

BIOTECHNOLOGY AND INDUSTRIAL MICROBIOLOGY - REVIEW



Bacterial-derived surfactants: an update on general aspects and forthcoming applications

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Abstract

The search for sustainable alternatives to the production of chemicals using renewable substrates and natural processes has been widely encouraged. Microbial surfactants or biosurfactants are surface-active compounds synthesized by fungi, yeasts, and bacteria. Due to their great metabolic versatility, bacteria are the most traditional and well-known microbial surfactant producers, being *Bacillus* and *Pseudomonas* species their typical representatives. To be successfully applied in industry, surfactants need to maintain stability under the harsh environmental conditions present in manufacturing processes; thus, the prospection of biosurfactants derived from extremophiles is a promising strategy to the discovery of novel and useful molecules. Bacterial surfactants show interesting properties suitable for a range of applications in the oil industry, food, agriculture, pharmaceuticals, cosmetics, bioremediation, and more recently, nanotechnology. In addition, they can be synthesized using renewable resources as substrates, contributing to the circular economy and sustainability. The article presents a general and updated review of bacterial-derived biosurfactants, focusing on the potential of some groups that are still underexploited, as well as, recent trends and contributions of these versatile biomolecules to circular bioeconomy and nanotechnology.

Keywords Bacteria · Bioeconomy · Biosurfactant · Extremophiles · Nanotechnology

Introduction

Surfactants can be defined as a class of chemical compounds commonly present in soaps and detergents. These molecules have a polar domain (ionic, anionic, or amphoteric) and an apolar domain (composed of a hydrocarbon chain); thus, their amphiphilic character allows reducing the surface tension of liquids acting at the interface of fluids of different polarities such as oil/water, besides having the ability to form emulsions [1–3]. Most surfactants are obtained by chemical synthesis from petroleum derivatives and have been widely used in various industrial sectors (petrochemical, pharmaceutical, food, agrochemical, and hygiene/cosmetic) as detergents, emulsifiers,

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Marcia Nitschke nitschke@iqsc.usp.br adhesives, flocculants, foaming agents, demulsifiers, and penetrants [4]. Currently, the production of surfactants exceeds 7.5 million tons, generating an income of over \$41 billion [5].

The growing environmental concern combined with the new environmental control laws culminated in the search for more sustainable alternatives to replace existing surfactants [6]. It is known that, from their manufacture to their final disposal, chemical-derived surfactants might cause diverse environmental damage. For example, the presence of sulfate and phosphate groups, common in several types of surfactants, contributes to an increase in the amount of such ions in the aquatic environment, which causes undesirable growth of planktonic organisms and surface aquatic plants, resulting in the eutrophication of the system. In addition, the discharge of surfactants into water bodies also causes a decrease in the surface tension of the water and an increase in the solubility of organic compounds present, as well as preventing the entry of sunlight due to the formation of foam. As a result, aquatic plants, algae, and cyanobacteria do not photosynthesize, and the degree of oxygen solubility drops dramatically, damaging the aquatic ecosystem and causing the death of several species [7]. Another aspect to

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consider is the use of non-renewable raw materials (mainly petroleum derivatives) for the production of surfactants, a non-sustainable practice from an environmental point of view.

The search for microorganisms that produce compounds of economic interest, including natural surfactants, has driven biotechnology in recent decades. The biosurfactants (BS) are molecules of microbial origin that present a great structural diversity, consisting of metabolic by-products of bacteria, fungi, and yeasts [8]. This property is particularly common among microorganisms capable of degrading water-insoluble hydrophobic compounds, such as petroleum [9]. Like synthetics, BS also exhibit surfactant, emulsifying, wetting, foaming, solubilizing, and dispersing properties [10, 11], besides presenting advantages such as biodegradability, low or no toxicity, and stability over a wide range of temperature, ionic strength, and pH [12].

Despite the benefits, the BS present high production cost, which disfavors their widespread use and replacement to synthetic surfactants [13], highlighting the relevance of the search for alternatives that can reduce such economic barriers. The circular bioeconomy concept, which has emerged in the last years, encompasses the production of renewable resources and their conversion into bio-based products by using efficient strategies to minimize waste generation [14]. Such scenario favors microbial surfactants, since their production using residues or byproducts from agro/food industry can contribute to reduce costs and waste generation.

In recent years, BS have also emerged as sustainable ingredients in nanotechnology, where they are utilized in the synthesis of metal and organic nanoparticles; they also provide self-assembly structures to encapsulation, functionalization, or templates and act as emulsifiers in nanoemulsions [15].

Some well-known bacteria are described in the literature as capable of synthesizing BS that exhibit useful properties for pharmaceutical, food, cosmetic, and agriculture applications [16]. Regardless of significant advances in this field, the BS-producing potential remains scarce or unknown for most bacterial species. Factors such as habitat, physiological, genetic, and biochemical characteristics of bacteria may influence the production, composition, as well as physicochemical properties of BS [17–19]. Thus, the search for BS synthesized by strains adapted to extreme environments, along with endophytic and diazotrophic strains, is advantageous since they can reveal new surfactant molecules with unique properties.

This article presents an overview on general and applied aspects of bacterial-derived BS highlighting their production by novel and few-exploited bacterial strains, as well as, their contribution to circular bioeconomy and prospective uses in nanotechnology.

Bacterial-derived surfactants

The first record regarding BS is attributed to Jarvis and Johnson who, in 1949, elucidated the structure of rhamnolipids of *Pseudomonas aeruginosa* [20, 21]. Later, in 1968, Arima and collaborators described a new compound synthesized by the bacterium *Bacillus subtilis*, which presented high surface activity, thus receiving the name surfactin [22].

Around 1972, during the first conference at the United Nations, the concept of sustainable development arises proposing maximum environmental preservation in the midst of industrial activities, thus meeting human needs without compromising the availability of resources for future generations [6]. Thereafter, the concern about consuming environmentally friendly products emerges, which has driven market sectors to give greater prominence to biotechnology, as an input for sustainable production. Within this context, studies regarding the production and use of natural surfactants have been gaining more and more attention [23].

Most microbial surfactants described in the literature are derived from bacteria such as *Pseudomonas* sp., *Bacillus* sp., and *Acinetobacter* sp. [24]. Studies show that the natural role of BS is associated with the emulsification of hydrophobic substrates, improving the availability of nutrients necessary for their growth [25]. Moreover, BS are also associated with antibiotic function favoring the survival of the producing strains [26] as well as, with the attachment/dissociation of cells to surfaces, motility, *quorum sensing*, and biofilm formation [1].

Unlike synthetic surfactants, which are classified according to their polar group, biosurfactants are categorized according to their biochemical nature and microbial origin (Table 1).

Bacteria of the genus *Pseudomonas* sp. (especially *Pseudomonas aeruginosa*) are producers of rhamnolipids, one of the most extensively studied and characterized glycolipid BS. They can reduce the surface tension of water to considerable values and present excellent potential for bioremediation of environments contaminated with oil and/or heavy metals [28]. They are synthesized as a mixture of homologs, being of greater predominance the mono-rhamnolipids (mono RL) and di-rhamnolipids (di RL)—the first containing a rhamnose linked to two molecules of β -hydroxydecanoic acid and the second consisting of two rhamnose attached to two molecules of β -hydroxydecanoic acid. Besides, the fatty acid chain can vary with respect to the number of carbons and presence of unsaturation [29].

Cyclic lipopeptides are another well-known class of bacterial surfactants. The majority of lipopeptide BS are

 Table 1
 Examples of some types of surfactants and their bacterial origin

Biosurfactant class	Structure	Bacteria	
Glycolipids	Rhamnolipids	Pseudomonas spp. P. aeruginosa Bulkholderia spp.	
	Trehalolipids	Rhodococcus erythropoli Mycobacterium spp.	
Lipopeptides	Viscosin	Pseudomonas fluorescens	
	Surfactin	Bacillus subtilis	
	Iturin, fengycin	Bacillus spp.	
	Lichenysin	Bacillus licheniformis	
	Polymyxin	Bacillus polymyxa	
Phospholipids, fatty acids, and neutral	Fatty acids	Corynebacterium lepus	
	Neutral lipids	Nocardia erythropolis	
lipids	Phospholipids	Thiobacillus thiooxidans	
Polymeric surfactants	Emulsan	Acinetobacter calcoace- ticus	
	Biodispersan	Acinetobacter calcoace- ticus	
Particulated surfactants	Vesicles	Acinetobacter calcoace- ticus	
	Cells	Cianobacteria	

Adapted from: Chen et al. [27]

synthesized by *Bacillus* and *Pseudomonas* strains [30]. Surfactin is a representative lipopeptide from *B. subtilis*, widely studied for its excellent emulsification, antibiotic, and surface activity [31, 32]. Typical structure of surfactin comprises a β -hydroxy fatty acid chain, containing thirteen to fifteen carbons, linked to a peptide ring with seven L and D amino acids [33, 34]. Other cyclic lipopeptides, like iturin, fengycin, and lichenysin also derived from *Bacillus* strains, differ from surfactin in their peptide moiety [35]. Equally, *Pseudomonas*-derived lipopeptides, as viscosin and putisolvin, consist of a short oligopeptide linked to a fatty acid tail [35] exhibiting high surface and biological activity [36].

The genus *Acinetobacter* is described to synthesize complex polymeric surfactants of high molecular weight, known as bioemulsifiers. Emulsan and Biodispersan are employed in the formation of oil/water type emulsions and in bioremediation processes [19, 37, 38]. The main classes of producing bacteria, the types of biosurfactants, as well as the chemical structures of these compounds, are shown in Table 2.

Other bacterial genera, such as Acidovorans, Agrobacterium, Alcaligenes, Aeromonas, Comomonas, Corynebacterium, Cycloclasticus, Flavobacterium, Microbacterium, Moraxella, Micrococcus, Neptunomonas, Nocardia, Paracoccus, Pasteurella, Polaromonas, Ralstonia, Sphingomonas, and Stenotrophomonas, have been described as capable of growing in hydrophobic environments and acting in oil biodegradation [39] and, therefore, are also prospective biosurfactant producers.

There are several techniques that, when used together, can be efficient in the prospecting of microorganisms producing surfactant compounds. Among the most employed methods are the emulsification index, droplet collapse, and surface tension measurement [42]. The emulsification index evaluates the emulsifying capacity of the BS by measuring the height of the emulsion formed after mixing a sample containing the BS and a hydrophobic source [43]. Droplet collapse, like the emulsification index, is a qualitative technique, described by Bodour and Miller-Maier [44], which aims to verify the destabilization of the oil droplet in the presence of the BS. In this method, an aliquot of the culture medium supernatant is deposited on a solid surface containing an oil drop. The presence of the surfactant is verified by the spreading or collapse of the oil drop, indicating the reduction of forces, or interfacial tension between the oil drop and the solid surface [44].

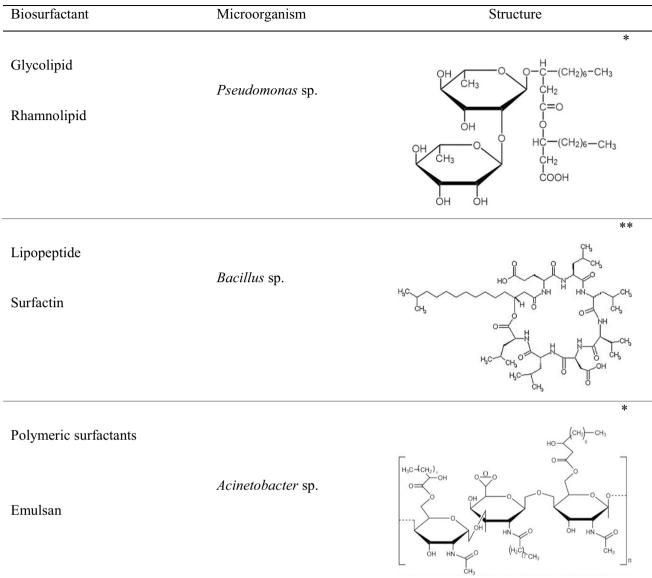
Surface tension measurement is a quantitative technique widely employed in monitoring BS production since it is reduced in the culture medium as the microorganism synthesizes and excretes the product [45, 46]. Some authors indicate that surface tension must present reduction values greater than 20% compared to the control medium for the BS to be considered active [47].

Underexplored bacterial groups as source of novel BS

As described above, the research in BS production is still limited to some well-known bacterial genera. Screening from molecular analysis accompanied by partial PCR amplification and sequencing of the ribosomal 16S rRNA gene, as well as other genes involved in the synthesis and regulation of biosurfactant production, has been little observed in the last decade [48, 49]. Thus, molecular biology, biochemistry, microbiology, computational biology, and environmental science become indispensable in the search and description of novel species of bacteria producing these biocompounds [49]. Most genetic studies involving biosurfactant-producing strains involve the genus *Pseudomonas*, probably due to its commercial potential and a wider range of information already present in the literature regarding these strains as producers of natural surfactants [50, 51].

Microorganisms, such *Streptomyces* sp. especially bacteria of the *Actinomycetes* group, are known to produce a pool of interesting metabolites like antibiotics, antifungals, and enzymes, but have been recently also described as producers of BS. Santos et al. [52] evaluated the potential for BS production by a *Streptomyces* sp. DPUA1559 strain, isolated from lichens from the Amazon region in Brazil. The

 Table 2
 Main types of bacterial BS and their representative chemical structures



*Adapted from: Abadia [40]

**Adapted from: Santos et al. [41]

BS produced showed a reduction of the surface tension of the culture medium, from 60 to 27.14 mN /m, emulsification index of 89 and 95% for motor oil and frying waste oil, respectively; a CMC value of 10 mg/mL, no toxicity to *Artemia salina*, and to seeds of lettuce and cabbage. In addition, BS presented high stability under extreme conditions of pH, temperature, and salinity. In a similar study using a strain of *Nocardiopsis* B4, isolated from marine sediments on the west coast of India, Khopade et al. [53] described the production of an efficient BS able to reduce the surface tension to 29 mN/m showing good stability in a wide range of pH and salinity, besides thermal stability under temperature conditions between 30 and 100 °C.

More recently, two marine actinobacterial strains, *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8, isolated from marine sediment in Gulf of Suez in Egypt, were described to produce very stable BS useful in pharmaceutical and antifouling manufacturing [54]. The stable behavior against high salt concentrations and temperatures, commonly described to the BS synthesized by actinobacteria, indicate their potential as a reservoir of novel natural surfactants. However, there is still a lack of exploratory studies that allow the expansion of the scope of applicability of the BS derived from these classes of bacteria.

Industrial processes frequently involve exposure to extreme conditions of temperature, ionic strength, pH, and

solvents; thus, finding compounds stable and active in such harsh conditions is an important issue. Despite advances in studies with microorganisms isolated from extreme environments, reports of BS production using such microbiota are still scarce; however, the physiological characteristics and adaptive capacity of extremophiles make them excellent candidates for the mining of novel surfactants [17].

From the studies relative to the isolation of thermophilic BS-producing bacteria, most were obtained from hot springs, deserts, volcanos, oil reservoirs, and composting. Regardless of their isolation from natural hot environments, the production of BS is, in general, conducted under mesophilic conditions [55]. Few attempts were done by using high temperatures of cultivation for BS production.

A lipopeptide BS produced by Aneurinibacillus thermoaerophilus strain MK01 isolated from a landfill was able to reduce surface tension of water to 43 mN/m, with a CMC of 1.21 mg/mL. The strain was cultivated at 45 °C producing around 5 g/L of BS that showed stability to temperature (20–90 °C) and pH range from 5 to 10 [56]. A thermophilic Ochrobactrum intermedium isolated from a hot spring (60 °C, pH 8.6) in Iran was able to produce a BS stable and active under alkaline conditions and temperatures (4–90 °C). Authors suggest the BS is suitable for laundry detergent formulations where high activity under alkaline conditions is needed [57]. Arifiyanto et al. [58] reported the isolation of a thermophilic *Streptomyces* sp. from a volcano mud that was able to grow and synthesize BS, with antimicrobial activity, when cultivated at 70 °C.

Reports of BS production by cold-adapted microorganisms inhabitants of deep oceans, polar regions, snow, and glaciers are yet scarce. However, BS derived from psychrophiles, once active at low temperatures, can be suitable for bioremediation of pollutants in cold environments; as anti-freezing agents; for recovery of natural gas hydrates; for enhancing the flow of biodiesel and detergents. In addition, they are synthesized without the need of heat, therefore using a green and low-energy-impact process [59]. A screening study pointed out that the ability to synthesize BS is regularly present in microbial populations of soil and sediments from polar regions [60] once they are involved in the uptake and solubilization of hydrocarbons [61]. Besides, psychrotolerant strains of well-known BS producers, such as Pseudomonas, Burkholderia, Rhodococcus, and Bacillus, are frequently isolated from cold habitats [62–64].

Malavenda et al. [64] reported the isolation of 199 psychrotolerant bacteria from Arctic and Antarctic shoreline sediments, and 18 isolates were selected for their ability to grow and produce BS at 15 °C and 4 °C, in the presence of crude oil as carbon source. The biosurfactant-producing isolates were identified as *Rhodococcus* sp. (14 isolates), *Pseudomonas* sp. (2 isolates), *Pseudoalteromonas* sp., and *Idiomarina* sp. Preliminary chemical characterization revealed that most strains produced a glycolipid-type BS with potential to remediation of hydrocarbon-contaminated cold environments.

Halophilic strains are usually found in saline and hypersaline environments where NaCl concentration is higher than in seawater (3.5% NaCl) such as salt lakes, salterns and hypersaline seas, sediments, soils as well as, in salted foods and brines [65].

The halophilic bacteria *Halomonas* sp BS4 was isolated from solar salt works in India. The bacterial strain showed optimum growth at 8% NaCl and synthesized a BS with antimicrobial and anticancer properties [66]. Further work of the same group reported the isolation of a *Kokuria marina* able to produce a lipopeptide-type BS in culture medium supplemented with 10% NaCl [67].

Besides bacteria, archaea are usually predominant in hypersaline and other extreme environments, but their exploitation is already limited. BS production by an extreme halophilic archaeon Haloferax sp. MSNC14 was evaluated in the presence of different hydrocarbon substrates. The strain showed ability to grow on linear (n-heptadecane) and isoprenoid (pristane) alkanes, and in polyaromatic hydrocarbon (phenanthrene) in the presence of 22.5% NaCl at 40 °C. Authors conclude that the BS-producing ability of such strain plays a role in the bioavailability of insoluble hydrocarbons, facilitating their uptake and their biodegradation at high salt concentrations [61]. Two bacterial and seven archaeal representatives were isolated from salterns in Argentina. The strains were cultivated under different temperatures (30 and 55 °C) and salinities (2.5 and 5.0 M NaCl), and their emulsification ability was determined. Most archaea and both bacteria, Salinibacter ruber and Salicola sp., showed ability to produce a BS more stable than commercial surfactants [68].

Production of BS under extreme pH conditions is also scarce in the literature. Elazzazy et al. [69] described the isolation and BS production by a *Virgibacillus salaries* strain. The maximal BS yield was attained when the bacteria were cultivated at pH 9.0, 40 °C, and 4% salinity. Surface tension was reduced to 29 mN/m after 3 days of growth, and the BS was partially characterized as a lipopeptide. Such alkaline active BS can be especially suitable for detergent industry and bioremediation in marine environments.

Production of biosurfactants by an acidophilic mycobacterium isolated from a sulfur storage site in Russia was described. BS activity was detected under aerobic growth using several hydrocarbons under extremely acidic conditions (pH 2.5). According to the authors, the isolated mycobacterium was the first known acidophilic hydrocarbonoxidizing surfactant producer. In addition, the BS derived from the acidophilic isolate showed significant surface activity and stability within a wide range of pH, temperature, and salinity [70]. The *Stenotrophomonas maltophilia* strain recover from a mining site in Saudi Arabia was able to produce a BS that assisted the degradation of polycyclic aromatic hydrocarbons (PAHs) under acidic conditions at 30 °C. The strain was able to degrade low (anthracene, phenanthrene, naphthalene, fluorene) and high (pyrene, benzopyrene, and benzofluoranthene) molecular weight PAHs in mineral salt medium at pH 2, with removal rates of up to 95% and 80% respectively, suggesting it is suitable for the treatment of contaminated wastewater [71].

Although BS derived from extremophiles are attractive for biotechnology, the main challenge to overcome is the difficulty to simulate natural condition to cultivate such strains even at a laboratory or industrial scale [72]. Energy expenses, equipment adaptation, and specific waste treatment impact final production cost; thus, their economics may be not feasible comparatively to both BS derived from mesophilic strains and synthetic surfactants [72]. Most work regarding the isolation and selection of BS-producing bacteria utilize traditional culture-dependent techniques; however, culture-independent methods can help to overcome problems related with cultivation of extremophiles [17]. In this sense, the use of molecular biology and bioinformatics tools allows rapid identification of the genetic potential and BS genes present in an extreme environment sample without the necessity of cultivating the microorganisms [59]. The use of omics techniques is a promising approach to discovery and further application of biomolecules derived from extremophiles [73].

Diazotrophic bacteria that live in consortium with various types of plants are able to degrade toxic compounds inside the roots [74]. These microbial populations are also exposed to several toxic organic molecules that occur naturally in the soil, such as phenols, terpenes, alkaloids, and products of anthropogenic origin, besides other molecules found in the soil from the degradation of lignin and humic acids [75]. Thus, the biodiversity of such microorganisms may represent a potential source for biodegradation of environments contaminated with organic pollutants and, consequently, the production of BS [76]. It is important to highlight that it is highly desirable that microorganisms are harmless, especially aiming the application of BS in the food, pharmaceutical, and cosmetic industries. In this sense, diazotrophic bacteria represent a very promising alternative [77, 78].

BS production by diazotrophic strains of *Azorhizobium* sp. and *Sinorhizobium meliloti* was conducted using apple/cashew juice and vegetable oil as carbon sources [79], and the obtained BS showed high surface activity (~ 32 mN/m) and emulsification activity of 69%.

Some reports about the use of diazotrophic bacteria in hydrocarbon degradation have also been reported. Vargas et al. [80] isolated two strains of *Coccobacillus* sp. capable of producing rhamnolipids using kerosene as the only carbon source. The BS also showed emulsification index of 50% for

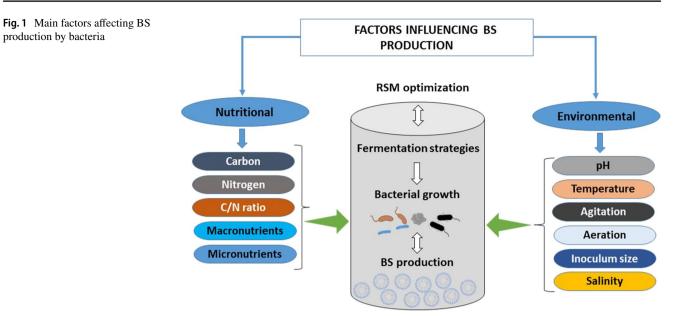
kerosene and diesel oil and 70% for motor oil. The bacteria also showed, through the bioenhancement strategy, ability to remediate soil, and the total hydrocarbon content was reduced by 80%, declining from 120 g/kg soil to less than 24 g/kg soil after 16 months of treatment [80]. In the work of Dashti et al. [81], the crude oil absorption capacity from the olive pomace, a residual by-product of the olive oil industry, was investigated. The authors observed that the by-product in question was able to absorb an amount above 40% of its weight in oil. It was identified in the residue the presence of a diverse bacterial population that showed ability to grow in mineral medium containing Hg²⁺ and oil as the only carbon source, thus exhibiting not only biodegradation capacity but also resistance to the heavy metal. After isolation of the colonies, molecular and phylogenetic analyses of the bacteria were performed, and sequencing of the 16S rRNA and nifH genes allowed the identification of the diazotrophic genera, including Rhizobium sp., Sinorhizobium sp., and Bradyrhizobium sp. The presence of these genera among the hydrocarbonoclastic strains suggests evidence of the capacity, almost unexploited, of these microorganisms in the production of BS with potential use in several applications, including the bioremediation of waters and soils contaminated with oil and heavy metals.

Biosurfactant production

General aspects

As with any cell-based bioprocess, the production of BS is influenced by nutritional and environmental parameters. Concerning nutritional factors, the carbon, nitrogen, phosphate, and mineral sources may impact the quantity, quality, and type of BS [82]. Carbohydrates, hydrocarbons, oils, and fats are typical C sources providing bacterial growth and BS accumulation. Both inorganic (nitrate and ammonium salts) and organic (peptone, meat, and yeast extracts) fonts can be utilized to supply nitrogen to the culture medium. It is important to point out that N-limiting conditions (or high C/N ratios), although decrease bacterial growth, increase BS production by *Pseudomonas* sp. [83]. Micro and macro elements also play an essential role in BS synthesis. Manganese, Iron, and Magnesium, for example, are cofactors to enzymes involved in surfactin production by Bacillus subtilis [84]. In general, the availability of Fe and Mn salts is considered critical in enhancing the growth and production of lipopeptide-type surfactants.

Environmental conditions, such as salinity, pH, temperature, time of incubation, inoculum size, aeration, and agitation rates, also affect BS productivity [82]. However, such optimal conditions varied among microorganisms and have to be defined for each specific process. The production of BS



depends on the levels and type of nutrients and culture conditions; thus, the combination of these several factors should be studied together to reach competitive yields (Fig. 1). In this view, the application of surface response methodology and other statistical methods to optimize BS production are determinants to their widespread utilization [85].

Most data in literature describe bacterial BS production at laboratory scale using shake flasks and low volumes; however, some reports using bioreactors are available [86]. Stirred tank reactors (STR) operated in batch or fed-batch mode are usually applied for BS production [87-89]; however, conventional fermentation is dependent on the supply of dissolved oxygen by stirring and aeration that, when in high levels, can lead to shear stress damaging the cells and also to excessive foaming that reduces the efficiency of the process [90]. In this sense, foam-controlling systems [91], bubble-free membrane reactors [92], and solid-state fermentation [93] have been proposed as alternative strategies to BS production. Particularly solid-state fermentation (SSF) is advantageous by the possibility to use sustainable waste materials, smaller volume of fermentor, reduced recovery and energy costs, and low contamination risks [94]; nevertheless, some bacterial strains do not develop properly in such systems because of the heterogeneity and limited transfer rate [94].

Waste-based BS: role in circular bioeconomy

Among the major challenges of BS production on an industrial scale is the high cost of production associated with low productivity, which hinders their widespread application to replace synthetic surfactants [95–97]. However, it is important to point out that for environmental and agricultural purposes, such as bioremediation and pesticide degradation, BS can be applied in their raw form which allows for reducing costs since purification is not required.

For commercialization of large volumes of microbial surfactants to be fully achieved in some industrial fields, it becomes necessary to search for optimization methods that promote an increase in production yield, more efficient techniques for BS separation and purification, development of hyper-producer strains, or strategies to reduce the expenses of the process [98, 99]. Regarding the latter, the search for alternative low-cost substrates for the cultivation of the microorganism has gained attention, since the raw material is a factor that represents 10–30% of the overall cost of BS production [95, 97, 100, 101]. Among the alternatives proposed as substrates, residues and by-products from agro-industrial processes are predominant.

The traditional linear economy model assumes an infinite supply of natural resources and an unlimited capacity of nature to convert waste and pollution [102]; thus, this model is no longer suitable. A circular bioeconomy highlights the 4R concept recycle, reuse, recover, and reduce maximizing the value of bio-based resources replacing fossil-based raw materials [103, 104]. The use of organic sources, such as food and oil processing wastes, besides contributing to economics of BS production, also allows the reuse and valorization of substrates in a sustainable way [97, 105]. Moreover, the residues generated by the BS bioprocess can be reutilized as organic fertilizer completing the circular loop without generation of additional waste [97]. Within this context, use of agro-industrial wastes or by-products as alternatives to support BS production has been extensively reported and reviewed in last decades [106-110]. Although the idea is not new, the emergence of circular bioeconomy

gave an additional boost to the production of BS using waste-based substrates since this approach fulfills the actual market demands opening novel perspectives to innovation and competitiveness [97, 110].

Lignocellulosic materials (bran, husks, straw, bagasse); fruits and vegetables processing wastes (peels, stalks, wastewater); starch-rich residues (rice, potato, cassava wastewater); oil processing wastes (soapstock, lard, tallow, and oil mill effluents); dairy and sugar wastes (whey, buttermilk, molasses); cooking oil wastes; and even municipal solid wastes are proposed as substrates to BS production (Table 3), and some recent examples are discussed below.

Das and Kumar [117] isolated a strain of *Pseudomonas azotoformans* from an oil-contaminated soil; the bacterium produced a high yield of rhamnolipids using potato peel and sugarcane bagasse as carbon sources. The BS obtained showed no toxicity, was stable under high salt concentration

(6% w/v NaCl) and temperature (90 °C), reduced the surface tension of the medium from 65 to 30.5 mN/m, and promoted recovery of up to 36.56% of oil from sand under high salt condition. The authors concluded that BS can be a strong candidate for tertiary oil recovery in reservoirs with high salinity, for bioremediation of saline soils, as well as for applications involving extreme environmental conditions.

The potential of using agroindustrial waste in consortium (brewery wastewater, beet molasses, apple peel extract, and carrot peel extract) as alternative carbon sources for BS production by two *Bacillus subtilis* strains (KP7 and I'-1a) was investigated by Paraszkiewicz et al. [100]. Both strains were able to produce BS in all culture media prepared with the waste. However, higher production yield was observed for *B. subtilis* KP7 grown on medium containing carrot peel extract supplemented with 0.5% yeast extract, producing about 140.6 mg/L of surfactin. In media containing

Table 3 Examples of agro-industrial wastes utilized as substrates for production of biosurfactants and their potential applications

Agro-industrial sector	Residue type	Bacteria	Biosurfactant	Potential applications	Reference
Starch-based	Cassava flour wastewater and corn waste oil	Serratia marcescens UCP 1549	-	Removal of burned engine oil	[111]
Sugar	Sugarcane bagasse	Bacillus safensis J2	-	Enhanced oil recovery/ bioremediation	[112]
Soy	Refinement wastes: soybean acid oil, soybean deodor- izer distillate and soybean soapstock	Pseudomonas aerugi- nosa MR01	Rhamnolipid	-	[113]
Fruit	Orange peel	Bacillus licheniform- isKC710973	Lipopeptide	Solubilization of oil	[114]
Fruit	Cashew apple juice	Bacillus subtilis	Lipopeptide	Bioremediation of oil con- taminated soil	[115]
Starch-based	Rice mill polishing residue (RMPR)	Bacillus subtilis MTCC 2423	Surfactin	Removal of copper ions by foam separation	[116]
Starch-based	Bagasse and potato peels	Pseudomonas azotofor- mans AJ15	Rhamnolipid	Bioremediation of oil- contaminated saline soil and enhanced oil recovery	[117]
Brewery	Brewery wastewaters (BW)	<i>Bacillus subtilis</i> (KP7 e I'-1a)	Surfactin and Iturin	-	[100]
Milk	Lactoserum	Enterobacter sp., Pseu- domonas aeruginosa, Bacillus pumilus, and Rhizobium sp.	-	Bioremediation and oil recovery	[118]
Oil	Waste cooking oil (WCO)	Pseudomonas aeruginosa MTCC7815	Rhamnolipid	Oil biodegradation	[119]
Oil	Soybean waste frying oil	Streptomy- ces sp. DPUA1566	Lipopeptide	Bioremediation	[101]
Oil	Waste sunflower oil	Burkholderia thailandensis	Rhamnolipid	-	[120]
Oil	Palm oil effluent and crude glycerol	Bacillus subtilis TD4	Lipopeptide	Biodegradation of oil industry waste	[121]
Alcohol	Distillery wastewater (DWW)	Pseudomonas aeruginosa SRRBL1	Rhamnolipid	-	[122]
Cellulose	Liquor from sisal pulp hydrolysis	Bacillus subtilis	Surfactin	Bioremediation	[123]

apple and carrot peel extracts as the main carbon sources, iturin production by *B. subtilis* I'-1a reached 428.7 mg/L as opposed to 73.3 mg/L in conventional medium [100]. Yanez-O Campo et al. [124] reported the use of cooking oil and coffee wastewater as carbon sources for BS production by bacteria isolated from an anaerobic digestion sludge and obtained a yield of 3.7 g/L of glycolipids. The BS reduced the surface tension of the culture medium from 50 to 29 mN/m.

In medium containing palm oil refining waste as carbon source, the production of rhamnolipid by *Pseudomonas aeruginosa* PAO1 reached 0.43 g/L. BS reduced the surface tension to 29 mN/m with critical micellar concentration (CMC) of 420 mg/L [125]. The use of waste cooking oil was also investigated for BS production by *Pseudomonas* SWP-4. The authors reported that the oil favored the synthesis of BS, achieving yields of 13.93 g/L of rhamnolipids, showing an emulsification index of 59% with hexadecane, reduction of interfacial tension of n-hexadecane from 29.4 to 0.9 mN/m, water surface tension from 78 to 24.1 mN/m and CMC of 27 mg/L [50].

Cellulose pulp from sisal (Agave sisalana) was evaluated as a substrate for the simultaneous production of nanofibers and biosurfactant. The liquor deriving from acid and enzymatic hydrolysis of sisal cellulose, after separation of resulting nanofibers, was utilized as carbon source to provide growth and surfactin production by a Bacillus subtilis strain. The surfactin obtained in acid hydrolysate-based medium generated surface tension (ST) of 29.8 mN/m, interfacial tension (IT) of 5.7 mN/m, and a CMC of 1394.0 mg/L, whereas, with enzymatic hydrolysate, surfactin showed a ST of 28.7 mN/m, IT of 3.8 mN/m, and a CMC of 64.0 mg/L. The synthesized BS demonstrates potential for bioremediation of diesel oil-contaminated areas and similar properties when compared to standard surfactin [123]. The combined production of a bio-based nanomaterial and BS from cellulosic substrates represents an interesting strategy to valorization of biomass while contributing to circular bioeconomy.

Although the use of agroindustrial residues has advantages, some disadvantages also need to be considered. The production, separation, and purification of BS from wastes may become more expensive compared to the use of conventional sources due to the complexity and heterogeneity in their composition. The presence of impurities can hinder conventional analytical methods, requiring the development of specific methods and strategies for BS separation and analysis. Another disadvantage is the need for pre-treatment and the difficulty in storing, preserving, and transporting the raw materials [110]. Besides these factors, some solid and/ or liquid wastes present in their composition complex structures that are difficult to be degraded by microorganisms, and the toxicity of some components can also preclude their use in various bioprocesses. Therefore, the use of alternative sources for production of BS should be accompanied by technical and economic feasibility studies.

Industrial applications of biosurfactants

Environmental use of BS: biodegradation and bioremediation of pollutants

BS can be applied in several industrial sectors; however, their main use is associated with the crude oil industry and due to the high capacity of these compounds to solubilize hydrocarbons. Heavy fractions of oil are more viscous and sediment on the bottom of storage tanks, making their removal difficult by the traditional pumping method. The removal of such fractions requires manual washing with organic solvents, which besides time-consuming and dangerous method, involves high economic cost for its execution and causes concern due to the destination of the washing wastewater [126].

Traditional EOR (enhanced oil recovery) technology is based on the use of heat, miscible gas injection, and chemical compounds, such as surfactants, to remove residual oil present in natural reservoirs that are difficult to access because they are infiltrated into the pores of the rocks [127,128]. MEOR (microbial enhanced oil recovery) is an alternative that employs microorganisms and/or their metabolites to recover some of the remaining oil in their reservoirs. MEOR technology can be applied in two ways: in situ and ex situ. The in situ method occurs by inserting biomoleculeproducing microorganisms that lead to EOR or by injecting previously selected nutrients to stimulate the synthesis of the active molecules by bacteria naturally present in the site (Fig. 2A). In the ex situ method, microbial products are produced outside and then introduced in the site [129]. The best strategy to be adopted will depend on some variables such as reservoir conditions, temperature, pressure, pH, porosity, salinity, available nutrients, and presence of local native microorganisms [24]. Among the various types of microbial metabolites that play important roles in this technology (biosolvents, biopolymers, and biogases), BS are the most prominent [24, 130]. Like synthetic surfactants, BS are effective in reducing the interfacial tension between oil/ water and oil/rock and promoting the reduction of capillary forces that normally mobilize oil in rock pores; in addition, the emulsifying capacity of these compounds facilitates the formation of emulsions between oil/water, stabilizing the desorbed oil, allowing its removal more easily (Fig. 2B) [131, 132].

Some advantages that justify the employment of BS in oil recovery (ex situ) are biodegradability and low toxicity, reducing damage to marine biota, low cost of production using agro-industrial wastes, and the possibility of testing

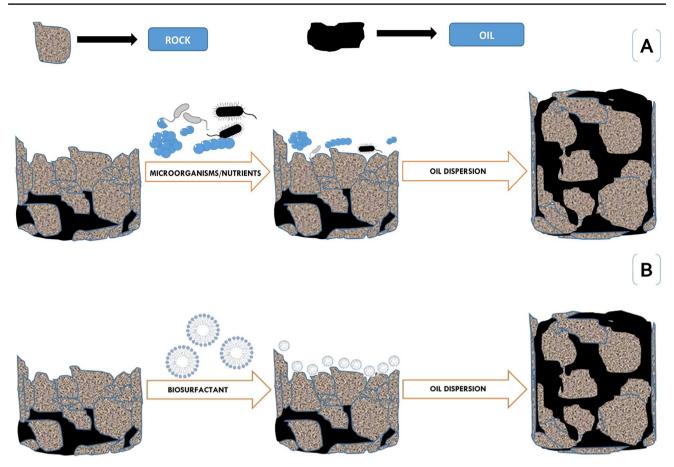


Fig. 2 Schematic of the use of microorganisms/nutrients in MEOR (in situ) (A)/Schematic of the use of BS in MEOR (ex situ) (B)

the stability and effectiveness of the compound in the laboratory, i.e., before its application in the field, thus raising the success rates of the technique [133]. Despite the advantages, BS-MEOR method also presents some challenges such as cost-effectiveness compared to the use of synthetic surfactants; high initial investment for the acquisition of large-scale bioreactors; a large amount of BS required for field application (a pronounced problem when we take into account the losses that usually occur during BS extraction and purification); and the lack of published data for field testing [134]. In contrast to the ex situ BS-MEOR technique, in situ BS-MEOR has some advantages due to the lower cost tied to its use and is a preferred choice of application. However, the in situ method also presents limitations such as difficulty to perform control treatments under field conditions, difficulty to promote the growth of useful native microorganisms and control harmful ones (such as sulfate-reducing bacteria-SRBs), and difficulties to select strains adapted to the extreme ambient conditions of the reservoirs (pH, temperature, salinity, pressure). Furthermore, the amount of BS produced cannot be controlled, and only aerobic and/or facultative anaerobic bacteria can be used in MEOR applications [135, 136].

Bioremediation aims to promote biological treatments to reduce, to acceptable levels, several types of environmental contaminants. Usually, the most common types of soil and water contaminants are related to accidents with oil spills and waste improperly deposited in containers, generating harmful effects [137]. Other types of contamination involve the excessive use of pesticides in agriculture, textile dyes, and poorly managed landfills [138-140]. Most of these contaminants have low solubility in water, making it difficult and costly to remove them from the environment [141]. Despite the presence of several current technologies that use physical and/or chemical processes for the decontamination of these sites, from the environmental point of view, the use of BS characterizes an ecologically more appropriate and effective alternative for the treatment of soils and aquatic environments contaminated with complex organic compounds [142, 143]. Some studies report the use of rhamnolipids and surfactin synthesized by the bacteria Pseudomonas aeruginosa and *Bacillus methylotrophicus*, respectively, in the process of bioremediation of soils impacted by oil spills, highlighting a performance similar to synthetic surfactants, but still limited to their use on a large scale due to production costs [47, 144–146].

In agriculture, biosurfactants also find a wide range of applications. They can improve the quality of agricultural soil through the biodegradation of organic pollutants, promote plant growth through their antimicrobial activity, and increase the interaction between plants and beneficial microorganisms [49]. BS also have advantages as substitutes for synthetic surfactants in the formulation of agricultural pesticides, since they are used as a carbon source by microorganisms present in the soil [49, 147]. Biological surfactants can also enhance the degradation of chemical pesticides found in agricultural soil. In a study involving Pseudomonas sp. B0406, Reyes and collaborators evaluated the ability of this strain to increase the solubility of two pesticides: endosulfan (ED) and methyl parathion (MP). The crude extract of Pseudomonas B0406, which presented as an anionic glycolipid, showed a critical micellar concentration of 1.4 g/L and a surface tension of 40.4 mN/m. The extract increased the solubility of pesticides from 0.41 to 0.92 mg/L for ED and from 34.58 to 48.10 mg/L for MP. Solubilization of these pesticides by BS may be the first step for their degradation; thus, the results suggest the effectiveness of the BS in improving the solubility of both pesticides in water and consequent bioremediation of the pollutants [140].

The effect of surfactant produced by *Bacillus velezensis* MHNK1 on the biodegradation of atrazine was investigated. *B. velezensis* MHNK1 produced an anionic biosurfactant that reduced surface tension from 72.1 to 33.2 mN/m with CMC of 40 mg/L and emulsification index of 85.2%. The bacteria showed 87.1% biodegradation of atrazine in 5 days, and the herbicide was completely degraded after 4 days by using a combination of *B. velezensis* MHNK1 (2%) and surfactin (2 CMC), showing the efficiency of this combination to increase the scope of application in bioremediation of sites contaminated with this herbicide [148].

A bacterium capable of growing on methamidophos as the sole carbon source was classified as belonging to the genus *Leuconostoc* sp. The genus *Leuconostoc* comprises lactic acid species (BAL), recognized for their biotechnological properties that give them potential for use in the food industry, mainly in the manufacture of fermented products [149, 150]. Among these properties is the probiotic potential and the production of several antimicrobial compounds, including BS [151, 152]. Another study identified *Arthrobacter*, isolated from a pesticide-contaminated site, as a rhamnolipid producer using endosulfan and its oxidated form endosulfan sulfate (26.65 ppm), as carbon sources. The surface tension of the medium was reduced to 37 D/cm, suggesting that its ability to degrade endosulfan and endosulfate would be related to the production of biosurfactants [153].

A recent approach suggests the use of microbial BS to improve the digestion of fats/oils by ruminant animals. Being an economically viable energy source, fats/oils are usually present in the diet of these animals; however, their use is limited due to the low physiological capacity of the animal to digest high concentrations of these compounds [154].

Human and natural processes often also result in the entry and accumulation of inorganic pollutants such as heavy metals in the environment, causing the contamination of soil and water, both surface and groundwater [155]. The literature points out a promising scenario for the use of bacteria and their biocompounds in the recovery of areas impacted by metals [156, 157]. Among these biocompounds, BS have proven efficient in an attempt to remediate sites impacted by the presence of potentially toxic metals such as cadmium, mercury, zinc, arsenic, and lead [158]. This process occurs from the formation of a complex between the metal and micelles and can be recovered later by means of precipitation technique or membrane separation [52, 159]. Current literature points to glycolipids as the main class of BS used in heavy metal remediation, with rhamnolipid being the most studied [158, 160]. Aşçi et al. [161] described a 91% recovery of Cd and 87% of Zn using rhamnolipid at a concentration of 25 mM. Yang et al. [162] investigated the ability of a glycolipid-like BS, isolated from the bacterial strain Burkholderia sp. Z-90, to remove heavy metals in contaminated soil samples. The compound showed removal of 44.0% for Zn, 32.5% for Pb, 52.2% for Mn, 37.7% for Cd, 24.1% for Cu, and 31.6% for As, respectively. In a study with *Pseu*domonas sp. CQ2, Sun et al. [163] reported the efficiency of the BS from such bacterium, in decreasing heavy metals present in soil. The BS favored the removal of 78.7, 65.7, and 56.9% for Cd, Cu, and Pb respectively, and also reduced the surface tension of the medium from 72.5 to 27.4 mN/m and showed excellent stability in various pH, temperature, and salinity ranges.

Food and health applications of BS

Surfactants have several applications in the food industry and play an essential role in food consistency and textures. Their use allows the control of fat globules agglomeration and stabilization of emulsions by reducing the surface energy between two immiscible phases, besides changing the rheological properties of wheat dough, being widely employed in baking [164, 165]. Surfactant agents also improve aerated systems and increase the shelf life of starch-containing products [166–168]. Regular monitoring of the presence of heavy metals in food is of essential importance to avoid the potential health risks due to their excessive accumulation in the human food chain. Anjum et al. [169] reported the removal of a considerable amount of cadmium present in some vegetables such as potato (47%), radish (62.5%), onion (61.03%), and garlic (73%) after washing with a BS produced by Bacillus sp. MTCC 5877. In a similar work, a lipopeptide produced by *Bacillus* sp. was utilized to wash vegetable samples (cabbage, carrot, and lettuce) previously contaminated with heavy metals reducing levels of copper, lead, cadmium, zinc, and nickel from 59 up to 87% [170].

In view of the growing concern about sustainability and health, the current trend among cosmetics consumers is the search for products based on natural ingredients that provide equal or superior benefits to chemical-based ones. In this sense, BS are potential candidates in the cosmetics area [171]. The emulsifying function is perhaps the most important in cosmetic formulation; however, the moisturizing power and good compatibility with the skin are also prominent features [12, 171]. Among other advantages, the antimicrobial and antioxidant activities demonstrated by many BS are indispensable for the personal care industry [172]. Several hygiene and cosmetic products can incorporate BS in their formulation, such as anti-dandruff shampoos, toothpastes, face creams, and repellents, among others [12, 172]. Studies highlight that natural surfactants of the glycolipid class such as rhamnolipids, by presenting good emulsifying activity and low CMC, can be advantageous to replace synthetic surfactants in cosmetic products [171, 173]. However, before incorporating BS in such formulations, it is necessary to evaluate toxicity in cells and/or animals [172]. Ferreira et al. [174] evaluated the ability of a glyco-lipopeptide, produced Lactobacillus paracasei, as a stabilizing agent for an emulsion based on almond essential oil and grape seed extract. The emulsion containing 10 g/L of the BS showed good stability and lower cytotoxicity than SDS (sodium dodecyl sulfate), a surfactant commonly used in industry. The data suggest that BS produced by L. paracasei can be used as a natural ingredient in cosmetic formulations.

The biological activity demonstrated by BS is widely explored in therapeutic applications not only because of their inherent bioactivity but also for their natural status, low toxicity, and biocompatibility [175]. The antimicrobial, anti-tumoral, anti-adhesive, antibiofilm, anti-inflammatory, immune-modulatory, and immune-suppressive properties of BS have been extensively described and reviewed in the literature [154, 176, 177].

Several bacterial glycolipids and lipopeptides are reported to show antimicrobial activity against pathogenic bacteria, fungi, and viruses [177] in addition to inhibiting their adhesion and biofilm formation in abiotic and biotic surfaces [178, 179]. Although the mechanism is not fully elucidated, the antimicrobial activity of BS is attributed to their disturbance in the integrity of cytoplasmic membrane, leading to the formation of pores and ion channels, increased permeability, metabolite leakage, and cell death [180].

The antiviral activity of BS, especially against enveloped viruses, makes them potential candidates to develop strategies to prevent COVID-19. Since coronavirus has an envelope and also capsid and surface spike proteins, BS treatment can disrupt lipid envelope or interact with capsid proteins [181, 182]. Within this context, the application of BS in antiviral protective clothes and coating films to minimize the lifespan of viral particles on surfaces and reduce transmission is a promising perspective [15].

Another interesting feature is the control of insects by using BS [183, 184]. The surfactant produced by Bacillus amyloliquefaciens was able to inhibit the growth of A. *aegypti* mosquitoes in the larval and pupal stages [185]. Silva et al. [183] evaluated larvicidal, insecticidal, and repellent properties of rhamnolipids against A. aegypti. Application of 800 mg/L of RL eliminated all mosquito larvae in 18 h, while 1000 mg/L resulted in 100% mortality of adults. The repellent activity of rhamnolipid was also observed and was associated by the authors with the odor of this BS being recognized by A. aegypti as unfavorable. In a similar study, Prabakaran et al. [186] reported that di-rhamnolipid inhibited the growth of Culex quinquefasciatus, A. aegypti, and Anopheles sp. The BS was highly toxic to pupae of mosquitoes, especially Anopheles sp., and Aedes aegypti, showing potential in the control of dengue, chikungunya, yellow fever, and malaria vectors. These results point to the potential of using BS to combat vectors related to diseases that affect humans and animals.

Biosurfactants in nanotechnology

Due to their amphiphilic and self-assembly nature, surfactants are key molecules in the synthesis of nanostructures where they act as stabilizers, growth control agents, templates, and modifiers [187]. Increasing demand for green technologies to replace hazardous chemicals stimulates the use of biological methods using plants, microbes, or their metabolites for the synthesis of nanomaterials [188]. Several reports concerning microbial synthesis of nanoparticles (NP) are available in literature. In general, metal NP are synthesized by enzymatic reduction of gold, iron, silver, platinum, and other metal salts; hence, the microbial metabolism provides a suitable environment (intra or extracellularly) to assist the NP synthesis [189, 190].

Currently, the use of microbes as nanofactories is considered less effective because it is difficult to control the size and shape of NP along with the complexity to separate NP from cells and medium [188]. Among microbial-derived metabolites with potential for synthesis of nanostructures, natural surfactants have emerged as green and eco-friendly candidates [15]. BS can replace chemical surfactants during metal NP preparation even by chemical reduction or microemulsion techniques. In the former, BS is adsorbed on the surface of metal NP acting as capping agent to prevent agglomeration, favoring their dispersion and stabilization in solution [191]; in the latter, the capacity of BS to form micelles is exploited to obtain uniform and stable NP [192]. Considering the importance to avoid hazardous chemicals in green synthesis, efforts have been made to introduce BS in the preparation of different types of nanostructures.

Most studies concentrate on silver and gold NP preparation using BS and their further application in the biomedical field, predominantly to control pathogens.

A lipopeptide BS extracted from *Bacillus vallimortis* was utilized for the synthesis of silver NP which showed antimicrobial activity against E. coli, S. aureus, and Listeria monocytogenes [193]. Another study describes the preparation of stable silver NP using a lipopeptide isolated from a Bacillus subtilis CN2 strain. The presence of the BS reduced the average size, homogeneity, and long-term stability of the NP and resulted in remarkable antibacterial activity against Pseudomonas aeruginosa and Bacillus subtilis strains comparatively to the NP prepared without the BS [194]. Another approach using biogenic synthesis by BS-producing microbes was also described. In this case, microbial growth is performed, and the resulting cell-free medium is utilized as starting material for NP synthesis after the addition of a metal precursor. Cubic silver NP with an average size of 15.40 nm were obtained using culture filtrates of a surfactin producing Brevibacillus brevis. According to the authors, surfactin acted as reduction and stabilization of the NP, which exhibited potential for the treatment of Gram-negative infection in wounds [195].

An environmental application of BS-synthesized metallic NP was described by Hazra et al. [196] who reported that rhamnolipids were effective capping and stabilizing agents for developing stable and biocompatible ZnS NP useful as nanophotocatalyst in the textile industry and for wastewater and effluents treatment. The inherent physicochemical and biological activity demonstrated by BS also allow their incorporation as coatings (functionalizing) agents to improve or modify the properties of NP. The presence of a RL shell in Ag and Fe₃O₄ NPs changed surface hydrophobicity enhancing their anti-biofilm activity against *P. aeruginosa* and *S. aureus*. Authors suggest RL-coated NP as potent alternative to develop novel antibacterial coatings and wound dressings [197]. Functionalization of iron oxide NP with rhamnolipids reduced their toxicity and provided a more selective and biodegradable NP able to effectively remove methyl violet dye showing potential to detoxify wastewater streams from hazardous pigments [198].

The utilization of BS in the synthesis of natural organic NP is yet scarce in literature; however, this approach is considered more eco-friendly once it reduces the use of metals and petrochemicals that can accumulate in the environment [190]. Synthesis of hybrid biopolymer-biosurfactant NP was recently reported by Marangon et al. [199] showing that the presence of rhamnolipid reduced the size and polydispersity of chitosan NP and improved the antimicrobial activity against S. aureus both planktonic and biofilm. Authors hypothesized that high density of polycationic chitosan in the hybrid NP improves electrostatic interactions favoring the release of RL close to bacterial surface helping disruption of the cell envelope and subsequent access of antimicrobials to their targets. The low cytotoxicity and high antimicrobial potential demonstrated by the chitosan-RL NP can be advantageous to the design of novel strategies to control S. aureus in the pharmaceutical and food industries. A protein/polysaccharide/surfactant NP containing zein, propyleneglycol alginate, and rhamnolipid was designed to deliver

Application	Product/sector	BS type/formulation	Reference
Household detergent	Cleaning agent	Lipopeptide	[205]
Toothpaste	Health care	Lipopeptide	[206]
Antitumoral	Health biomedical	Surfactin	[207]
Phytopathogen control	Agriculture	Rhamnolipid	[208]
Emulsifier and antioxidant	Food	Lipopeptide	[209]
Wound dressing	Health biomedical	Lipopeptide + Gelatin nanofibers	[210]
Emulsions	Cosmetic	Glycopeptides	[174]
S. aureus control	Health biomedical	Chitosan/Rhamnolipid NP	[199]
Oil recovery	Environmental	Rhamnolipid + Silica NP	[211]
COVID 19 control	Health biomedical	Several BS	[182]
Bioactive carrier	Health biomedical	Rhamnolipid liposomes	[202]
Biofilm control	Food	Rhamnolipid	[212]
Mosquitoes control	Environmental/health	Rhamnolipid	[183, 186, 213]
Antimicrobial/active packaging	Food	Nisin-rhamnolipid liposomes	[204]
Edible coating	Food	Rhamnolipid-chitosan film	[214]

Table 4 Recent proposed utilization of bacterial-derived biosurfactants

NP nanoparticle

of curcumin. The presence of BS increased the encapsulation efficiency of curcumin and improved the photo-stability and bioaccessibility, important features to the successful delivery of hydrophobic nutraceuticals in food and supplements [200].

The molecular self-assembly character of BS can also be advantageous for development of active nanostructures useful as carriers of active agents. Surfactin nanospheres containing the anticancer drug doxorubicin showed stronger cytotoxicity against resistant human breast cancer cells compared to free doxorubicin. Enhanced cellular uptake and superior tumor inhibition with fewer side effects were observed suggesting surfactin nanocarrier as promising alternative to overcome multidrug resistance in cancer chemotherapy [201].

Rhamnolipid liposomes loaded with bioactive peptides were claimed to design of novel antimicrobial systems for use in medicine [202] and agriculture [203]. Rhamnolipidfunctionalized liposomes (rhamnosomes) were prepared and loaded with nisin to improve antimicrobial and antibiofilm character of liposomes and the efficacy of nisin. The resulting nanovesicles showed higher antimicrobial activity than liposomes and enhanced the activity of nisin against *L. monocytogenes*, *S. aureus*, *E. coli*, and *P. aeruginosa*. Around 80% reduction in biofilm biomass was observed due to its improved binding with the bacterial surface.

Rhamnosomes can offer an innovative and viable solution for the sustained release of antimicrobial peptides to ensure food preservation [204]. BS emerged as sustainable ingredients for the formulation of nanostructures once their physicochemical and biological properties, combined with natural status, are advantageous over synthetic surfactants. Some examples of recent applications of bacterial-derived BS described in literature are summarized in Table 4, highlighting the emergent trend of using such molecules in nanotechnology being incorporated into the formulation of diverse types of nano-based structures.

Conclusions and future outlooks

The application of BS extends from the traditional use, in the bioremediation of pollutants, to the development of nanostructures for use in different industrial sectors. The versatility of these molecules, coupled with their status as "green products," has stimulated their use, and interest has been growing more and more in recent years. Bacteria represent the largest group of microorganisms involved in the production of BS; however, many potential genera remain little explored for this purpose. Extremophilic, diazotrophic, and endophytic bacteria are prospective candidates for the discovery of new surfactant molecules with unique characteristics, due to their adaptation to peculiar environmental and ecological conditions. Efforts in the search for new biosurfactant-producing strains and the development of technologies to enhance their economics may stimulate investments in their production on a large scale. The sustainable production of BS from agro-industrial wastes fulfills the principles of circular bioeconomy, a growing demand of society. The increasing interest in the application of BS in nanotechnology suggests that the potential of these molecules is still far from being exhausted. Moreover, the discovery of new bacterialderived molecules showing surfactant activity may serve as models for enzymatic synthesis of a novel generation of bio-inspired detergents.

Author contribution MAMD brought up the idea, did the literature search, and wrote the manuscript. MN wrote topics and critically revised the manuscript.

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