



## Biosurfactants: Production, properties, applications, trends, and general perspectives

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### ABSTRACT

Biosurfactants have become attractive microbial products in the emerging biotechnology industry due to their advantages over synthetic surfactants in terms of environmental sustainability, global public health, and the concerns of industries to produce environmentally friendly goods. The amphipathic structure of biosurfactants with hydrophilic and hydrophobic moieties enable these molecules to play a key role in emulsification, foam formation, detergency, and oil dispersion activities, which are desirable traits in different industries. Several types of biosurfactants are commercially produced for applications in the pharmaceutical and cosmetic industries while others have promising roles in the food, petroleum, and agricultural industries. In this paper, we offer an extensive review of knowledge on microbial biosurfactants accumulated over the years. We also discuss current and promising industrial applications of biosurfactants as well as the advantages and challenges for their development and applications.

### 1. Introduction

Surfactants are chemical compounds composed of amphipathic molecules containing hydrophilic and hydrophobic moieties that partition at physical interfaces. The non-polar moieties are often hydrocarbon chains, while the polar moieties can be a cationic, anionic, non-ionic, or amphoteric molecules. This combination of hydrophobic and hydrophilic moieties enables surfactants to reduce surface and interfacial tensions and form microemulsions, in which hydrocarbons are solubilized in water or vice-versa [1]. The most effective way to characterize a surfactant is through measuring the attraction force between the molecules of liquids, therefore grading the surfactant on its ability to affect surface and interfacial tensions. Effective surfactants lower surface tensions, facilitating interactions between molecules of differing polar natures [2]. Critical Micelle Concentration (CMC) is defined as the minimum concentration of surfactant required to achieve

the lowest surface tension. Upon reaching CMC amphipathic molecules are aggregated with the hydrophilic portions positioned towards the outside of the molecule and the hydrophobic portions towards the inside, as illustrated in Fig. 1 [2]. After reaching the CMC values, further addition of surfactant will not affect a further reduction in surface tension, as such surfactants with a low CMC are judged as being advantageous for applied usage compared with those with higher CMC values [3].

The four general grouping of surfactants are based upon the polarity of the molecules head group, these are: anionic, cationic, nonionic, and zwitterionic. Anionic surfactants carry a negative charge and these are the most commonly available surfactants of those both synthetically and naturally produced [4]. Anionic surfactants have applications in personal care products and soaps as they are very effective in cleansing systems [5,6]. They are also used in the oil industry, agriculture, health, remediation, and bioprospecting because of their wide range of

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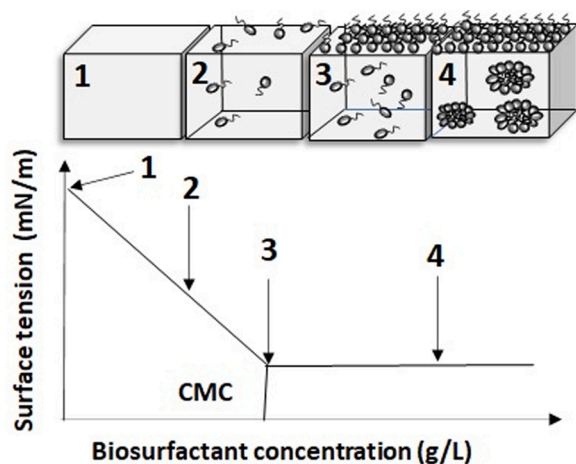


Fig. 1. Steps in the reduction of surface tension due to addition of surfactant until reaching the CMC and micelle formation.

hydrophilic–hydrophobic balance (HLB) values, emulsification properties, and their excellent ability to reduce surface tensions. Cationic surfactants are well suited for applications on surfaces with a negative charge; thus, they are used as anti-corrosion agents, flotation collectors, hair conditioners, fabric softeners and bactericides. Nonionic surfactants possess uncharged hydrophilic head groups, these molecules have good activity in low-temperature detergents and emulsifiers. They also have low irritating effects on organic tissue. Zwitterionic surfactants are amphoteric with poor cleansing and emulsifying properties but have excellent dermatological properties and skin compatibility [4]. As such zwitterionic surfactants are used in manufacturing shampoos and cosmetics.

Most synthetic surfactants are derived from the petrochemical industry and can therefore be produced at a low cost and high yield. However, this form of production is widely seen as unsustainable and contrary to the United Nations sustainable development goals, many national government strategies to build a sustainable green economy, and consumer pull which favors products generated in a sustainable fashion. Additionally synthetic surfactants often have toxicity and biocompatibility issues, and cause harm to ecosystems further limiting their application [7]. Biobased surfactants include microbial biosurfactants which are generated as metabolic products during bacterial or fungal fermentation [1]. Biosurfactants are natural products, generated in a sustainable fashion and are environmentally benign. Additionally, in comparison to synthetically derived surfactants, biosurfactants have reduced toxicity [8]. Biosurfactants are widely seen as a favorable replacement to their synthetically produced counterparts, as such their application and the proportion of the overall surfactant market they occupy has been increasing over the past 15 years [9]. Much like their synthetic counterparts, biosurfactants comprise of a wide variety of chemical structures. They are produced by microorganisms cultivated on both insoluble (oils, residues, and hydrocarbons) or soluble (carbohydrates) substrates [10,11]. Replacing synthetic surfactants with biosurfactants would reduce lifetime CO<sub>2</sub> emissions by 8%, leading to the avoidance of an estimated 1.5 million tons of CO<sub>2</sub> released into the atmosphere [1,12,13]. Currently, biosurfactants occupy about 10% of the total world production of surfactants (approximately ten million tons per year). These natural surfactants have applications in the petroleum, food (as emulsifiers), pharmaceutical (formulation of moisturizers, creams, and medicines), medical (antimicrobial agents), agricultural (fertilizers), and civil (waste and sewage treatment) industries [14].

The first research into microbial biosurfactant production took place in the 1960s and subsequent research carried out over the past 60 years has since enabled the development and commercialization of numerous

products containing these biomolecules [15]. Has a general result of the growing interest in sustainability during the last decade, studies aimed at the production of biosurfactants have intensified due to the desirable characteristics of these compounds, such as biodegradability, low toxicity, specificity, and stability under adverse environmental conditions (low temperature, extreme pH, and high salinity) [16]. Furthermore, biosurfactants can be produced from renewable substrates, offering economic advantages, and contributing to the biodegradability of the molecule [17].

Although extremely efficient, currently commercialized biosurfactants have a higher production cost than their synthetic counterparts [1,2]. This however can be reduced depending on the substrates used during fermentation and production yields of differing biosurfactant producing microbial strains [18,19]. Additionally, as most microbial strains produce a mixture of different biosurfactant compounds, the degree of purity required for some applications, such as in the pharmaceutical and medical fields can be a limiting factor for their application [20]. The development of strategies that enable the production and subsequent application of biosurfactants on an industrial scale is of fundamental importance. This review provides a state-of-the-art look at the most recent knowledge on biosurfactants and biotechnological strategies, offering a description of concepts, properties, classifications, and industrial applications. As the topic of microbial biosurfactants is highly complex and has generated a sizable bulk of literature, plant-derived biosurfactants are not included in this current review.

## 2. The classification of biosurfactant molecules

The average molecular mass of a biosurfactant ranges from 500 Da to 1500 Da, as such they are broadly grouped into low molecular weight and high molecular weight biosurfactants. Low molecular weight biosurfactants are more effective in reducing the surface tension at the air-water interface and the interfacial tension at the oil-water interface, whereas the higher molecular weight ones are most effectively used in stabilizing oil-in-water emulsions [21]. Molecular structure and microbial production source are the most important criteria for the classification of biosurfactants. Lipoproteins and lipopolysaccharides are high molecular weight biosurfactants, they are often referred to as bio-emulsifiers [22]. Glycolipids, lipopeptides, and phospholipids are low molecular weight compounds and are classically referred to as biosurfactants [23,24]. This review will mainly concentrate on these low-molecular weight biosurfactant compounds.

The most widely investigated class of low molecular weight biosurfactants are glycolipids. Glycolipid structure is comprised of a hydrophilic carbohydrate moiety connected to hydrophobic fatty acid chains of variable lengths through an ester group [25]. Intern glycolipid biosurfactants are further characterized based on the structure of hydrophilic carbohydrate moiety they possess with rhamnolipids, trehalolipids, mannosylerythritol lipids and sophorolipids being the most prevalent subclasses. Rhamnolipids comprise of one or two fatty acid chains ranging from 8 to 16 carbons in length linked to one or two rhamnose sugar molecules [25]. The main producer of rhamnolipids is the Gram-negative bacterium *Pseudomonas aeruginosa*, however further research has shown other bacteria species to be actively producing rhamnolipid biosurfactants [26–29]. The types of rhamnolipids produced depends on the strain, the carbon source used and the culture conditions. Rhamnolipids constitute one of the most interesting classes of biosurfactants because of their advantageous characteristics. Several renewable substrates such as used oils or wastes from the food industry has been reported as suitable carbon sources. Rhamnolipids can reduce the water/air surface tension from 72 mN/m to values close to 30 mN/m, as well as the water/oil interface tension from 43 mN/m to values around 1mN/m. The critical micelle concentration of pure rhamnolipids and their mixtures depends to a great extent on the chemical composition of the various species and varies from 50 to

200 mg/L [2]. The biosynthesis of rhamnolipid in *P. aeruginosa* is driven by three enzymes; RhlA generates the fatty acid pre-cursor molecular for rhamnolipids; RhlB adds the rhamnose to generate mono-rhamnolipids; RhlC utilises mono-rhamnolipids as substrate, adding a second rhamnose to form di-rhamnolipids. In *P. aeruginosa* the *rhlA* and *rhlB* genes are located on a single operon and expression is regulated by an acyl homoserine lactone mediated quorum sensing system expressed from the genes *rhlI* and *rhlR* located on the same operon. In *P. aeruginosa* the *rhlC* gene, which is also under the regulation of the *rhlI/R* quorum sensing system is located separately on the bacterial genome, however in other rhamnolipid producing bacteria such as *Burkholderia* species this is not the case with all three biosynthesis genes located together [30,31].

Sophorolipids consist of a hydrophilic disaccharide sophorose which is comprised of two monomers connected by  $\beta$ -1,2 bonds, the sophorose is intern connected by a glycosidic bond to C16 or C18 hydroxylated fatty acid chains that can be either acetylated or non-acetylated [32,33]. Sophorolipid congeners either exist as a lactonic or acidic form, with each form possessing differing application. Sophorolipids are synthesized by yeasts such as *Starmerella bombicola* [32]. The surface tension of these biomolecules presents values around 33 mN/m and interfacial tension of 5 mN/m between n-hexadecane and water. *Starmerella bombicola* is considered one of the most productive strains, being able to produce large yields of sophorolipids (on average 300 g/L) [34,35]. Due to this high yield of production sophorolipids are the class of microbial biosurfactant that has progressed furthest toward commercial application.

Trehalolipids contain trehalose disaccharides associated with a fatty acid (mycolic acid), have high structural diversity, and are mainly produced by species of the genera *Rhodococcus*, *Nocardia*, *Mycobacterium* and *Corynebacterium* [32]. Trehalolipids from *Arthrobacter* spp. and *Rhodococcus erythropolis* are able to lower surface and interfacial tensions in culture broth to 25–40 and 1–5 mN/m, respectively [2].

Mannosylerythritol lipids (MEL) are among the most promising biosurfactants and are abundantly produced from vegetable oils by *Pseudozyma antarctica*. MELs are characterized by mannose sugar linked to a fatty acid and are subdivided according to the length of the hydrophobic fatty acid chain and degree of saturation and/or acetylation in the C4 and C6 positions of the monosaccharide [14].

Other classes of low-molecular weight biosurfactants are the lipopeptides, phospholipids polymeric surfactants. The Gram-positive bacterium *Bacillus subtilis* produces compound called surfactin, a cyclic lipopeptide consisting of seven long-chain hydrophobic amino acids (13–15 carbons in length and a loop of seven amino acids such as L-asparagine (Asn), L-leucine (Leu), glutamic acid (Glu), L-leucine (Leu), L-valine (Val) and two D-leucines connected via lactone linkage) [23]. Surfactin is known to be one of the most potent biosurfactants reported. More than 30 types of surfactin have been discovered with different amino acids and fatty acid residues. Surfactin molecules however remains identical depending upon the chiral sequence [36]. Surfactin has anti-bacterial, anti-viral, anti-fungal and anti-mycoplasma activities suitable for health-related applications and can act as an efficient emulsifier, stabilizer, and surface modifier in the food industry [37]. It reduces surface tension from 72 to 27 mN/m with a concentration less than 5% by volume [38] and has also showed a low critical micelle concentration (CMC) and hence has been explored for use in extensive different potential applications. [39]. The biosynthesis of surfactin typically produced by *Bacillus subtilis* is driven by the *urfA* operon which encodes the four open reading frames necessary for the building of the megaenzyme surfactin synthetase, that recognizes the seven amino acids in the surfactin molecule [40]. Therefore, SrfAA incorporates the amino acids Glu, Leu, and D-Leu; srfAB incorporates Val, Asp, and D-Leu; whereas SrfAC functions in the incorporation of Leu. In addition, *srfAD* or *srfD* encodes a thioesterase that initiates the surfactin biosynthesis. The activation of the *urfA* operon is regulated by the gene *comA*, which is a transcriptional activator able to bind DNA [41].

Phospholipids are produced in large quantities during the growth of

bacteria and yeasts on n-alkanes. *Acinetobacter* spp. and *Thiobacillus thiooxidans* are known to synthesize phospholipid biosurfactants [32]. Emulsan and liposan are polymeric biosurfactants. These compounds serve as emulsifying agents and can be synthesized by bacteria as well as yeasts of the genus *Candida* [24,32]. The literature offers reports on the application of liposan as an emulsifier in the food and cosmetic industries [4]. Table 1 presents the main classes of biosurfactants and their microbial sources.

### 3. Physio-chemical properties of biosurfactants

Synthetic surfactants and biosurfactants share several properties, such as a reduction in surface tension, foaming capacity, emulsification, stabilizing ability, solubility, and detergency [2,23,74]. However, biosurfactants have been shown to either outperform or possess additional properties that make them more attractive than their synthetic counterparts.

As previously discussed, the efficiency of a surfactant is measured by their CMC. The CMC of most biosurfactant compounds ranges between 1 and 2000 mg/L, this is often dependent on the molecular structure of the biosurfactant in question [75]. A biosurfactant with optimal surface and interfacial activity can reduce the surface tension of water from 72 to below 35 mN/m and reduce interfacial tension (oil/water) from 40 to 1 mN/m [76]. The majority of biosurfactants have lower CMC, surface, and interfacial tension values compared to their synthetic counterparts therefore making them more efficient and effective when used in similar applications.

As biosurfactants are natural products produced by microorganisms colonizing a variety of environmental niches, they can maintain their

**Table 1**  
Main classes/subclasses of biosurfactants and microbial sources.

Class	Subclass	Microbes uses	References	
Glycolipids	Rhamnolipids	<i>Pseudomonas aeruginosa</i>	[42–44]	
		<i>Pseudomonas cepacia</i>	[10]	
		<i>Pseudomonas</i> sp.	[45]	
		<i>Lysinibacillus sphaericus</i>	[46]	
		<i>Serratia rubidaea</i>	[47]	
		<i>Nocardia farcinica</i>	[48]	
	Trehalolipids	<i>Rhodococcus</i> sp.	[49]	
		<i>C. bombicola</i>	[50]	
		<i>Candida sphaerica</i>	[51]	
	Sophorolipids	<i>Starmerella bombicola</i>	[52,53]	
		<i>Cutaneotrichosporon mucoides</i>	[54]	
		<i>Pseudozyma aphidis</i>	[55]	
		Not informed		
Mannosylerythritol lipids	<i>Meyerozyma guilliermondii</i>	[56]		
	<i>Saccharomyces cerevisiae</i>	[57]		
	<i>Candida utilis</i>	[58]		
	<i>Marinobacter hydrocarbonoclasticus</i>	[59]		
	Lipopeptides	Surfactin	<i>Bacillus subtilis</i> / <i>Bacillus nealsonii</i>	[60–63]
			<i>Bacillus licheniformis</i>	[64]
		Lichensyn	<i>Bacillus licheniformis</i>	[65]
			<i>Pseudomonas azotoformans</i>	[66]
		Not informed	<i>Bacillus velezensis</i>	[67]
			<i>Bacillus pseudomycoloides</i>	[68]
<i>Virgibacillus salarius</i>			[69]	
<i>Bacillus cereus</i>	[70]			
<i>Bacillus pumilus</i>	[71]			
Phospholipids	<i>Halomonas</i> sp.	[60]		
	<i>Thiobacillus thiooxidans</i>	[24]		
	<i>K. pneumoniae</i>	[62]		
Polymeric Surfactants	Liposan	<i>Candida lipolytica</i>	[24]	
			[72]	
	Rufisan			
	Emulsan	<i>Acinetobacter lwoffii</i>	[73]	
	Alasan	<i>Acinetobacter radioresistens</i>		

effectiveness even under adverse conditions. Biosurfactants have been shown to maintain their physio-chemical activity at high temperatures and within a pH range of 3–12. Biosurfactants can also tolerate saline concentrations up to 10% (w/v), whereas synthetic surfactants are mostly inactivated with  $\geq 2\%$  NaCl [2]. Santos and co-workers [77] demonstrated that the surface tension reduction and emulsification capacity of the biosurfactant produced by *C. lipolytica* remained unchanged for 120 days in the presence of NaCl (1–5%), in the pH range from 5 to 9 and at temperatures of 40 and 50 °C. They also showed that the biosurfactant produced by *Streptomyces* sp. was effective over wide ranges of temperature (4–120 °C), pH (2–12), salt concentration (2–12%) and heating time at 90 °C (10–120 min) [78].

The high diversity in molecular structure of different biosurfactant congeners results in functional activities that are depended on and often unique to specific molecular structure. Biosurfactants have the ability to self-assemble and form micelles, this allows them to have morphologically different structures from each other and increases their specificity. Spherical, rod-like, and wormlike micelles can be formed by biosurfactants. This feature is of considerable interest for applications in the food, cosmetic, and pharmaceutical industries as well as in the detoxification of different pollutants and the demulsification of industrial emulsions [2]. Small differences in congener molecular structure can also render significant difference in functionality. For example, sophorolipid congeners can possess the same length and chemical structure of fatty acid side chain but either be lactonic or acid by nature. The lactonic form of these congeners have been shown to have strong antimicrobial properties that are lacking in the acidic form.

The composition of biosurfactants makes them more biocompatible and biodegradable compared to their chemical counterparts. Studies have described the biodegradability of biosurfactants without addition of external microbial biomass, while varying the temperature, pH, and biodegradation time [79]. The presence of biosurfactants can also enhance biodegradability by solubilizing pollutants, as described by Silva et al. [80], who simulated a bioremediation process in sand and seawater samples. In both cases, oil degradation rates were higher than 90% in the presence of the biosurfactant and the producing species. Luna et al. [81] described that the biosurfactant from *Candida sphaerica* acted as a solubilizer of hydrocarbons in sea water by accelerating growth of the indigenous microorganisms. The literature also discusses the role of biosurfactants for enhanced biodegradation of motor oil from contaminated soils, as described by Chaprão et al. [82]. Regarding digestibility, the chemical nature of biosurfactants, which includes mainly glycolipidic and lipoprotein structures, make biosurfactants important compounds for use in the pharmaceutical, food and cosmetic industries [83].

Synthetic surfactant compounds are utilized in remediation and the treatment of effluents. As such they can be expelled into industrial wastewater. When this industrial wastewater is released, (either purposefully or accidentally), into a natural water body the presence of these synthetic surfactants can pose a threat to natural marine or freshwater ecosystems [84]. The degree of harm caused by this phenomenon varies depending on the concentration of synthetic surfactant, which increases over time [85]. If the concentrations of these released surfactants in the environment reach high concentrations, toxicity will accumulate in animals through the food chain, reaching humans through food consumption [85]. In contrast, as biosurfactants are natural products of microbial fermentation, they are significantly less toxic to aquatic flora and fauna and are more easily biodegraded by microorganisms in water and soil environments [84,85]. This increased biocompatibility is a favorable property to industries who are increasingly replacing synthetic surfactants with biosurfactants.

#### 4. Factors that affect biosurfactant production

Biosurfactants are produced either through excretion or adhesion to cells. The main physiological role of biosurfactants is to increase access to or allow microbial cells to grow on insoluble substrates through the

reduction in surface tension between phases, making hydrophobic substrate more available for uptake and metabolism. Different uptake mechanisms of these substrates are described. The direct uptake of dissolved hydrocarbons in the aqueous phase, direct contact between cells and large hydrocarbon droplets, and the interaction with emulsified droplets (emulsion) have been described. Biosurfactants are also involved in the adhesion of microbial cells to hydrocarbons. Microorganism cell adsorption to insoluble substrates and the excretion of surfactant compounds allow growth on these carbon sources [2].

Obtaining optimal biosurfactant yield poses a challenge, as several factors exert an influence on microbial growth and metabolism during fermentative production. Finding the ideal combination of substrates for a defined culture medium to facilitate intracellular diffusion and the production of compounds of interest has been the subject of numerous investigations [2,23,86]. To obtain optimal biosurfactant production from a selected strain of microorganism, it is important to define the culture conditions. Factors to consider include sources of carbon and nitrogen, concentration of the lipophilic substrate, micronutrients availability, inoculum size, temperature, pH, aeration and agitation speed [76]. Although most biosurfactant producing microorganisms generate these compounds under more restrictive conditions, the growth phase in which the highest production rate is achieved (exponential or stationary phase) should also be investigated [2]. The chemical and physical parameters of the fermentation process can be optimized using statistical methods, which provide the opportunity to study the effects of interactions between the different variables in search of the optimal culture conditions for the maximum production of biosurfactants at the lowest possible costs [87,88].

To produce biosurfactants economically, the process must integrate production and downstream processing. Mechanisms to improve production such as innovative statistical approaches (e.g. surface methodology), Artificial Intelligence (AI) based technique like Artificial Neural Intelligence coupled with Genetic Algorithm (ANN-GA), along with using recombinant bacterial strains must be considered. Recently Ambaye et al. [89] concluded that the use of genetically engineered microbial strains, cost-effective substrate(s), optimized media, improved fermentation process, better downstream processing and purification of end products using well developed statically models can represent commercially viable biological and engineering solutions to achieve cost-effective large scale industrial biosurfactants production for the substantiality of the environment.

##### 4.1. Carbon source, nitrogen source and carbon / nitrogen (C/N) ratio during fermentation

Carbon source is the primary variable to consider in the production of biosurfactants, as it directly influences the growth of the microorganism as well as the structure and yield of the target biosurfactant molecule. A variety of carbon sources have been used to generate biosurfactants, these include malt, molasses, animal fat, vegetable oils, oil residues, petroleum products, dairy products, and distillery residues [90,91]. Interestingly there are many studies that are investigating using carbon sources derived from waste products of other industrial processes to generate biosurfactants through fermentation [92]. However, a number of these studies can be questioned as they utilize methods of both characterizing and quantifying the produced biosurfactant that have fundamental flaws. Twigg and co-workers recently published a comprehensive review that provides a critical analysis of techniques used in the identification, up scaling, and functional analysis of biosurfactants [76]. If found to be optimal utilizing waste products would cut the cost of biosurfactant production and increase their sustainability through contribution to a circular economy. Both hydrophobic and hydrophilic carbon substrates can be used to produce biosurfactants. However, there are reports of higher productivity in yeasts, such as *Torulopsis bombicola*, *S. bombicola* and *C. lipolytica*, when hydrophobic, or a combination of different hydrophilic and hydrophobic sources are

used at concentrations above 5% (e.g. glucose and vegetable oil) [2]. Molecular weight of the carbon source also exerts an influence on the yield of biosurfactant, which is reported to increase when the medium is supplemented with glycerol, an organic compound consisting of only three carbons metabolized by bacteria of the genus *Bacillus* and *Pseudomonas aeruginosa* [93].

A suitable nitrogen source must also be chosen for the production of biosurfactants. Nitrogen source exerts a significant influence on the growth of microorganisms and contributes to the synthesis of metabolites of interest such as biosurfactants. Nitrogen source can be of an inorganic origin, such as ammonium nitrates and sulfates, or organic, such as urea, amino acids, and yeast extract [28]. The choice of nitrogen source depends on the composition of the medium and the producing microorganism [69,94]. An example of where nitrogen source effects biosurfactants production has been identified in *Bacillus* spp. Increased surface activity or emulsifying activity of *Bacillus* biosurfactants is linked to the isolated use of organic or inorganic nitrogen sources during growth [95]. When both organic (yeast extract) and inorganic (ammonium nitrate) sources are added simultaneously to the medium assimilation of the inorganic source is slower and simulates limiting nitrogen conditions, thereby facilitating the production of biosurfactants with lower surface tension results [96,97].

The availability of other nutrients such as phosphorus, manganese, sulfur, iron, and their ratio, especially C:N, C:Fe, and C:P, affect the biosurfactants fermentative processes [98]. Therefore, it is imperative to optimize these parameters to enhance the production of biosurfactants for obtaining cost-effective products so that they can be applied in industry at a large scale. The Carbon / Nitrogen (C/N) ratio is very important to the productivity of the biosurfactant production process, as high ratios of carbon to nitrogen contribute to a reduction in microbial growth and, consequently, direct the cell metabolism to favor the increased production of metabolites, such as biosurfactants. The literature describes culture media with varied C/N ratios, with the aim of increasing the biosurfactant yield, demonstrating that nitrogen must be present, but at lower concentrations for greater productivity [93]. Examples of optimized C/N ratios were described by Jimoh and Lin [93], as 7:1 for improved biosurfactant yield by *Pseudomonas aeruginosa* F23, 22:1 for biosurfactant production by *Pseudomonas nitroreducens* and 10:1 for the biosurfactant from *Virgibacillus salaries* KSA-T. The effect of oil-to-glucose ratio has also been investigated, as described by Pansiripat et al. [99], who found an optimum ratio of 40:1 for the biosurfactant produced by *Pseudomonas aeruginosa* SP4 grown in a mineral medium with palm oil and glucose.

#### 4.2. Physical variables that influence cell growth and biosurfactant production

The literature describes a wide range for physical variables which can be evaluated in the optimization of biosurfactant production. These physical variables are again dependent on the microbial species used and the composition of the culture medium. An optimum pH range for the generation of biosurfactant compounds ranges between 5.7 and 7.8 [93]. Temperature throughout growth is also a physical factor that requires attention, as a small difference can significantly affect the production of biosurfactants. The optimal temperature range for the generation of glycolipid biosurfactants from species of *Candida* is 27–30 °C [2]. Generation of rhamnolipid biosurfactants from *P. aeruginosa* occurs at 37 °C, on the other hand, biosurfactant production from bacterial strains isolated from environmental sources such as the open ocean are often optimal at lower temperatures (22–30 °C) [28, 29]. Aeration of the culture during the growth cycle by modulation of shake-flask rotational speed between 50 and 250 rpm was found to effect biosurfactant production. Faster rotation, and therefore greater aeration favors the production of biosurfactants with higher yields and high surfactant activity (surface tension less than 28 mN/m) [100,101]. Aeration of the culture is also an important factor to be considered when

scaling fermentative production of biosurfactants up from shake-flask to bioreactor scale [76]. Depending on the ideal microbial growth phase for the production of biosurfactants, (exponential or stationary), incubation time can range from a few hours to several days to obtain maximum productivity. In the majority of cases optimal biosurfactant production from a bacterial strain varies between 18 and 48 h of growth, however extended times ranging between 88 and 120 h, and up to 11 days have been described [57,102,103]. Finally, higher inoculum size can lead to higher yields in less time, as a higher cell density favors the maximum productivity of metabolites but is also important to determine an inoculum concentration that does not lead to the depletion of nutrients and consequent reduction in microbial activity [47]. Inoculum size and the growth phase of the inoculating culture is also an important factor for consideration when scaling up biosurfactant production via microbial fermentation past the laboratory scale [76].

#### 4.3. Genetic engineering strategies for enhancing biosurfactants production

The application of genetic engineering and recombinant DNA technology to produce biosurfactants has gained prominence in recent years due to their potential of in many industrial processes [89]. According to Satpute et al. [104], little information about microbes were used to produce biosurfactants in their cloning, functional characterization, degradation and molecular characteristics. As a result, a new area of research has emerged for scientists to develop new microorganisms using recombinant DNA technology to improve the production and efficiency of biosurfactants in various industrial applications. Improving the microbial strain through recombinant DNA technology can provide not only higher yields at lower costs, but also more efficient biomolecules due to the modification of their chemical properties. Biosurfactants obtained from this technology can also withstand extreme environmental conditions such as high temperature, presence of salt and pH variations [105]. Kandasamy et al. [106] compared the production of biosurfactants from olive oil using recombinant *Escherichia coli*. The pSKA clones containing the BioS gene, srfA showed that the production of biosurfactants was improved compared to the biosurfactants obtained from their parent strain of *Bacillus* sp. SK320. Sekhon et al. [107] also reported a similar result for the biosurfactant produced by microbial cloning. Biosurfactant production was doubled in a recombinant strain compared to its parent strain [89].

Biosynthesis and genetic engineering strategies have been especially used for enhancing surfactin production and generating novel surfactin variants since its low productivity largely limits its commercial application [108]. Recently, a successful example of the modularization of metabolic pathways for improving titre and yield in biotechnological production has been reached by Wu et al. [109]. They developed a systematic engineering approach to improve the biosynthesis of surfactin. They increased the final surfactin titre to 12.8 g/L, with a yield of 65.0 mmol/mol sucrose (42% of the theoretical yield) in a metabolically engineered strain. According to the authors, these findings may pave the way for the commercial production of surfactin.

### 5. Pathways for biosurfactant production

According to Sylatk and Wagner [110], the biosynthesis of a surfactant occurs through four different routes: (a) carbohydrate and lipid synthesis; (b) synthesis of the carbohydrate part while the synthesis of the lipid part depending on the length of the chain of the carbon substrate in the medium; (c) synthesis of the lipid part while the synthesis of the carbohydrate part depends on the substrate employed; and (d) synthesis of the carbohydrate and lipid parts, which are both dependent on the substrate. Therefore, the chemical structure of the carbon source used during microbial fermentation can alter the biosynthesis of the surfactant. Diverse metabolic pathways dependent upon the nature of the main carbon sources employed in the culture medium are involved

in the synthesis of precursor molecules which are intern utilised for biosurfactant production. For instance, when carbohydrates are the only carbon source to produce a glycolipid, carbon flow is regulated in such a way that both lipogenic pathways (lipid formation) and the formation of the hydrophilic moiety through the glycolytic pathway are suppressed by the microbial metabolism [2]. To produce lipids when a simple carbohydrate is utilised as a carbon source, glucose is oxidised through glycolysis and converted into a fatty acid, which is one of the precursors for the synthesis of lipids. When a hydrocarbon is used as the carbon source, however, the microbial mechanism is mainly directed to the lipolytic pathway and gluconeogenesis, thereby allowing its use for the production of fatty acids or sugars [2].

## 6. Renewable natural resources used in biosurfactant production

The generation of agro-industrial byproducts is growing rapidly. In 2019, the industrial activities of bioethanol production, animal slaughter, as well as the processing of cassava, oil palm, and milk together generated more than four billion liters of wastewater [111]. To increase the sustainability of these agro-industrial processes it is therefore urgent to either reduce this wastage or to utilize these waste products and / or effluents in processes that can generate other meaningful products such as surfactants. The food production industry should be explored with regards to the use of its residues, effluents, and byproducts [2,111]. The production of biosurfactants via microbial fermentation can be achieved using many of these industrial wastes. Studies have demonstrated that biosurfactants can be produced from a variety of substrates; including but not limited to hydrophobic mixtures, solvents, hydrocarbons, vegetable oils, dairy products and brewing restudies. The literature describes a number of waste products employed in biosurfactant production, such as vegetable oils, oily effluents, starchy effluents, animal fat vegetable fat, vegetable cooking oil waste, soapstock, molasses, dairy industry waste (whey), corn steep liquor, cassava flour wastewater, oil distillery waste and glycerol [2].

Examples of feedstocks that have been used in the production of biosurfactants by microbial species can be seen in Table 2. The increased

production costs associated with biosurfactants compared to their synthetic counterparts can be mitigated to the point of making biosurfactant production economically viable using these low-cost raw materials derived from other industrial processes [2,93]. It is however important that low-cost raw waste materials should meet the nutritional needs of the microorganisms, offering an appropriate balance of carbohydrates and lipids for the functioning of microbial metabolism to ensure the successful production of the biosurfactant of interest. Feedstocks that provide this balance and contain significant amounts of other micro-nutrients, such as magnesium, manganese, phosphorus, iron, and sulfur, can further reduce the cost of biosurfactant production. In addition to nutritional aspects, the availability of residues, transportation, storage costs, need for pre-treatment, degree of purity, and the physical state of the residues must be considered to guide the choice of the most suitable components for production. As each raw material has its own particularities, the activity of each microorganism occurs in a specific way, which explains the fact that the same raw material is suitable for the production of an effective biosurfactant by one microorganism but not another [112]. The reuse of industrial waste in the production of valuable compounds has assumed a great importance in recent times, not just for the economy of any commercial production process, but also for waste management. However, the use of industrial waste cannot only be sustained by the low cost of these raw materials, other factors are important such as the stability, availability, and variability of each component must be considered. Finally, variability represent a major limitation for use of waste products at an industrial level for the generation of biomolecules such as biosurfactants, since the structures and properties of these biomolecules must remain constant and well-defined.

## 7. Extraction, purification, and characterization

Downstream processing currently accounts for 60–80% of the total cost of obtaining biosurfactants, and as such optimization of processing techniques are fundamental to enabling biosurfactants to be economically and competitively integrated into the market. Different factors, such as the location of the molecule (intracellular, extracellular, or bound to the cell), ionic charge, and solubility significantly affect

**Table 2**  
Raw materials used for biosurfactant production.

Raw material	Microbe	Nitrogen source	Fermentation mode and conditions	Fermentation scale	Biosurfactant type	Biosurfactant concentration (g/L)	Reference
Waste soybean oil (2.0%)	<i>Bacillus cereus</i> UCP 1615	Peptone (0.12%)	Batch mode, 28 °C, 1.0 vvm, 48 h, 250 rpm	50-L bioreactor	Lipopeptides	4.70	[70]
Sugarcane molasses (5%)	<i>Bacillus subtilis</i> RSL-2	No nitrogen sources	Batch mode, 41 °C, 180 rpm, 168 h	Shake flasks		12.34	[113]
Waste sunflower oil (4.0%)	<i>Bacillus amyloliquefaciens</i> RHNK 22	NaNO <sub>3</sub> (0.05%)	Batch mode, 37 °C, 180 rpm, 48 h	Shake flasks		0.80	[114]
Clarified cashew apple juice (4–9%)	<i>Bacillus subtilis</i> LAMI005	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (0.10%)	Batch mode, 30 °C, 180 rpm, 72 h	Shake flasks		0.32	[115]
Trub (residue from brewing industry) (2.0%)	<i>Bacillus subtilis</i>	Yeast extract (0.7%) and peptone (0.09%)	Batch mode, 1.0 vvm, 30 °C, 225 rpm, 24 h	5 L- Bioreactor		1.11	[116]
Vinasse (5.0%)	<i>Bacillus pumilus</i> CCT 2487	NaNO <sub>3</sub> (0.7%)	Batch mode, 30 °C, 200 rpm, 96 h	Shake flasks		27.70	[117]
Sugarcane molasses (5%) and waste soybean oil (5.0%)	<i>Candida bombicola</i> URM 3718	Corn steep liquor (3.0%) and	Batch mode, 28 °C, 180 rpm, 120 h at	Shake flasks	Glycolipids	25.00	[118]
Olive oil (4.0%)	<i>Pseudomonas aeruginosa</i> M408	NaNO <sub>3</sub> (0.50%)	Batch mode, 28 °C, 200 rpm, 96 h	Shake flasks		12.00	[119]
Waste canola oil (2%)	<i>Pseudomonas cepacia</i> CCT6659	Corn steep liquor (3%) and NaNO <sub>3</sub> (0.20%)	Batch mode, 28 °C, 250 rpm, 60 h	Shake flasks		8.00	[120]
Sunflower oil soapstock waste (3.0%)	<i>Pseudomonas aeruginosa</i> LB1	NaNO <sub>3</sub> (0.40%)	Batch mode, 30 °C, 150 rpm, 72 h	Shake flasks		7.3	[121]
Whey (15.0%), vinasse (1.0%) and sucrose (2.0%)	<i>Lactococcus lactis</i> CECT-4434	Yeast extract (0.75%)	Batch mode, 37 °C, 100 rpm, 24 h	Shake flasks	Glycolipo-peptide	0.11	[122]

**Table 3**  
Biosurfactant recovery techniques and their advantages.

Process	Biosurfactant Property Responsible for Separation	Advantages
Acid precipitation	Biosurfactants become insoluble at low pH values.	Efficient in crude biosurfactant recovery, low cost
Organic solvent extraction	Biosurfactants are soluble in organic solvents due to the hydrophobic end.	Efficient in crude biosurfactant recovery and partial purification, reusable nature
Ammonium sulphate precipitation	Salting-out of polymeric or protein-rich biosurfactants	Effective in isolation of polymeric biosurfactants
Adsorption to wood-activated carbon	Biosurfactants are adsorbed to activated carbon and can be desorbed using organic solvents.	Highly pure biosurfactants, cheaper, reusability, recovery from continuous culture
Adsorption to polystyrene resins	Biosurfactants are adsorbed to polystyrene resins and subsequently desorbed using organic solvents.	Highly pure biosurfactants, cheaper, reusability, recovery from continuous culture
Centrifugation	Insoluble biosurfactants are precipitated due to centrifugal force.	Effective in crude biosurfactant recovery, reusability
Ion-exchange chromatography	Charged biosurfactants are attached to ion-exchange resins and can be eluted with buffer.	High purity, fast recovery, reusability
Foam fractionation	Biosurfactant form and partition into foam.	Useful in continuous recovery processes, high purity of product, recovery from continuous culture
Ultrafiltration	Biosurfactants form micelles above their critical micelle concentration (CMC), which are trapped by polymeric membranes.	Fast, one-step recovery, high level of purity, reusability

Source: Adapted from Santos et al. [2].

extraction and purification procedures [94]. Several techniques can be used to recover biosurfactants from microbial fermentation, the most reported of which is liquid phase solvent extraction using a variety of organic compounds (e.g. chloroform-methanol, dichloromethane-methanol, butanol, ethyl acetate, pentane, hexane, acetic acid, and ether) [76]. However, the large volume of solvents required in this process, in addition to increasing costs, are harmful to the environment, making liquid phase solvent extraction disadvantageous [74]. Extraction technologies such as adsorption to wood-activated carbon or polystyrene resins, centrifugation, ion exchange chromatography, foam fractionation, and ultrafiltration are being investigated as viable alternative to liquid phase solvent extraction that are more environmentally benign. Another advantage achieved using these extraction technologies are that one can obtain highly pure biosurfactants at lower cost, faster, and the extraction materials can be reused [2]. Foam fractionation has been one of the most studied separation techniques in recent years since it is a solvent-free method that separates biosurfactant molecules adsorbed to air bubbles in the culture medium. Foam formation in the culture broth during biosurfactant production interferes with the mass and heat transfer processes, thereby affecting productivity. However, foam also assists in the continuous removal of product, and therefore production and recovery processes can be accomplished in a single stage [123]. Continuous foam fractionation helps prevent the accumulation of product that could otherwise inhibit biomass growth and product formation and facilitates biosurfactant production in fed-batch or continuous mode operations [2]. Bages-Estopa et al. [124] described an inexpensive and environmentally friendly strategy to

separate trehalolipids from emulsified fermentation broth through foam fractionation, reaching 23–58% of the total trehalolipids.

An interesting strategy to improve biosurfactant concentration was used by Bustos et al. [125], who showed that cells of *Lactobacillus pentosus* subjected to sequential fermentation and extraction processes with phosphate buffered saline were able to regenerate biosurfactants after various fermentative and extractive cycles, reaching the highest biosurfactant concentration reported for *L. pentosus* growing on glucose.

Following extraction, the crude biosurfactant mixture can be purified to remove unwanted contaminants, the methodologies for carrying this purification are discussed and individually evaluated by Twigg et al. [29]. Once purified, biosurfactants can be identified and characterized by a range of techniques, such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), high-performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR), matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and nuclear magnetic resonance (NMR) [29,47].

One of the most extensive techniques used to identify unknown ingredients found in biosurfactants is TLC. This basic and inexpensive technique is carried out in a sheet of aluminium, glass, or plastic covered with silica gel as adsorbent material and gives the first clue on the presence or absence of either groups such as carbohydrate, lipids, and protein in either a crude or purified sample. As described by Ambaye et al. [89], a more precise version of TLC approach is high-performance thin-layer chromatography (HPTLC), which is more accurate for data obtained as compared to TLC. HPLC is another method used for the separation extraction, performance, and separation of the biosurfactant samples, which allows separation in large volume and saves time in the downstream process. In some cases, HPLC and TLC are both used interchangeably to determine the purity of the separated components. Mass spectrometry, which is coupled with either gas (GC-MS) or liquid chromatography (LC-MS) recognizes the chemical bonds and structures of biosurfactant compounds. As described by Jimoh and Jin [93], the process also gives both qualitative and quantitative analysis of the compounds, which differentiates its usage to HPLC as it gives the molecular mass determination in Daltons [126].

In most cases, the hydrophobic portion of the biosurfactant compound is revealed by GC-MS while LC-MS identifies hydrophilic moiety structural composition. Electrospray ionization has also been employed for the ionization of several biosurfactants compounds before the molecular mass analysis [127]. Reports have shown the usage of tandem mass spectrometry as a great tool in analyzing complex compounds. This allows efficient differentiation among diverse homologs and isoforms existing within a mixture of compounds. Liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LCESI-MS/MS) is a delicate method for the identification of biomolecules with low concentrations, secondary metabolites, and a crude extract of natural origin. These procedures have reduced the possibility of inaccurate characterization. The technique is also cost-effective, reduces time, and energy required for identifying biosurfactants compounds. MALDI-TOF joined to mass spectrometry allows the documentation of integral compounds due to its ability for soft ionization [128]. Although MALDI-TOF examinations are costly, it is quick, giving high-resolution data for the basic characterization of biosurfactants compounds [126]. NMR is also used efficiently to identify the molecular structure of biosurfactants that contain hydrogen and proton components. It also detects the composition and purity of the sample as well as its structural composition. FTIR analytical technique, has also been used to determine the general chemical structure of biosurfactants at a low cost [89].

## 8. Biosurfactants applications

### 8.1. Oil industry

Total oil consumption in 2018 was 99.5 million barrels per day, a

1.5% increase over 2017 [129]. At the current consumption rate, light and medium oils are expected to become increasingly scarce, leading to an increase in the dependence on heavy and extra-heavy oils. Furthermore, total oil reserves worldwide are expected to be depleted within the next 40–45 years [4]. As such the oil industry constantly seeks improvements in technologies to ensure efficient extraction processes to increase production extending the exploration time of reserves and allowing access to residues of oil trapped in the pores of the rocks, estimated to correspond to approximately 60% of the oil in reservoirs [2]. The oil recovery process is generally carried out by three methods: primary, secondary, and tertiary [130]. Natural and induced pressure are part of the primary and secondary methods, respectively, while enhanced oil recovery (EOR) operations constitute the tertiary method. EOR utilizes heat, the injection of miscible gas, and interestingly synthetically derived surfactants to increase oil production and prolong the life of depleting reservoirs [131]. Microbial enhanced oil recovery (MEOR) involves the replacement of synthetic surfactants with biologically derived secondary metabolites such as acids, biopolymers, enzymes, gases, solvents, and the most promising biosurfactants for recovering secondary oil from sediments [15,74,132,133]. During MEOR, microorganisms that produce biosurfactants are introduced to the oil reservoir along with nutrients to stimulate microbial growth [134,135]. Biosurfactants are efficient at mobilizing immobile hydrocarbons by promoting the reduction in surface tension between the oil and rock, which reduces the capillary forces that obstruct the movement of oil through rock pores [32,136].

Alvarez et al. [137] carried out MEOR simulations with the biosurfactant produced by *B. amyloliquefaciens* and achieved a petroleum hydrocarbon recovery rate of greater than 90%. Khondee et al. [138] achieved 100% oil recovery using the foam fractionation technique with the biosurfactant produced by *Bacillus* sp. GY19. Using biosurfactants from strains of *B. subtilis* strains, Gudiña et al. [139] demonstrated the efficient recovery of residual oil from reservoirs exploited for long periods, reporting increased recovery rates of 6–25% for heating oil, 16–24% for viscous paraffin oil, 13–18% for light Arabic oil, and 15–17% for heavy crude oil. Rhamnolipid produced by *P. aeruginosa* contributed to a 50.45% recovery rate of medium weight oils, corresponding to an 11.91% improvement promoted by the presence of the microorganism, which was a better recovery rate than that achieved with the synthetic surfactants evaluated in the study [140]. The use of biosurfactants in MEOR can however be a controversial topic as the amounts of biosurfactants required to extract oil residues trapped in the porous rock may render the process uneconomical. Additionally utilizing compounds whose main value proposition is to replace synthetic chemicals derived from the petrochemical industry for oil recovery is seen as counter intuitive [10,15].

The same properties that are advantageous for oil exploration can be used for oil clean up and bioremediation often necessary because of the occurrence of accidents and consequent environmental contamination by hydrocarbons [141,142]. Bioremediation approaches show that biosurfactants are an excellent ecological alternative to synthetic surfactants, as they can maintain a high rate of biodegradation in contaminated soils. Compared to their synthetic counterpart, biosurfactants can be released in situ where they can carry out their effects with less subsequent handling effort and are technically efficient [16]. Biosurfactants improve the dispersion of contaminants in the aqueous phase and enhance the bioavailability of the hydrophobic substrate to microorganisms for the subsequent removal of such pollutants by biodegradation, with the added advantages of low toxicity and biocompatibility [70]. Soil washing using biosurfactants and the removal of hydrophobic contaminants can also take place in two different ways: the first, occurs below the CMC of the surfactant, while the second, called solubilization, occurs at the higher concentrations. In the first case, the surfactant molecules accumulate at the soil-pollutant or water-soil interface and change the system's affinity for water [2, 16]. In addition, its adsorption on the contaminant surface causes

repulsion between its main groups and soil particles, favoring the release of pollutants from the soil. In the second case, an incorporation of contaminants in micelles favors their partition in the aqueous phase. Pollutants partitioned into micelles can be recovered and demulsified, or even electrochemically destroyed or adsorbed on activated carbon, while the wash solution containing the surfactant can be recycled, which reduces remediation costs [16].

Jadhav et al. [143] reported that the addition of a biosurfactant produced by *Oceanobacillus* sp., increased the biodegradation of crude oil to up to 90%. Mouafi et al. [144] obtained satisfactory dispersion and emulsification of motor oil in water using the biosurfactant produced by *B. brevis*. The application potential for the remediation of soil contaminated with oil has been demonstrated in several studies. Surfactin is among the various surfactants used in biotechnological decontamination processes, with removal rates greater than 85% using biomolecules produced by *B. licheniformis* and 88% using those produced by *B. subtilis* [81,136,137]. Other biosurfactants produced by species of *Bacillus*, *Pseudomonas*, and *Candida* have also been successfully used in soil remediation [82,145–150]. Hentati et al. [151] recently showed that the glycolipidic biosurfactant from *Pseudomonas aeruginosa* was able to remove hydrocarbons from polluted soil. The biosurfactant was also more efficient than the tested chemical surfactants. Again, as with MEOR, there are questions regarding the ability to produce enough biosurfactants via microbial fermentation to carry out bioremediation over the large areas of hydrocarbon contaminated land or ocean associated with either prolonged industrial use or accidental release.

It is important to note that, although many studies have shown that biosurfactants are an attractive choice for removing and/or improving the degradation of hydrophobic contaminants in soil, they, and their synthetic counterparts, may have no influence or even delay degradation through inhibition of microbial metabolism [16,82]. Thus, the degree of toxicity and the permitted doses must be evaluated prior to application of these biomolecules in soils treatment.

## 8.2. Detergent industry

The detergent market includes products for personal care, home cleaning, and heavy industrial cleaning. The surfactant compounds used in formulations within this industry are usually derived from the petrochemical industry. For instance, disinfectants that most widely used surfactant in the personal care and home care sectors during the COVID-19 pandemic is derived from crude oil, making biodegradation difficult and posing considerable toxic potential to aquatic environments [152]. Replacing these synthetically derived surfactants with biosurfactants is increasing becoming an attractive commercial option [153]. This situation has driven the search for ecologically appropriate products, such as biodegradable detergents that are straight-chain (non-branched) organic compounds, which enable efficient microbial degradation [1]. A strategic way to produce more sustainable detergents is to replace synthetic surfactants with green surfactants, such as biosurfactants and preferably those that are effective at low temperatures and/or in hard water [154,155].

One of the main properties of biosurfactants in this sector is their emulsifying capacity, which is necessary for detergent activity. In addition to this property, others are similar to commercial detergents and can be applied in the detergent and laundry industries [93,156]. Bouassida et al. [157] reported greater efficiency in the ability to reduce vegetable oil and coffee stains using a lipopeptide from *B. subtilis* SPB1 compared to commercial detergents. Fei et al. [35] found that surfactin from *B. subtilis* HSO121 can be used in the same way as chemical surfactants, with the added advantages of low toxicity and the absence of irritation, along with excellent emulsifying activity and wetting capacity, high compatibility, stability, biodegradability, and high foaming capacity. A study with the biosurfactant produced by *Ochrobactrum intermedium* MZV101 demonstrated its strong ability to remove oil from fabric [158]. A study by Liley et al. [159] investigated the performance



of a mixture of five ternary surfactants (octaethylene monododecyl ether, C<sub>12</sub>E<sub>8</sub>, sodium dodecyl 6-benzene sulfonate, LAS, and sodium dioxyethylene glycol monododecyl sulphate, SLES) surfactant/ biosurfactant mixture (mono-rhamnolipid- R1, di-rhamnolipid- R2, with C<sub>12</sub>E<sub>8</sub> / LAS / SLES) at low temperatures measuring adsorption properties by surface tension and neutron reflectivity. They concluded that the addition of the rhamnolipids provide a greater degree of tolerance to a temperature reduction from 25° to 10°C than is provided by the conventional surfactant mixtures. They also suggested that the incorporation of rhamnolipids in the detergent-based formulations would likely improve the operating range of detergent formulations at lower temperatures.

### 8.3. Food industry

The application of biosurfactants in foods has been an area of interest in recent years due to the increasing interest by consumers in sustainably produced ingredients and vegetarian and vegan food products. As some of these natural compounds do not have any adverse effects on human health due to their low toxicity, they can be used to improve formulations by changing the viscosity or altering textural aspects as well as inhibiting the growth of some pathogenic microorganisms, which enhances the shelf life, quality, and safety of food [160,161]. Thus, foods incorporated with biosurfactants derived from microorganisms with a Generally Regarded as Safe (GRAS) status may extend resistance to deterioration by oxidation due to the antioxidant action of some biosurfactants as well as stability in the presence of variations in acidity, alkalinity, and temperature. The literature describes numerous biosurfactants with thermal stability under adverse conditions of pH and salt concentration [57,58]. Among microorganisms recently reported for the production of biosurfactants include the yeasts *Starmerella bombicola*, *Candida sphaerica*, *C. lipolytica*, *C. utilis*, *Saccharomyces cerevisiae* and *Meyerozyma guilliermondii*, which have potential as producers of compounds with emulsification and surfactant activities as well as antimicrobial and antioxidant properties [14]. The low toxicity of microbial surfactants meets consumer needs for more natural foods with fewer artificial and chemically synthesized compounds. Thus, biosurfactants can replace additives currently found in foods, which can be harmful to long-term health when consumed in excess [162]. Bakery products (breads, cakes, cookies, and muffins) [163,164] and salad dressings [102], stand out among the applications studied, in addition to applications in flavoring oils and ice cream to control solubilization and consistency as well as intensify aromas [165,166]. Despite the various application possibilities in foods, numerous studies are required to obtain a viable application with the adequate performance of functions in food complex matrices under different processing conditions. For an economically viable application, it is important to develop strategies that employ these biomolecules at the lowest possible concentration for maximum performance [118,167].

### 8.4. Cosmetic industry

The global trend in cosmetic industry is focused on the development of products with more natural and renewable active ingredients to replace or reduce the use of synthetic raw materials. The cosmetic industries are also addressing challenges such as allergies, hair loss, skin and eye irritation caused by chemical surfactants in some formulations, which in addition to affecting humans and animals, can also affect soils and groundwater, causing harm to the environment [168]. Another factor that strengthens this trend is the growing movement that advocate a more conscious relationship with aesthetic care, which, in addition to being linked to well-being, is based on sustainability and clean formulas, influencing consumers, and putting pressure on the beauty and hygiene industry to reduce and, where possible, replace ingredients that fail to fulfil these criteria [20].

Large companies in the cosmetic sector have an average of around

10,000 different cosmetic products and reformulate 25–30% of these products each year. About 10% of these reformulations depend on new active ingredients for the market or the industry. Such companies introduce up to 80 new ingredients into their product portfolio each year [169]. In this context, biosurfactants constitute an option for meeting the demand for new ingredients. With their renewable, biodegradable, low-toxic or non-toxic nature, biosurfactants pose minimal risks to humans and the environment, which is in line with the interest of the emerging consumer market and, consequently, the cosmetic industry. Investments in the applied research of these biomolecules has a considerable chance to result in direct applicability in reformulations and the development of safer innovative cosmetics [170]. The properties inherent to biosurfactants, such as foaming, wetting, dispersing, and solubilizing, are essential in cosmetics. Foaming is also a desirable property for applications in shampoos, soaps, and shaving creams. Wetting capacity enables water-in-oil creams to penetrate the skin more easily. Dispersing and solubilizing capacity is needed to incorporate pigments into various products, such as hair dyes and nail polishes [171–173].

Microbial biosurfactants have properties that are applicable to the cosmetic industry, including antimicrobial, skin surface moisturizing and low toxicity properties of glycolipid and lipopeptide biosurfactants in general which could make them suitable substitutes for chemical surfactants in current cosmetic and personal skincare pharmaceutical formulations [83]. Specific effects have also been reported such as moisturizing properties (mannosylerythritol lipid), antiviral and antibacterial action (trehalolipids), increased dissolution of immiscible compounds in water (sophorolipids), moisturizing and stabilizing properties (emulsan), photoprotective potential (amino acids similar to mycosporin), foaming (surfactin), and mucosal re-epithelialization (rhamnolipids) [31,32]. The German chemical company Evonik, which managed to develop biotechnological methods to produce microbial biosurfactants on an industrial scale in 2010, is currently developing technologies for the production of rhamnolipids for application as foam promoters in cosmetic products, confirming the applicability and the interest of the cosmetic industry in the use of biosurfactants as new active ingredients in formulations [174].

### 8.5. Medicinal and pharmaceutical industry

Biosurfactants have been used in the medicinal and pharmaceutical industries in different therapeutic applications due to their antimicrobial, anti-adhesive, and enzyme-inhibiting properties [175]. Some of the main fields of research on these biomolecules in medicine and pharmacy involve gene-releasing biosurfactants, drugs, as well as antiviral and antitumor activity [23]. Such properties enable alternative therapeutic approaches to the prevention and treatment of diseases and infections as well as reducing the adherence of pathogenic microorganisms [32]. Giri [176] reported that biosurfactants, such as glycolipids and lipopeptides, can damage the cell membrane, leading to lysis and consequent apoptosis, thus inhibiting the proliferation of cancer cells. Surfactin exhibits antimicrobial, antitumor, and anti-mycoplasma properties. Lipopeptides were also reported to have applications in wound healing through their free radical scavenging properties, helping to prevent inflammation as well as improving tissue formation and epidermal differentiation [177]. Another application is in oral health where rhamnolipids or sophorolipids biosurfactants from *Pseudomonas aeruginosa*, *Burkholderia thailandensis* and *Starmerella bombicola* were reported to contribute to oral hygiene through the elimination of bacterial biofilms or inhibition of other bacterial cultures in the oral cavity [178–181].

### 8.6. Nanotechnology

The application of biosurfactants in nanotechnology basically consists of the potential of these molecules for the synthesis of nanoparticles, as they can act both as reducing agents and stabilizers,

especially for silver particles. This is due to the increasing need for "green" alternatives to currently used chemical methods, which require high pressure, temperature, and energy, in addition to forming toxic byproducts [178,182]. Thus, biosurfactants constitute an alternative that favors an efficient eco-friendly process, with no energy consumption and with the absence of harmful compounds [183]. Some microorganisms, such as the bacterium *Bacillus subtilis*, have the ability to produce gold and silver nanoparticles within (intracellular synthesis) and outside (extracellular synthesis) their cells with reported biological activities [184]. Biosurfactants produced by the bacteria *Pseudomonas aeruginosa* and *Brevibacterium casei* have also demonstrated promising results by respectively facilitating the stabilization of nanoparticles in microemulsions [185] and reducing the formation of nanoparticle aggregates, with the maintenance of uniform morphology for more than two months [186]. Studies with silver nanoparticles synthesized by microorganisms indicates important applications in oil recovery (greater than 50%) through the reduction in surface tension and viscosity as well as antimicrobial action, but greater expansion is needed for these Nano biotech applications in bioremediation [187].

### 8.7. Agriculture

The versatile properties of biosurfactants also enabled them to be used in agriculture, mainly to replace synthetic surfactants in formulations of pesticides and agrochemicals, favoring the expansion of "green chemistry" in this sector in response to the need to reduce/eliminate negative impacts on the environment and human health due to the excessive use of chemical compounds [188,189]. Literature reports that

rhamnolipid and lipopeptide biosurfactants are associated with an improvement in soil quality, which is important for the production of crops, as these natural compounds serve as bioremediation agents of soils, surface water, groundwater, and waste streams contaminated with hydrophobic organic compounds, such as polycyclic aromatic hydrocarbons [190,191] and metals [192,193]. Due to their antimicrobial activity, biosurfactants from the lipid classes of mannosylerythritol, rhamnolipids, and lipopeptides can also be used as biopesticides to control different pests, pathogens, phytopathogenic fungi, and weeds [194–196]. Lipopeptides produced by *Pseudomonas putida* and *Pseudomonas fluorescens* respectively cause the lysis of *Phytophthora capsici* zoospores, which cause the "damping off" of cucumbers [197] and inhibit the growth of phytopathogens, such as *Pythium ultimum*, *Fusarium oxysporum*, and *Phytophthora cryptogea* [198,199]. Other lipopeptides and some glycolipids have also showed promising results in this field regarding the inhibition of the action of aphids, mosquitos, and harmful toxins produced by the fungus *Aspergillus parasiticum* in peanut, cotton, and corn crops, preventing microbial infections and pest infestations [4, 32]. Biosurfactants and biosurfactant-producing microorganisms can also serve as nutrients for plants (carbon source) and aid in the absorption of essential substances for their proper development through the efficient distribution of metals and micronutrients in the soil and production of biofilm on roots, while also protecting plants from harmful substances [188,200,201]. Table 4 shows a summary of uses of biosurfactants in different industries.

**Table 4**  
Applications and roles of biosurfactants in industries.

Industry	Biosurfactant types	Application	Role of Biosurfactants	References
Petroleum	Rhamnolipids, sphorolipids and lipopeptides	Enhanced oil recovery Crude oil pipelines/transport	Emulsification of oils, lowering of interfacial tension, de-emulsification of oil emulsions, solubilisation of oils, viscosity reduction, dispersion of oils, wetting of solid surfaces, spreading, detergency, foaming, corrosion inhibition in fuel oils and equipment.	[4,10,15,16]
Environment	Rhamnolipids, sphorolipids and lipopeptides	Bioremediation Oil spill cleanup operations Soil remediation and flushing Treatment of wastewater Heavy metal remediation Biofouling	Emulsification and de-emulsification of oils, lowering of interfacial tension, dispersion of oils, solubilisation of oils, wetting, mobilization, spreading, detergency, foaming, corrosion inhibition in fuel oils and equipment; binding, desorption, and mobilization of heavy metals.	[2,4,10,15-17,88]
Mining	Glycolipids and polymeric surfactants	Heavy metal cleanup operations Soil remediation Flotation Heavy metal recovery	Wetting and foaming, collectors and frothers, removal of metal ions from aqueous solutions, soil and sediments, heavy metals sequestrants, spreading, corrosion inhibition in oils.	[2,12,16,20]
Agriculture	Rhamnolipids, sphorolipids and lipopeptides	Biocontrol Fertilisers Plant protection	Wetting, dispersion, suspension of powdered pesticides and fertilisers, emulsification of pesticide solutions, facilitation of biocontrol mechanisms of microbes, plant pathogen elimination and increased bioavailability of nutrients for beneficial plant-associated microbes.	[2,82,155]
Food	Glycolipids, lipeptides and polymeric surfactants	Emulsification Functional ingredient	Solubilisation of flavoured oils, control of consistency, emulsification, wetting agent, spreading, detergency, foaming, thickener.	[2,14]
Medicine	Rhamnolipids, sphorolipids and lipopeptides	Microbiological Pharmaceuticals and therapeutics	Anti-adhesive agents, antifungal agents, antibacterial agents, antiviral agents, vaccines, gene therapy, immunomodulatory molecules.	[2,82,88]
Cosmetics	Sphorolipids, mannosylerythritol lipids, rhamnolipids and lipopeptides	Health and beauty products	Emulsification, foaming agents, solubilisation, wetting agents, cleansers, antimicrobial agents, mediators of enzyme action, antioxidant, moisturizing, healing, and skin toning properties.	[2,11,88]
Cleaning	Rhamnolipids, sphorolipids and mannosylerythritol lipids	Washing detergents	Detergents and sanitisers for laundry, wetting, foaming, spreading, solubilizing, corrosion inhibition.	[1,82]
Textiles	Biodispersant	Preparation of fibres Dyeing and printing Finishing of textiles	Wetting, penetration, solubilisation, emulsification, detergency, and dispersion, wetting and emulsification in finishing formulations, softening.	[89]
Nanotechnology	Rhamnolipids and lipopeptides	Synthesis of nanoparticles	Emulsification, stabilisation.	[12,20]

## 9. Formulation of packages

Research in the field of biosurfactant technology seeks to develop efficient products through formulations containing multi-components with different functions [156,202,203]. The application of “surfactant packages” is one approach along this line of multifunctional products that involves biosurfactants and bioemulsifiers working together. This is mainly employed in the petroleum industry for crude oil extraction, facilitating transport through pipelines, the cleaning of storage tanks, and the treatment of petroleum residues as well as serving as demulsification agents [204,205]. As a rule, packages are formulations containing a mixture of surface-active agents, compatible solvents, and other special chemical products selected according to the type of oil to be transported by the micelles, which may have other applications at the end of the process. Thus, the surfactant package must contain both water- and oil-soluble compounds, be capable of lowering interfacial tension, minimize foaming, be chemically compatible with other chemical additives, and produce microemulsions. The microemulsion system has high solubilization capacity and ultra-low oil-water interfacial tension, making it an ideal choice for practical applications, such as EOR [204].

Formulations containing a blend of a rhamnolipid, surfactin, and phengycin demonstrated 86% oil washing efficiency and emulsification activity in crude oil, suggesting potential application in EOR [206]. Ultimately, the emulsions formed (emulsanosols), which have a high concentration of oil, can be used as fuel without prior treatment. This can provide economic gains in the process, as it results in an incremental return on the investment [204,207]. Mixtures of glycolipids and lipopeptides with other substances have also been studied for applications in the fields of medicine, cosmetics [156,208,209] and food [210].

## 10. Economics of microbial biosurfactant production

### 10.1. Production cost

To be economically competitive in the market, biosurfactants need to have prices equal to or lower than their synthetic counterparts currently valued at approx. \$2/kg [2]. The high costs required to obtain biosurfactants are attributed to the acquisition of raw materials and the biomolecule recovery processes, estimated to account for between 10% and 80% of the total production cost [211]. The upstream (selection of microbial strains, culture media, and sterilization) and downstream (separation and purification of products) steps are part of the flow of obtaining any biomolecule of commercial interest [93,211]. However, downstream steps are responsible for the largest part of the cost of biotech products [212]. It costs 10–12 times more to produce the same amount of biosurfactants compared to synthetic surfactants, which hinders the establishment of these biomolecules in the surfactant and related markets [213]. Any project for the production of biological products usually includes an economic assessment involving the estimated capital investment, operating costs, and profitability analysis. When considering biopharmaceuticals, the cost is very high (between US \$20 and US\$500 million), as R&D expenses must be included for all unsuccessful products and a high degree of purity is required. The operating cost of a biochemical plant is the sum of all expenses, i.e., raw materials, labor, utilities, waste disposal, general expenses, etc., divided by the annual operating cost and the annual production rate to obtain the unit production cost of the bioproduct (in \$/kg). In obtaining biosurfactants, some products cost \$1.0/kg and others cost more than \$10,000,000/kg to produce [211], depending on different variables inherent to the production process of each biomolecule. The market price of rhamnolipids is between US\$1.5 and \$1500/g, depending on the purity level and manufacturer. The cost of lipopeptide biosurfactants, which are produced in small quantities but have potential applications in the pharmaceutical and cosmetic industries, is approximately US\$20 to US\$130/mg [219]. The current prices of some biosurfactants compared to

the synthetically derived surfactant sodium dodecyl sulfate (SDS) are shown in Table 5.

When biosurfactants are produced in a batch fermentation process, the cost is mainly linked to the inputs driving the fermentation and purification processes [214]. Soares da Silva et al. [215], calculated the cost of the biosurfactant isolated from *Pseudomonas cepacia* using food waste as substrate for production with a reported yield of 40.5 g/L of glycolipid in a 50-L fermenter. They estimated a cost of about \$20/kg for the obtained biosurfactant, considering both production and purification. A key approach in this production system involves increasing product yield by optimizing fermentation through factorial planning experiments. One possible approach to produce biosurfactant is to utilize solid state fermentation with substrates derived from other industrial processes [19]. In two days of fermentation using a simple downstream process with 5.58 g of soy flour and 3.67 g of rice straw, 50.01 mg/g of dry lipopeptide was produced by *B. amyloliquefaciens* XZ-173 in solid-state fermentation [215]. *B. subtilis* SPB1 was grown in a mixture of olive leaf waste flour and olive cake flour also using solid state fermentation, leading to the production of 30.67 mg of lipopeptide per gram of solid substrate [216]. In addition, through solid state fermentation and using molasses as a substrate, Al-Dhabi et al. [214] reported a strain of *B. subtilis* producing 74 mg/g of biosurfactant [217]. These three cases show the feasibility of using solid-state fermentation to produce lipopeptides, saving operational costs related to submerged fermentation [218]. Solid state fermentation holds promise for biosurfactant production, but much optimization needs to be carried out to make this approach viable in the market. Little work has been done to analyze this fermentation process for biosurfactant production. Current productions are mostly constrained to batch or fed-batch production processes due to limitations in the sustained nutrient feeding strategy, biomass growth and heat and mass transfer reactions that limit the efficiency of the solid-state process. Therefore, more research is needed to create an integrated bioprocess for continuous biosurfactant production and recovery using low-cost waste biomass in solid state fermentation [19].

Despite the increased costs associated with biosurfactants, the market for biosurfactants currently represents 5% of the total surfactant market and has been showing a steady increase over the past decade [221]. The compound annual growth rate (CAGR) is the rate of return measured over the period of the investment. According to a new research report by Global Market Insights, Inc., biosurfactant market revenue is expected to register more than 5.6% CAGR by 2025, as attention to human safety will nurture the growth of this industry. Moreover, the market for rhamnolipid-based biosurfactants with applications in food processing is projected to have a more than 16.5% CAGR from 2020 to 2026 [222,223]. The actual continental biosurfactants demand is showed in Fig. 2 and the main biosurfactant-producing companies in the world market are summarized in Table 6.

### 10.2. Strategies for feasible commercial biosurfactant production

Reducing the total cost of producing biosurfactants as well as increasing their yield and productivity to make them economically

**Table 5**

Price of main biosurfactants and SDS marketed by MilliporeSigma (values obtained in September 2021).

Surfactants	Pack Size	US\$ (2021 Prices)
Rhamnolipids, 90%	100 g	\$233.00
Surfactin <i>Bacillus subtilis</i> , $\geq 98.0\%$ (HPLC)	10 mg	\$223.00
Rhamnolipids, 95% (90% Di-Rhamnolipid)	10 mg	\$429.00
Fengycin $\geq 90\%$	5 mg	\$570.00
Iturin A from <i>Bacillus subtilis</i> $\geq 95\%$ (HPLC)	5 mg	\$567.00
Saponin for molecular biology	250 g	\$398.00
Sodium dodecyl sulfate ACS reagent, $\geq 99.0\%$	100 g	\$146.00

Source: MilliporeSigma [220].

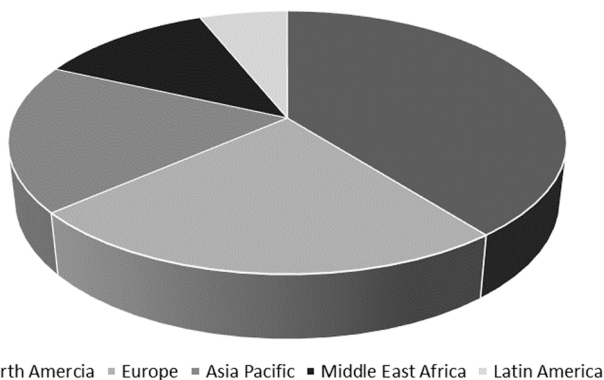


Fig. 2. Current continental biosurfactants demand. Adapted from Ambaye et al. [89].

attractive to the surfactant market depends on several key approaches. These include, selecting robust microorganisms; using cheap raw materials; optimizing culture media to increase the concentration of biosurfactant; the use of genetically modified microorganisms; the use of new statistical approaches to improve the fermentation process; and the development of novel low-cost downstream processes [18,93,207,224]. Table 7 offers a summary of the main strategies to make the commercial

production of biosurfactants viable.

## 11. Future research directions

Despite an enormous volume of research over the past two decades on the economics of biosurfactant production, their commercial success compared to their synthetic counterparts remains an economic challenge [32]. Several aspects still need to be investigated and many strategies can be combined to increase the industrial production of these biomolecules, as discussed in section 9.2. Producing biosurfactants under non-sterile conditions, for example, would significantly reduce production costs. The use of fortified and unprocessed waste substrates and the co-production of biosurfactant with other industrially economical products must be studied more critically, especially in large fermentation vessels. Furthermore, the downstream process and purification steps typically involves substantial costs, as also discussed above. Therefore, advancement in this field are urgently needed to reduce production cost [226]. To enable large-scale production, technical development in reactor design and process control are also required. Another field of research recognized for future work is the exploration of sustainability and life cycle assessment of biosurfactants. In this regard, research work should emphasize all phases, starting with production, extraction, and their applications [227]. Such studies should also consider some important aspects, such as issues related to toxicity,

Table 6

Commercial scale biosurfactants produced by different companies in the world with industrial applications.

Company	site	Biosurfactant	Application	Country
Jeneil Biosurfactant	<a href="http://www.jeneilbiotech.com/">http://www.jeneilbiotech.com/</a>	Rhamnolipid biosurfactants	Cleaning and oil recovery from storage tanks, EOR	USA
AGAE Technologies	<a href="https://www.agaetech.com/">https://www.agaetech.com/</a>	Rhamnolipid biosurfactants	Pharmaceuticals, cosmetics, personal care products, bioremediation (in situ and ex situ), enhanced oil recovery (EOR)	USA
Rhamnolipid Companies	<a href="http://rhamnolipid.com/">http://rhamnolipid.com/</a>	Rhamnolipid biosurfactants	Agriculture, cosmetics, EOR, bioremediation, food products, pharmaceutical products	USA
TensioGreen	<a href="http://www.tensiogreen.com/index.php">http://www.tensiogreen.com/index.php</a>	Rhamnolipid biosurfactants	Petroleum industry, cleaning, and oil recovery from storage tanks, EOR	USA
Logos Technologies	<a href="https://www.natsurfact.com/">https://www.natsurfact.com/</a>	Rhamnolipid biosurfactants	Petroleum industry, cleaning, and oil recovery from storage tanks, EOR	USA
Synthezyme	<a href="http://www.synthezyme.com/index.html">http://www.synthezyme.com/index.html</a>	Sophorolipid biosurfactants	Emulsification of crude oil, petroleum, and gas	USA
Paradigm Biomedical Inc	<a href="http://www.akama.com/company/Paradigm_Biomedical_Inc_a7bcb2680775.html">http://www.akama.com/company/Paradigm_Biomedical_Inc_a7bcb2680775.html</a>	Rhamnolipid biosurfactant	Pharmaceutical products	USA
Kanebo Cosmetics Inc.	<a href="http://www.kanebo.com/science/skincare/biosurfactants">http://www.kanebo.com/science/skincare/biosurfactants</a>	Mannosylerythritol lipid B (MEL-B)	Cosmetics	Japan
Allied Carbon Solutions (ACS) Ltd	<a href="https://www.allied-c-s.co.jp/english-site">https://www.allied-c-s.co.jp/english-site</a>	Sophorolipids	Agricultural products, ecological research	Japan
Kaneka Corporation	<a href="https://www.kaneka.co.jp/en/business/qualityoflife/nbd_002.html">https://www.kaneka.co.jp/en/business/qualityoflife/nbd_002.html</a>	Sodium surfactin	Cosmetics	Japan
Saraya Co. Ltd.	<a href="http://worldwide.saraya.com/">http://worldwide.saraya.com/</a>	Sophorolipid biosurfactants	Cleaning products, hygiene products	Japan
ZFA Technologies Inc.	<a href="http://www.zfatech.com/index.php?lang=en">http://www.zfatech.com/index.php?lang=en</a>	BERO biosurfactant	enhanced oil recovery (EOR)	China
Urumqi Unite Bio-Technology Co. Ltd.	<a href="https://unite-xj.en.alibaba.com/productlist.html">https://unite-xj.en.alibaba.com/productlist.html</a>	Rhamnolipids	Cleaning and oil recovery from storage tanks, EOR	China
Groupe Soliance	<a href="http://www.soliance.com/dtproduit.php?id=42">http://www.soliance.com/dtproduit.php?id=42</a>	Sopholiance S (Sophorolipid)	Cosmetics and pharmaceuticals	France
Lipofabrik	<a href="http://www.lipofabrik.com/">http://www.lipofabrik.com/</a>	Lipopeptides	Pharmaceutical products	France
TeeGene Biotech	<a href="http://www.teegene.co.uk/">http://www.teegene.co.uk/</a>	Rhamnolipids and lipopeptides	Pharmaceutical products, cosmetics, antimicrobials and anticarcinogen ingredients	UK
Sabo S.P.A.	<a href="http://www.sabo.com/sabo/home.php">www.sabo.com/sabo/home.php</a>	Sodium surfactin	Cosmetics	Italy
Ecover Eco-Surfactant	<a href="https://www.ecover.com/">https://www.ecover.com/</a>	ACS-Sophor / Sophorolipid	Oil recovery and processing, EOR; biofilm removing agent, biofilm growth inhibitor; detergent action	Belgium
Fraunhofer IGB	<a href="https://www.igb.fraunhofer.de/">https://www.igb.fraunhofer.de/</a>	Glycolipid and cellobiose lipid biosurfactants	Cleaning products, dishwashing liquids, pharmaceutical products (bioactive properties)	Germany
Evonik	<a href="https://household-care.evonik.com/">https://household-care.evonik.com/</a>	Rhamnolipids, sophorolipids	Cosmetics, cleaning products, dishwashing liquids,	Germany
BioFuture	<a href="https://biofuture.ie/">https://biofuture.ie/</a>	Rhamnolipid biosurfactants	Washing of fuel tanks	Ireland
EcoChem Organics Company	<a href="http://www.biochemica.co.uk/">http://www.biochemica.co.uk/</a>	Rhamnolipid biosurfactants	Dispersant of insoluble hydrocarbons in water	Canada
Soft Chemical Laboratories	<a href="http://www.probac.co.za">www.probac.co.za</a>	Surfactin	Cleaning products, dishwashing liquids	South Africa
MG Intobio Co. Ltd.	<a href="http://www.intobio.com">http://www.intobio.com</a>	Sopholine (Sophorolipids)	Cosmetics	South Korea

**Table 7**  
Summary of key strategies to make commercial biosurfactant production viable.

Sr. No	Strategies	Comments	References
1	Agro-industrial waste products	Agro-industrial waste, crop residues; animal fat and oil industries; by-products of the milk and distillery industry; waste from the petroleum processing industries; food processing by-products.	[18,93,207,224]
	Growth conditions	Improved production within the alkaline condition range; Slight variation in temperature; Agitation speed and aeration; The size of inoculum; Proper balance of carbon, nitrogen, phosphorus, and other trace elements to maximize yield; growth stimulators such as lactones.	[93]
2	Optimization for biosurfactant production by innovative statistical approach	RSM response surface methodology (RSM), Plackett-Burman design (PBD), central composite design (CCD) and central composite rotational design (CCRD)	[93]
3	Nanoparticles	Biosurfactant production is negatively altered by the presence of several metal salts. There is evidence that biosurfactant production is increased using low concentrations of metal salt nanoparticles, especially iron (Fe-NPs).	[93]
4	Co-production of biosurfactant (Biorefinery concept)	Biosurfactants production by yeasts using sugarcane bagasse hemicellulosic hydrolysate as new sustainable alternative for lignocellulosic biorefineries, Co-production of biosurfactant along with other commercially important compounds like enzymes.	[54,225]
5	Microbial engineering for the biosynthesis of biosurfactant	Mutant or recombinant strains for higher biosurfactant yields by altering the physiology and/or genetics of the microorganism. The modification of physiology includes the growth of the microorganism in the presence of different substrates.	[207]
6	Solid-state fermentation process	Overcome the foam production problem found.	[93,224]
7	Microbioreactors	Improved distribution of fermentation parameters, control of foam formation, and reduced expenses with the purification process.	[224]
8	Microorganism immobilization techniques	The growth stages and product formation are separated which can reduce costs by making product separation easier.	[224]
9	Stimulating biosurfactant production in situ	Induced microbial populations in places with high presence of oily products to produce biosurfactants.	[207]
10	High throughput screening using omics technology	Metagenomes will be screened for biosurfactant-	[207]

**Table 7 (continued)**

Sr. No	Strategies	Comments	References
		producing genes such as those involved in glycolipid or lipopeptide production.	

carbon footprint generation and resource depletion. To increase production, cheap raw material can be supplemented with readily available commercial components or with the use of stimulators to increase microbial growth and product yield.

The use of mutant strains, whose genome and metabolic information are known, should also be explored further [19]. By following the scaling process and increasing the annual production of biosurfactants, the price will decrease and allow entry of these biomolecules into the mass market. Adequate scaling up of most biosurfactant fermentation processes also requires a reliable system for foam control. Heavily foaming biosurfactants are desirable for manual cleaning applications such as dishwashers, glass cleaners, shower gels and others, but foaming also hinders large scale upstream production of biosurfactants. Bio-process engineering research has identified some results that should be further developed, such as foam fractionation, separation, implementation of foam traps or the addition of anti-foam agents. Some manufacturers report bad odour and color from biosurfactants that prevent them from being used in cosmetics and other personal care products. In this aspect, process development can be intensified to remove unwanted side products in sensory perception. Research should also focus on exploring suitable microbes with high-level metabolic activities through genetic engineering, molecular biology, and surface science, making biosurfactants most economically viable for application in different sectors.

Another important area to be considered is the proper understanding of these biomolecules and the method of monitoring and testing to select the best biosurfactant producers, which are still unknown. Many potent biosurfactants are lacking in their biomolecule data and hence hindered in their maximum usage [175]. Furthermore, research is needed to understand the biosurfactant pathway at the gene and species level using principles of genomics and proteomics [89]. Another important aspect concerns the safety of application of these biomolecules. Although the effects of biosurfactants on human health are not fully elucidated, advancing data to fill gaps in knowledge and technologies in this regard can help researchers to understand the potential of the applications. Biosurfactants have been extensively studied in relation to their anti-microbial activities. Other medicinal and industrially important characteristics of biosurfactants still need to be studied. In-depth studies of the anti-cancer and anti-biofilm activities of biosurfactants need to be carried out.

Most studies on biosurfactant properties to date have involved the whole product produced by a specific microorganism some after some clearing or purifying process. The produced biosurfactant products, however, are always a collection of several congeners some may be major and other minor fractions of a mixture which could typically vary from two to over twenty. Our recent investigations on separated congeners have shown that these congeners may have different properties or effects and can behave quite differently, and therefore observations of the mixed products basically reflects the overall characteristic of the mixture and not any of the individual congeners. This means that we need to purify these congeners and investigate their properties and characteristics independently of each other to make better use of the property sought. Thus, biosurfactants have a huge hidden potential not yet revealed. Accompanying the increased demand for bioproducts, most of the future research will be directed toward meeting a number of challenges to increase product efficiency, enhancing the productivity, and reducing the high costs of fermentation and downstream processing. It is also important to point out that without public support, the risk

seems too high for chemical companies to develop an economical production process for biosurfactants alongside established processes. For ensuring the industrial competitiveness in green chemistry, governments should create incentives promoting the economic production and thus the market entry of biosurfactants.

## 12. Conclusion

Biosurfactants are recognized for their potential in the development of commercial products. Their wide application favors innovative studies that take advantage of these molecules on different fronts of technology. Biosurfactants can be applied in wide and ever-expanding range of commercial sectors from the oil industry for the recovery of oils and bioremediation of environments to medical and pharmaceutical fields for their surface-active, antimicrobial, anti-adhesive, and anti-biofilm properties. Only some species of biosurfactant-producing microorganisms of the glycolipid class have achieved some satisfactory levels of metabolite production that can be used for the production and commercialization of products. Low yields and high production costs continue to be the main obstacles to large-scale production. Thus, agro-industrial byproducts are used to optimize the production process and lower costs, with the added advantage of contributing to environmental sustainability [221]. Advances in research have indicated the importance of yields and productivity improvements achieved through bioengineering strategies, changes in fermentation modes, and the statistical design of experiments. Thus, the use of low-cost substrates, the establishment of ideal production conditions for different bioproducts, the development of new purification methods, and securing high-yield strains can make the production of biosurfactants economically viable and pursuing purified products for high value specialized markets may also be a way forward.

## Declaration of Competing Interest

The authors declare no conflict of interests.

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