium by solvent extraction (chloroform-methanol, dichloromethane-methanol, butanol, ethyl acetate, pentane, hexane, acetic acid),^{17,182,206} precipitation (ammonium sulfate, ethanol, acetone),^{197,207} and foam fractionation by adsorption in air bubbles.^{100,119,182}

Figure 6 shows the production of microbial biosurfactants using plant biomass in agro-industrial by-products in biorefinery contexts.

As previously mentioned, microbial biosurfactants may be obtained with different chemical structures and physicochemical and biological properties, which presume a wide range of further applications.

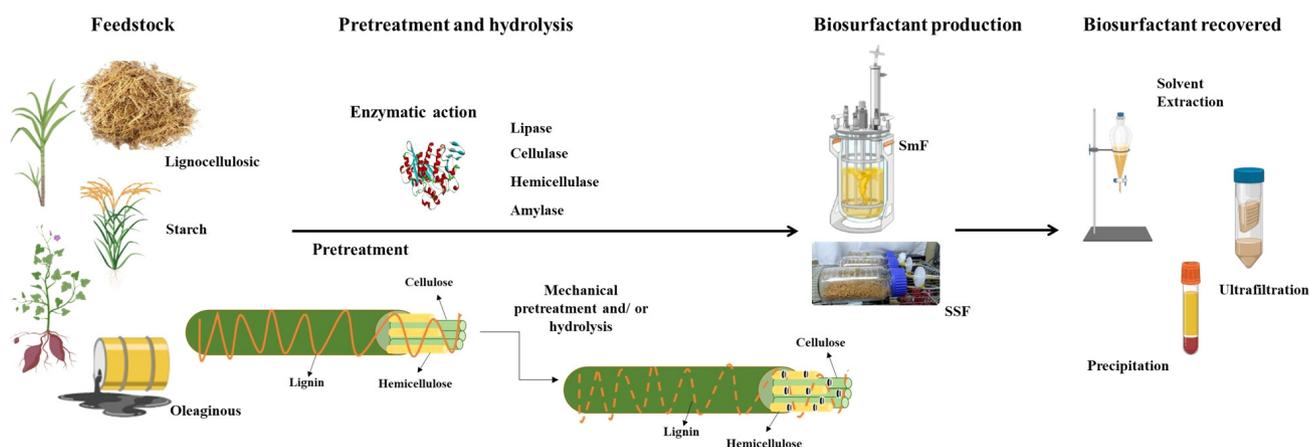


Figure 6. Microbial biosurfactant production in biorefinery using plant biomasses and agro-industrial by-products.

Table 3. Examples of commercial biosurfactants and their main applications

Country	Biosurfactant	Applications
USA	rhamnolipids	pharmaceutical products
	rhamnolipids	enhanced oil recovery (EOR), cleaning and oil recovery from storage tanks
	rhamnolipids	household cleaners, homecare detergents, agriculture, others
	rhamnolipids (chemically synthesized)	personal care, high tech services, cleaning agents, cosmetics
Germany/USA	green surfactant alkyl polyglucoside (APG)	cosmetics, industrial and institutional surface cleaning, hard surface cleaning
Belgium	sophorolipids	oil recovery and processing, EOR, anti-biofilm, detergent action
Germany	glycolipid and cellobiose lipid biosurfactants	bioactive properties in pharmaceutical products, cleaning products, dishwashing liquids
France	lipopeptides	agriculture and cosmetics
South Africa	surfactin	cleaning products
Japan	sophorolipid	cleaning products
	sodium surfactin	cosmetics
South Korea	sopholine (sophorolipids)	personal care products

Adapted from Santos *et al.*¹³² and Farias *et al.*²¹²

4. Applications of Microbial Biosurfactants

An increasing industrial interest to produce and commercialize biosurfactants has been observed during the last years. The main biosurfactant-producing companies are situated in North America, followed by Europe and Asia-Pacific (Table 3). However, as previously mentioned, an extensive commercialization remains limited due to the cost of production.²⁰⁸ In 2019, the commercialization of biosurfactants reached US\$ 1.5 billion,⁵ and the estimated forecast for their global market by 2024 is US\$ 58.3 billion, with an annual growth rate of 4.5%. The rhamnolipid-based biosurfactant market, for instance, has forecast to reach US\$ 145 million in 2026. This recent market growth may be explained by their broad potential applications in various industrial sectors, as already mentioned.^{110,209-211}

The Covid-19 pandemic led to an increased search for personal care products, industrial cleaning disinfectants,

and general medical and pharmaceutical products consisting of surfactants, which favors the product market.²¹¹ In the present moment, an increased interest on biosurfactant-based cosmetics, personal hygiene, medical and antimicrobial products has been observed. However, applications in the recovery of environmental damages, agriculture, and food products are also worth of mention and will be described in detail in the further section.

4.1. Remediation of environmental damages

The increase of environmental damages has drawn the attention of governments and civil society. Such tragedies are not only an ecological issue, but a threat to human health and the economy.²¹³ In 2015, the Mariana dam in Minas Gerais broke down in Brazil, and approximately 50 million m³ of mud containing iron ore were dumped into the terrestrial and aquatic territory, reaching the Atlantic Ocean.^{214,215} Another similar tragic event occurred in 2019, in the city of Brumadinho, Minas Gerais, Brazil, when approximately 12 million m³ of waste were dumped into the environment.²¹⁶

These events caused a severe impact on the environment, and a sludge rich in trace elements reached the rivers and was deposited and consolidated in the bottom as sediments. The presence of metallic elements in aquatic ecosystems is known to interfere in the food chain due to absorption and accumulation by living organisms.^{217,218} Some trace elements, such as Pb, Ba, Sr, As, Mn, Fe, and Al, have the potential to induce cytotoxicity and DNA damage,^{219,220} and the repeated exposure to such metals can cause cancer, disorders in the nervous system, and diseases in kidneys, liver, skin.^{221,222}

The conventional strategies applied in the recovery of metal-contaminated areas are limited due to operational costs, technical requirements, and secondary pollutant generation. Thus, bioremediation approaches using biosurfactant-producing microorganisms represent an eco-

friendly and economic alternative.²²³⁻²²⁵ The interaction of biosurfactants with metals involves the three main phases, namely (i) the isolation of heavy metals by sorption, (ii) the trapping of metals into the micelle through electrostatic interactions, and (iii) the recovery of micelles through precipitation or membrane separation methods^{226,227} (Figure 7).

Some studies demonstrated the efficiency of removing heavy metals from sludge and soil using biosurfactants, achieving recovery rates of 90-100% for Cu, Zn, Cr, and Cd.²²⁶ Therefore, efficient and compatible formulations that contain biosurfactants may allow to adopt robust plans for the remediation of affected ecosystems from mining activity.^{225,228}

In addition to environmental accidents caused by contamination with heavy metals, damage from oil spills is also observed in marine areas. In September 2019, for instance, large amounts of petroleum leaked and reached the entire northeastern and part of the southeastern coast of Brazil, being detected in more than 3000 km²²⁹ and affecting marine animals, seabirds, estuaries, mangroves, and seagrass meadows.²³⁰ People involved with tourism and fishing markets lost their revenues, jobs, and livelihoods.²³¹ The volunteers that participated in cleaning activities were exposed to volatile petroleum compounds that can cause redness, dermatitis, headache, nausea, swelling, respiratory symptoms, abdominal pain, and long-term health diseases.²³²

Again, biosurfactants are sustainable alternatives to remediate oil spills thanks to their high biodegradability potential, dispersing ability, foaming properties, and functionality in extreme environmental conditions¹³⁵ (Figure 7). When biosurfactant individual molecules are dispersed in the marine environment in the presence of hydrocarbons, they reduce the surface tension between water and oil.^{132,233} Moreover, biosurfactants can be present in the form of micelles, which allows the solubilization of hydrocarbons by the formation of droplets of oil in

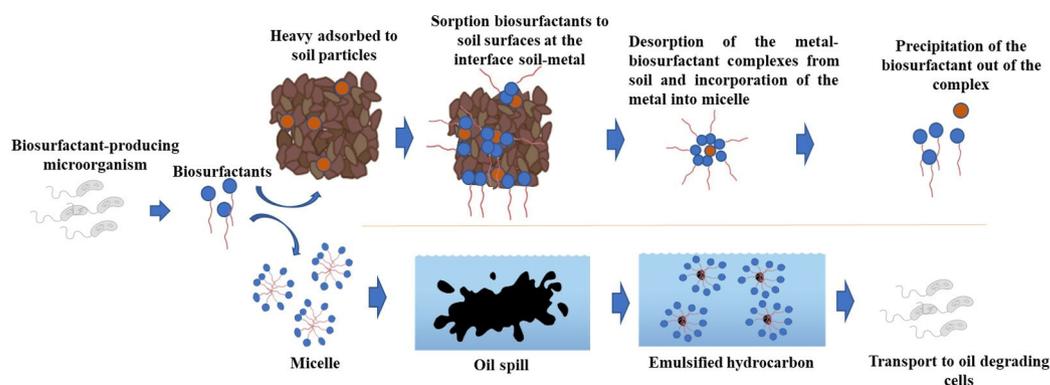


Figure 7. Mechanism of biosurfactant activity in toxic metal-contaminated soil and in marine oil spill.

the aqueous medium.^{118,234} Some changes in the cell membrane of degrading bacteria, such as size and proteins composition, allow a higher accessibility to hydrocarbons by microbial cells.^{235,236}

Biosurfactants can be additionally considered for the remediation of oil-contaminated soils. Within this context, the *in situ* biological remediation take place directly under natural conditions due to the metabolic ability of native microorganisms to survive in polluted environments.²³⁷ Actually, hydrocarbons are sources of carbon and energy to endure microbial activity and growth, in a cheap, environmentally friendly strategy.²³⁸ Furthermore, there is no need to collect and transport the contaminated soil. The production of biodegradable and less toxic microbial products have been used to improve the recovery of oil during extraction. Microbial enhanced oil recovery (MEOR) uses specific microbial strains to synthesize biosurfactants that are analogous to those used in chemically enhanced oil recovery (CEOR), which are capable of solubilizing residual underground oil. Chemical surfactants are more expensive, non-biodegradable and present loss problems due to adsorption to rocks.²³⁹ Thus, microbial surfactants arise as an alternative that significantly improves mobility in the porous medium and, consequently, increases oil production.²⁴⁰

4.2. Agriculture

Biosurfactants may have important applications in livestock and agriculture, as they can be applied, respectively, as food supplements to improve the digestibility of ruminants, and as antimicrobial and immunomodulatory ingredient.²⁴¹ In opposition to agriculture, the applications of biosurfactants for feed and animal nutrition is still minimally exploited. Alkyl polyglucosides, natural plant-derived biosurfactants, have shown positive effects on physiological and production parameters. Their ability to modify the ruminal fatty acids composition and to increase the activities of ruminal enzymes (such as carboxymethyl cellulase and xylanase) was demonstrated.^{242,243} Some studies also have suggested that a rhamnolipid produced by *Pseudomonas aeruginosa* may have similar effects due to the increase of xylanase activity and degradation rates of organic matter.²⁴⁴

The synthesis of phytohormones and the induction of resistance was modulated by the addition of rhamnolipids.^{245,246} This kind of biosurfactant also influences the microflora of the plants or soil, which regulates the growth, the removal of the contaminants from the soil and plant roots, and the mitigation of biotic and abiotic stress.²⁴⁷⁻²⁵²

Plant pathogens cause huge economic losses yearly, which may range from 10 to 40% depending on the crops, before or after harvest.^{250,253,254} Therefore, strategies of biocontrol are compatible with the crops and include the use of organisms that reduce the incidence of diseases through the competition with the pathogen for space and nutrients, the induction of the natural defense system of plants, and/or by the synthesis of antimicrobial substances.^{255,256} In this context, the effectiveness of pesticide formulations has been improved by the incorporation of surfactants, which can be used as additives or adjuvants to dispersions, as emulsification agents, and for better spreading. However, the use of chemical pesticides can be harmful to human and environmental health, and biosurfactants produced by microorganisms can be used as sustainable alternatives that have great potential to offer the same benefits of the chemical counterparts.^{257,258} Another beneficial effect of biosurfactants involves their role as antibiosis modulators of antagonistic microorganisms in the management of plant diseases.^{249,259}

Biosurfactants of microbial origin can be also used in fine applications, such as in medical, cosmetic, and food areas, where a higher degree of purity is required.

4.3. Medicine and pharmaceutical applications

The chemical structure of biosurfactants provides stability, micelle forming ability, biological compatibility, and low toxicity, which are important properties that can be exploited in the medical field. In this context, glycolipids and lipopeptides, derived from *Candida* species are widely investigated. Some authors reported an antimicrobial effect of rhamnolipids against Gram-positive bacteria, which is not observed in Gram-negative bacteria because their plasma membrane acts as a barrier, preventing the entrance of biosurfactants into the cells.^{260,261} Furthermore, rhamnolipids cause damage to the cell membrane by inserting acyl tails, generating cell leakage of cytoplasmic components.²⁶² Previous studies^{263,264} demonstrated the action of a rhamnolipid against *E. coli* and *S. aureus*, precisely by this mechanism, causing cell death. The process occurs by the interaction of the nonpolar region of the cell membrane with the hydrophobic and hydrophilic moieties of the biosurfactant.

On the other hand, due to the resistance of microorganisms to antibiotics and the emergence of new diseases, such as Covid-19, researchers are focusing on the use of biosurfactants as therapeutic agents. In fact, the coronavirus pandemic is a real example of the need for fast expansion of drug development, and the current scenario offers opportunities for biosurfactants to be used as part of

drug delivery systems and hygiene products formulations. In this sense, their amphiphilic nature allows a further potential application as anti-inflammatory and antiviral agents. In the specific case of the SARS-CoV-2 virus, when entering the host cell, the structure of the biosurfactants could allow it to interact with the viral cell membrane and to get inside the lipid membrane, causing changes in permeability through the formation of ion channels or disruption of the membrane system.²⁴

As an antiviral agent, biosurfactants can act on the viral membrane and promote the disruption of the outer coating.²⁶⁵ When the human body has contact with an infectious agent, the immune system activates the inflammatory response regulated by the enzyme phospholipase A2 (PLA2). The inhibition of the PLA2 could occur by the addition of the biosurfactant into the body, that could interact with the cells and macromolecular membranes, thus reducing the anti-inflammatory response.²⁴ Giri *et al.*²⁶⁶ tested an *in vitro* administration of surfactin in rat and fish models, which led to decreased pro-inflammatory cytokines and increased anti-inflammatory cytokines levels.

With respect to Covid-19, some patients that tested positive presented high levels of cytokines, causing the cytokine storm phenomenon correlated with the viral load.²⁶⁷ The increase in the cytokine concentration also causes the increase of some proinflammatory factors levels, such as interleukin-6 (IL-6) and interleukin-18 (IL-18). Biosurfactants as lipopeptides have shown to be effective in the reduction of the so-called cytokine storm. They act in the inflammatory response cascade, through the decrease in the production of cytokines such as Tumor necrosis factor- α (TNF- α), interleukin-1B (IL-1B), and IL-6, interleukin-2 (IL-2).^{24,268}

Furthermore, studies suggest the immune-adjuvant potential of biosurfactants. The cationic lipopeptide action in a vaccine containing an indigenous low pathogenicity AIV-H9N2 virus was demonstrated.²⁶⁹ Similarly, the adjuvant action of a surfactin synthesized by *Bacillus amyloliquefaciens* on hepatitis B surface antigens was observed when triggered humoral and cellular responses.^{270,271}

4.4. Cosmetics

The skin is the largest organ of the human body and serves as a barrier to prevent excessive loss of moisture from the inner body and the entry of toxic substances and pathogens.²⁷² The removal of lipids from the skin surface, skin irritation, and allergic reactions can be caused by the interaction of anti-bacterial preservatives with keratin or collagen and elastin.^{273,274} The composition of some biosurfactants with sugars, lipids, and proteins makes them

compatible with the membrane of skin cells, giving a high rate of permeability through the skin.²⁷⁵ The hydrolysis of triglycerides of fatty acid chain ends of biosurfactants could promote the adherence of the resident skin microorganisms discouraging the growth of pathogens through the maintenance of an acidic skin pH.²⁷⁶ In addition, the use of such compounds in creams, lotions, and shampoos formulations provides emulsification, foaming, wetting, and solubilizing functions.^{277,278}

The potential of different biosurfactants in the cosmetic industry is widely reported in literature, and glycolipids are highlighted in this context. The use of rhamnolipids as anti-wrinkle and antiaging in cosmetic products have been patented.²⁷⁹ The use of a rhamnolipid as part of the ingredients of a shampoo was proposed, and the results indicated that the scalp remained free from odor for three days thanks to the antimicrobial effect of the biosurfactant.²⁸⁰ Mannosylerythritol lipids (MELs) showed protective effects in cells against oxidative stress when tested in fibroblasts NB1RGB, which suggested their use in skin care formulations.²⁸¹ Sophorolipids are industrially produced and commercialized by companies to be used as humectants, and can therefore be found in lipsticks, and hair and skin moisturizers.^{282,283}

4.5. Food

The applications of biosurfactants in food are directly related to their stabilization, antiadhesive, and antimicrobial properties.²⁸⁴ The food industry establishes strict quality control on products that reach consumers, who are becoming more aware of the benefits of natural ingredients.^{285,286} New formulations are being developed to replace synthetic emulsifiers by compounds of vegetable origin, such as lecithin and Arabic gum.²⁸⁷ However, their properties vary when exposed to processes involving microwave cooking and irradiation. In addition to the functions of the emulsifiers in the food industry, such ingredients offer the right consistency and texture of food additives products and maintain the stability of liquid emulsions such as sauces dressings and beverages.^{233,287}

The thickening, stabilizing, and emulsifying properties make biosurfactants interesting food additives.²⁸⁸⁻²⁹⁰ In this sense, despite their potential for emulsification and surface tension lowering, there is no report of their application in commercialized food formulations up to the present moment.^{291,292} The use of rhamnolipids isolated from a marine source indicated the improvement of dough stability in bread and the increase of cake volumes.²⁹³

Some authors suggest that the improvement of the texture, creaminess, and quality of ice creams can

be reached by the incorporation of biosurfactants as emulsifiers.^{294,295} Biosurfactants isolated from bacteria present in dairy possess antimicrobial activity against bacteria, yeasts, and filamentous fungi. Hence, food shelf life is extended, and food safety is guaranteed through preventing food contamination.^{8,296} A combination of rhamnolipids with nisin inhibited thermophilic spores in ultra-high-temperature (UHT) soy milk, and rhamnolipids with natamycin were found to inhibit fungal growth when used in the processing of salad dressing.²⁹⁷

5. Conclusions

The search for environmental benign processes, and the increased concern with sustainability has boosted efforts on the exploitation of lignocellulosic biomass as a fundamental source of molecules. Herein, the constructs and functions of biosurfactants were presented, as well as the interactions that occur in biological systems that may benefit their implementation as sustainable key molecules for chemical, pharmaceutical, cosmetic and food industries. During the past years, insightful information was provided that demonstrate that biosurfactants can indeed replace fossil-based ones in the most diverse contexts, which hints at the possibility of rethinking the processes currently used. Evidently, the scale-up of products of biological origin requires efforts from all stakeholders, but the perspectives are optimistic for versatile molecules that can be successfully inserted in many industrial sectors, as it is the case for natural surfactants. The strategy additionally aggregates value for biorefineries, which is a desired characteristic in a society that might be progressively shifted to an eco-friendly platform.

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