

Review

Biosurfactants: Potential and Eco-Friendly Material for Sustainable Agriculture and Environmental Safety—A Review

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Abstract: With the present climate change and increasing world population, there is an urgent need to discover creative, efficient, and cost-effective natural products for the benefit of humanity. Biosurfactants are produced by various microorganisms that have several distinct properties compared to other synthetic surfactants, including mild production conditions, multifunctionality, higher biodegradability, and lower toxicity of living cells synthesis of active compounds. Due to their surface tension reducing, emulsion stabilizing, and biodegrading properties of these in place of chemical surfactants, they are generating huge demand in terms of research and usage. Biosurfactants are widely used in the food industry as food-formulation ingredients and antiadhesive agents as emulsifiers, de-emulsifiers, spreading agents, foaming agents, and detergents that find application in various fields such as agriculture, industrial sectors, and environmental recreation. Recent research focused more on heavy metal bioremediation from compost was achieved using biosurfactants-producing bacteria, which resulted in an improvement in compost quality. Although a number of studies on biosurfactants synthesis have been reported, very limited information on its cinematics and the consumption of renewable substrates are available. In this review paper, we made an attempt to critically review biosurfactants, their usage, research related to them, and challenges faced.

Keywords: biosurfactant; microorganisms; bacteria; bioremediation; biodegradation; environment



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1. Introduction

Biosurfactants are valuable, surface active and biologically efficient microbial amphiphilic molecules for different industries or processes. Microbes synthesize them and offer an alternative to chemically prepared conventional surfactants, especially when growing on water immiscible substrates. These molecules can be widely used in cosmetics, pharmaceutical and alimentary processes such as emulsifiers, humectants, preserving agents and detergents because of their structural diversity (e.g., glycolipids, lipopeptides, fatty acids, etc.), their low toxicity and biodegradability. In the fields of bioremediation and waste treatment, they are also ecologically safe. They can be made from different substrates, mainly from renewables, such as vegetable oils, distillery and milk waste; those are economical, but are not reported in detail. In this review, progress on the use of renewable substrates for the production and new applications of biosurfactants is reported.

Living cells synthesize surface-active compounds known as biosurfactants, which are produced by various microorganisms that have several distinct properties compared to other synthetic surfactants, including mild production conditions, multifunctionality, higher biodegradability, and lower toxicity. These compounds are primarily biosynthesized as secondary metabolites and play important roles in the growth and localization of their microorganisms.

Based on the chemical structure of their hydrophobic component, BSs are classified into four types: (1) glycolipid type, (2) fatty acid type, (3) lipopeptide type, and (4) polymer type. Due to their surface tension reducing, emulsion stabilizing, foam promoting, and biodegrading properties, use of these in place of chemical surfactants is a highly demanded area of interest. Thus, in hydrocarbon-contaminated sites, biosurfactants producing microorganisms accelerate bioremediation. Bacteria and yeasts synthesize most of these. Glycolipid, phospholipid, rhamnolipid, etc., are the biochemicals with great surface activity synthesized by these organisms. The hydrocarbon substrate is emulsified by the production of these chemicals for the facilitation of transportation into cells. A mechanism known as swarming motility is identified in the biosurfactants mechanism of action. The structure of a biosurfactant is composed of a hydrophobic tail and a hydrophilic head [1], otherwise composed of amphiphilic or hydrophobic peptides. Hydrocarbon uptake is highly related to the spontaneous release and function of biosurfactants. Thus, the maximum production is seen in the hydrocarbon-degrading microorganisms. Water-soluble compounds like glucose, etc., also seemed to produce biosurfactants in rare cases [2,3]. These compounds also have antibiotic properties that disrupt the membrane of the food competitive microorganisms. These biosurfactants show improved properties compared to a chemically synthesized surfactant, which enables them to be used in the process of oil recovery in an environmentally safer way. Their tolerance in extreme conditions, ease of culturing, high-scale production, eco-friendly nature, and diversified nature makes them efficient enough in the implementation of various fields, including microbial degradation. Antiadhesive agents and food-formulation components had long been used in the food sector. Biosurfactants stabilize emulsions by reducing surface and interfacial tension. Other activities of biosurfactants in food processing include controlling fat globule agglomeration, stabilizing aerated systems, enhancing the texture and shelf life of starch-containing goods, and improving the consistency and texture of fat-based products. Biosurfactants are used to manage consistency, prolong freshness, and solubilize flavor oils in bread and ice cream manufacture. They're also utilized in oil and fat frying as fat stabilizers and anti-spattering agents. The addition of rhamnolipid biosurfactant to bakery goods increases dough stability, texture, volume, and preservation, as well as butter cream, croissants, and frozen confectionary items' qualities. In light of changing climate circumstances and a growing global population, it is critical to investigate creative, efficient, and cost-effective natural products for the betterment of people [4–6].

Biosurfactants have seen a tremendous increase in research and development, as well as commercialization of biological agents in recent years. Microorganisms create a wide variety of amphiphilic metabolites, many of which are unique in their structure. Various ways for categorizing microbial biosurfactants, such as structural similarities, diameters, moieties, hydrophobicities, degree of change, and other physical and chemical parameters, are applied in addition to traditional surfactant classification methods. Aside from reducing surface stress, microbial surfactants may provide a number of other advantages. This combined effect of bioactivity and interfacial activity, which is highly dependent on the structure and composition of each molecule, provides the vast majority of biological structures with unique opportunities for pharmaceutical, agricultural, environmental, and other applications that have yet to be discovered. Apart from the well-known glycolipids and lipopeptides, there are several more classes, structures, and structural combinations of microbial biosurfactants.

Objectives:

1. Biosurfactants have emerged as potential molecules for drug delivery vehicles, medicinal applications, agricultural applications, and environmental safety, all of which provide economically appealing and scientifically novel applications.
2. The current study discusses biosurfactants and their production by bacteria, with an emphasis on their involvement in oil cleanup.
3. To explore novel biosurfactants those are commonly used for soil remediation.
4. To identify the notable biomolecules that potentially replace harsh surfactants now employed in pesticide manufacturing.
5. To determining the significance of environmental biosurfactants in plant growth promotion and other agricultural uses requires detailed investigation.
6. Emphasize the use of biosurfactants as eco-friendly and alternatives to synthetic surfactants.

This review offers an overview of microbial biosurfactants' diversity and classification by presenting both well-known and well-investigated examples, and covers the present reality of biosurfactant research, pointing the way toward the discovery and development of molecules with innovative structures and different functionalities for modern techniques.

2. Classification of Biosurfactants

Surfactants are one of the most diverse chemical groups used in different industrial processes. They have a competitive market, and producers will have to expand the production of surfactants in an environmentally friendly way. Incentives for environmentally friendly and cost-efficient biosurfactants have led to an increased interest in biological agents. The structural variety and functional features of biosurfactants make them an enticing compound class that may be employed for a broad range of industrial, environmental, and biotechnological applications. Screening methods make it simpler to find prospective bacteria that produce biosurfactants. A variety of purifying and analytical approaches are available for the characterization of biosurfactants. Biosurfactants are classified largely based on their chemical composition and source of generation. Based on their molecular mass, another classification with two major classes was also suggested [7]. Glycolipids and lipopeptides constitute the low molecular biosurfactants, whereas lipoproteins, lipopolysaccharides, and amphipathic polysaccharides constitute the high molecular mass biosurfactants. The low molecular biosurfactants lower the surface and interfacial tensions, whereas the other is highly efficient in stabilizing emulsions [8]. The other classes of biosurfactants include phospholipids, polymeric surfactants, and particulate surfactants [9]. The detailed list of classification is given in Table 1.

Table 1. Types of biosurfactants—according to the structure.

Glycolipids		Lipo-Peptides		Surface-Active Antibiotics		Fatty Acids/ Neutral Lipids		Polymeric Surfactants		Particulate Biosurfactants	
(i)	Rhamnolipids	(i)	Surfactin/Iturin/ Fengycin	(i)	Gramicidin	(i)	Corynomicolic Acids	(i)	Emulsan	(i)	Vesicles
(ii)	Trehalose lipids	(ii)	Viscosin	(ii)	Polymixin			(ii)	Alasan	(ii)	Whole Microbial Cells
(iii)	Sophorolipids	(iii)	Lichenysin	(iii)	Antibiotics TA			(iii)	Lipin		
(iv)	Manno- sylerythritol lipids	(iv)	Serrawettin					(iv)	Lipomanan		
		(v)	Phospholipids								

2.1. Glycolipid

Long-chain aliphatic acids combined with carbohydrates make up the glycolipids or hydroxy aliphatic acids. Rhamnolipids, sophorolipids, trehalolipids, and fructose-lipids are some of the glycolipids β -hydroxy fatty acids attached with different sugars are found in glycolipids whereas cycloheptapeptides with amino acids linked to fatty acids of different chain lengths are found in lipopeptides. Their solubility is seen in both polar and non-polar solvents [10–12].

2.1.1. Rhamnolipid

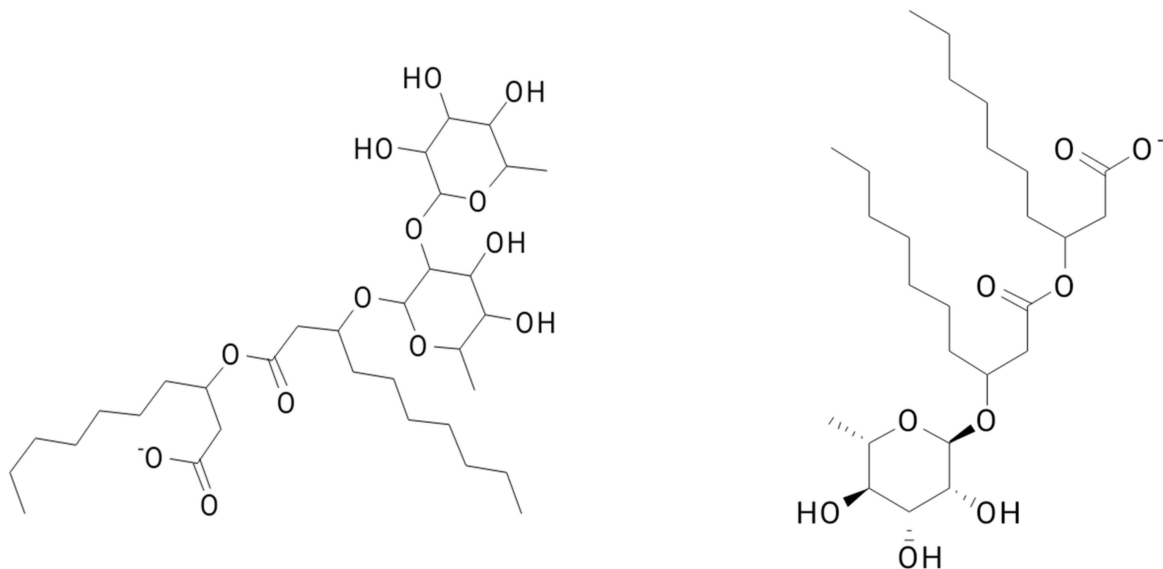
One or two molecules of rhamnose attached to one or two molecules of b-hydroxy decanoic acid make up the rhamnolipid. Thus far, seven homologs of rhamnolipids have been reported [13]. *P. aeruginosa* produces the following prominent substances: L-Rhamnosyl-L-rhamnosyl-b-hydroxy decanoyl-b-hydroxy decanoate and L-rhamnosyl-b-hydroxy decanoyl-b-hydroxy decanoate, which were referred to as rhamnolipid 1 and 2, respectively [9]. Against n-hexadecane the surface tension and the interfacial tension have been reduced to 30 mN/m and 1 mN/m, respectively, by the rhamnolipids from *P. aeruginosa* [14]. The rhamnolipids synthesized from *Pseudomonas aeruginosa* L2-1 showed 100% emulsification against soybean oil, and 69% of crude oil [15]. Rhamnolipid also showed significant antimicrobial activities against several microbes, thus playing a crucial role in the field of pharmaceuticals [16]. In few cases, heterogeneous mixtures of rhamnolipids are isolated from *Pseudomonas* sp. [17], whereas rhamnolipids produced from *Marinobacter* sp. have prospects for industrial applications [18]. Rhamnolipids have antimicrobial potential against *L. monocytogenes* [19]. Rhamnolipids in combinations of enzymes for improved cleaning wherein the weight percent of the rhamnolipid made up by mono-rhamnolipids can be a good detergent composition [20]. Rhamnolipids can be produced using sugars as carbon sources by *P. aeruginosa* [21]. DYNA270 produced by *Pseudomonas* sp. showed to be a promising factor in displacing heavy oily sludges from polypropylene coupons [22]. The desorption efficiency of phenanthrene is found to be increased by rhamnolipid, thus proving their application in the remediation of PAH contaminated soils in cold region [23]. The structures of rhamnolipid are given in Figure 1A–H.

2.1.2. Sophorolipids

Extracellular sophorolipids consisting of a mixture of different hydrophobic sophorosides constitute the sophorolipids. Surface-active glycolipids like sophorolipids are made up of a disaccharide sophorose unit glycosidically attached to hydroxylated fatty acid surface-active glycolipids [24]. They exist naturally in both open (acidic sophorolipids) and closed (lactonic sophorolipids) types. Furthermore, novel sophorolipids, sophorosides (SSs), and glucosides with promising metal coordination properties, have been formed [25], including novel bolaform sophorolipids and sophorosides [26,27]. The systemic and structural disparities among the sophorolipid substances have an impact on their physicochemical activities. Lactonic acetylated forms, for example, have the greatest biological activity, while acidic forms provide greater foam-forming abilities and water solubility [28]. Sophorolipids are manufactured across a number of companies in the United States, Europe, and Asia. It is used in sustainable safe cleaning goods and cosmetics [29]. This market success is mostly due to their high productivity. A fed-batch fermentation method will produce over 300–400 g/L of liquid [30].

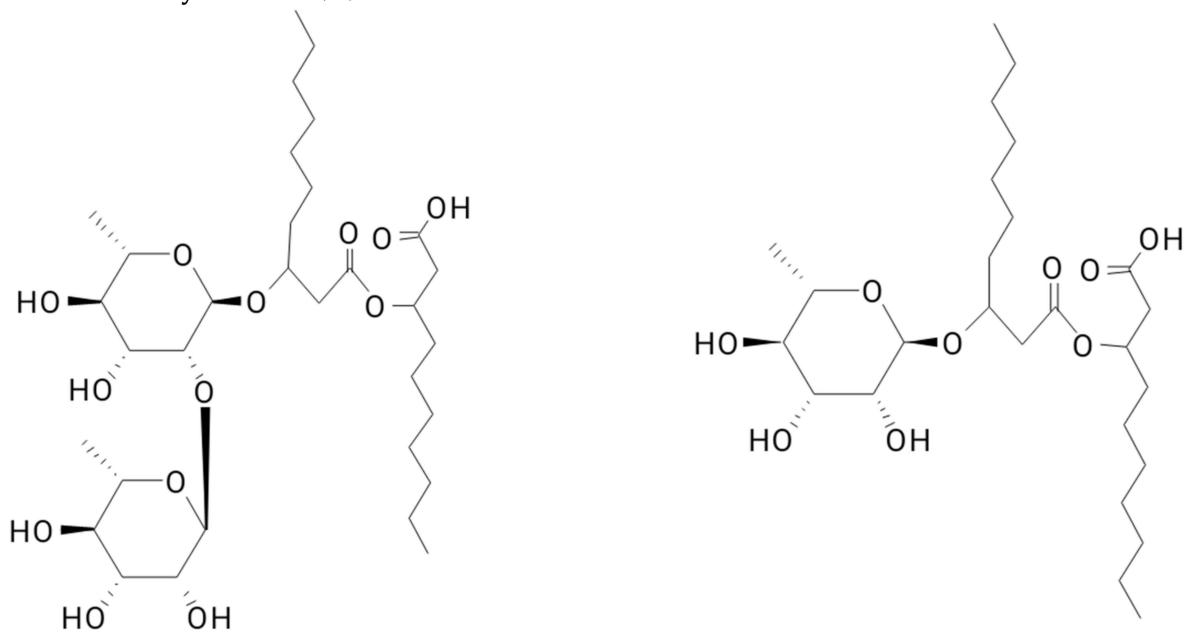
Certain species of *Torulopsis* has been reported to produce dimeric carbohydrate sophorose linked to 1,2 long chain hydrocarboxylic acids [8]. Though the emulsifying properties were not seen, surface and interfacial tension, and reduction capability has been reported [31,32]. The surface tension and the interfacial tension against n-hexadecane and water have been reduced to 33 mN/m and 5 mN/m by both lactonic and acidic sophorolipids with 10 mg/L of pure sophorolipid. Though the temperature and pH are changed, notable stability is seen. In the pH values of 6–9, and at temperatures 20–90 °C stable surface-active properties have been noted. Antibiotic resistance problem was tried to address the antimicrobial properties of Sophorolipids (SLs). *Staphylococcus aureus* growth was completely inhibited by the SL-tetracycline combination [33]. The growth of organisms like *Bacillus subtilis*, *Staphylococcus xylosus*, *Streptococcus mutans*, and *Propionibacterium acne* were completely inhibited by the sophorolipids produced by *Candida bombicola* ATCC 22214 [34,35]. Biofilm formation was also being disturbed by these sophorolipids [36]. Acidic sophorolipids are reported have higher antimicrobial properties against the nosocomial infective agents thus contributing a major part in the production of antimicrobial cream [37]. Sophorolipid produced by *Rhodotorula babjevae* YS3 shows a greater antifungal

activity when compared to others [38]. Bolaform sophorolipids may be an intriguing biological option, with potentially different characteristics. However, apart from certain “high-end” uses, the existing demand for industrially manufactured SLs is controlled by its usage in detergent applications by more than 90% [39]. The structure of sophorolipid is given in Figure 2A–D.



(A) L-rhamnosyl-L-rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate(1-)

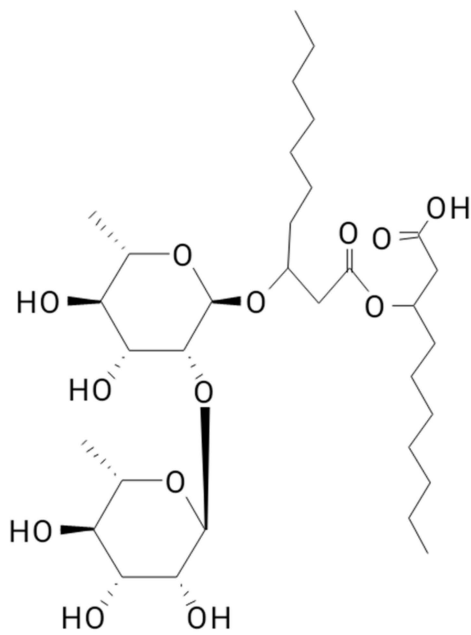
(B) L-Rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate



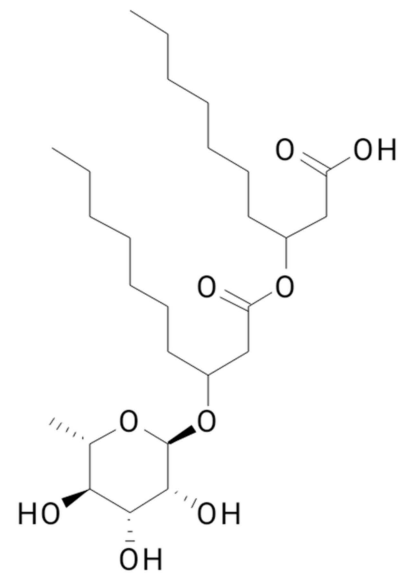
(C) 2-O- α -L-rhamnopyranosyl- α -L-rhamnopyranosyl- α -hydroxydecanoyl- α -hydroxydecanoic acid

(D) α -L-rhamnopyranosyl- β -hydroxydecanoyl- β -hydroxydecanoate

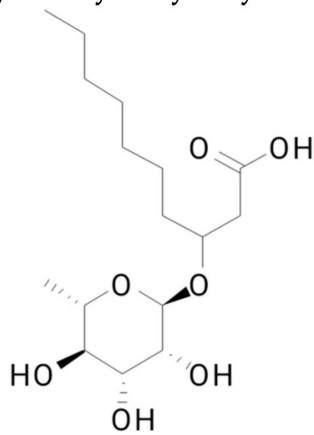
Figure 1. Cont.



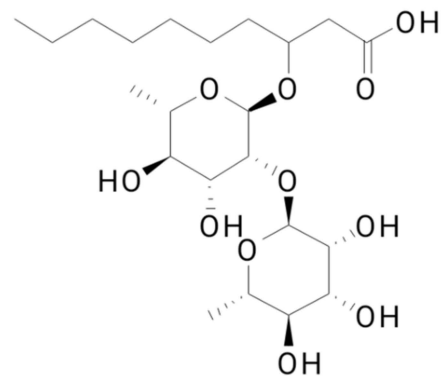
(E) 2-O- α -L-rhamnopyranosyl- α -L-rhamnopyranosyl- α -hydroxydecanoyl- α -hydroxydecanoic acid



(F) 3-[3-[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy]decanoyloxy]decanoic acid



(G) 3-[(6-Deoxy-Alpha-L-Mannopyranosyl)Oxy]Decanoic Acid



(H) 3-[(2-O-Alpha-L-Rhamnopyranosyl-Alpha-L-Rhamnopyranosyl)Oxy] Decanoic Acid

Figure 1. (A–H) The structures of rhamnolipid.

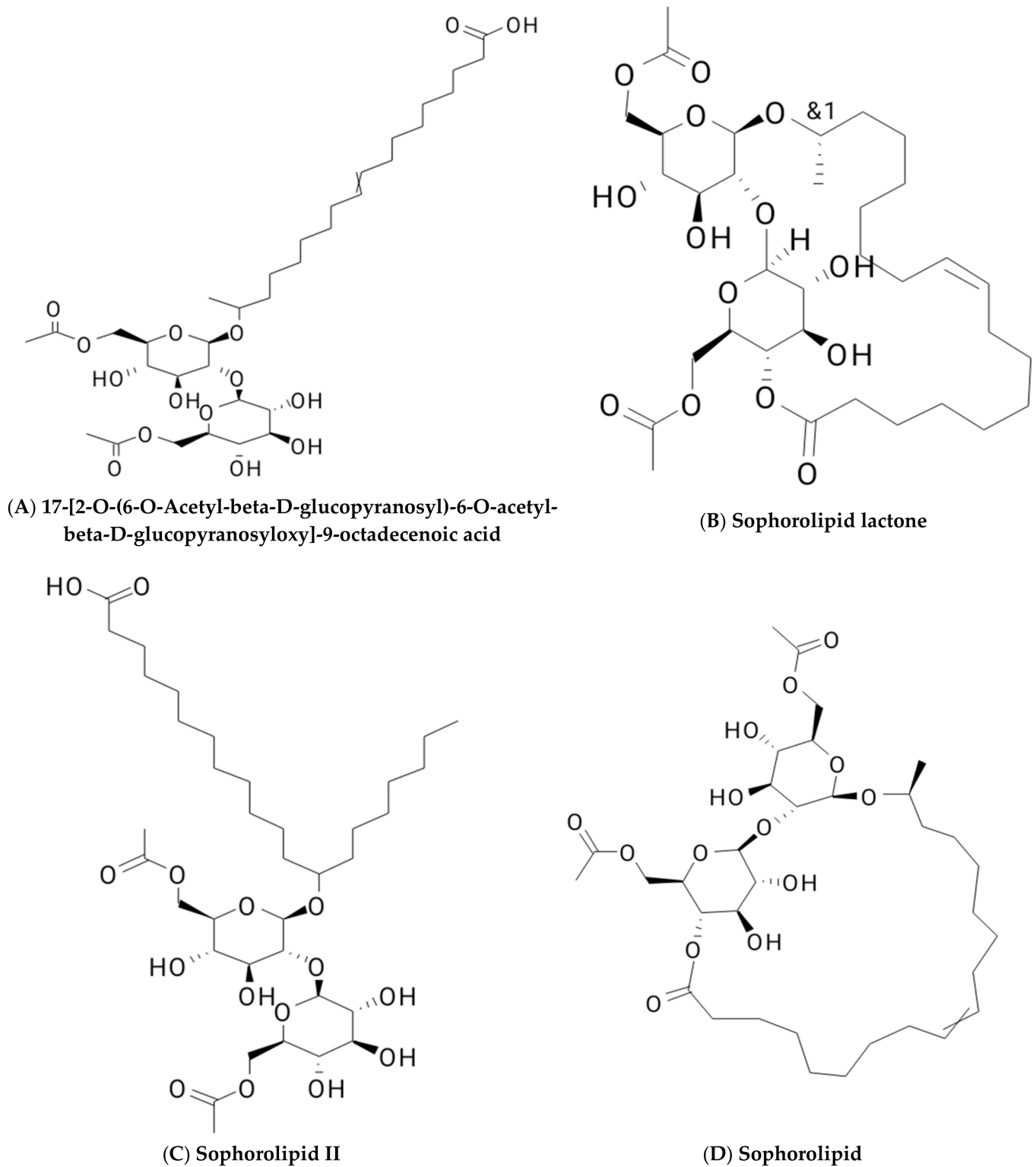


Figure 2. (A–D) The structure of sophorolipid.

2.2. Lipoproteins and Lipopeptides

Cyclic lipopeptides with 8–17 amino acids and a lipid portion constitutes the lipopeptides. They show heavy variation in their amino acid and lipid composition [9]. *Streptomyces* sp. DPUA1566 has potential applications either in bioremediation processes or in pharmaceutical and cosmetic industries, and it is from a low-cost waste source [40]. Lipoprotein synthesized from *Pseudomonas gessardii* shows prominent metal ion removing properties [41], similarly a

lipopeptide produced by *Bacillus atrophaeus* 5-2a showed high efficiency in crude oil removal [42]. The anthracnose disease caused by *Colletotrichum gloeosporioides* has been greatly inhibited by the lipoprotein produced by *Bacillus subtilis* CMB32 [43]. The structure of lipopeptides is given in Figure 3A,B.

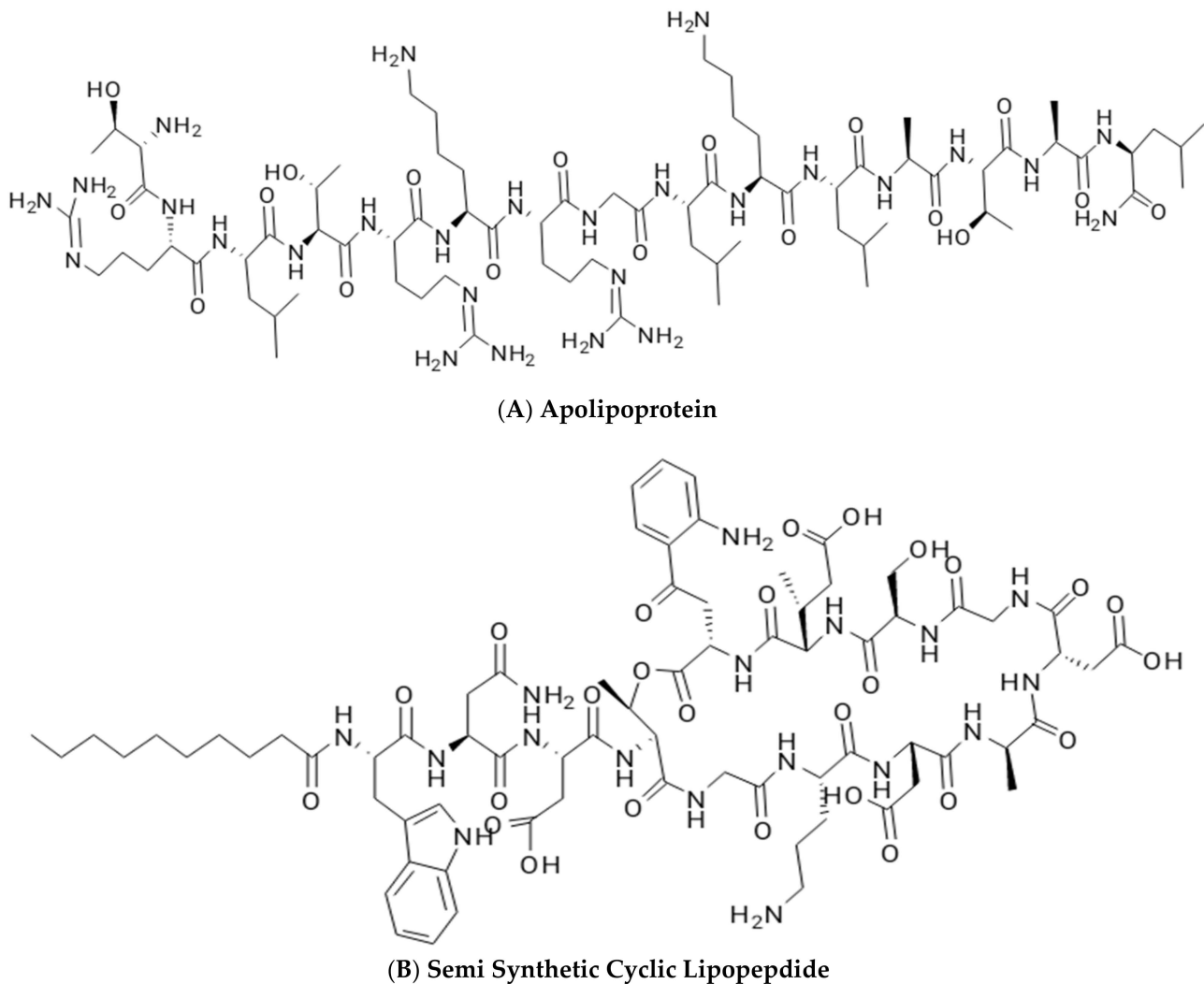


Figure 3. (A,B) The structure of lipopeptides.

Surfactin

Cyclic acidic lipopeptides constitute the surfactin. Out of all known biosurfactants, the surfactin produced by *B. subtilis*, is the most effective one. [44,45] Surfactin is attributed with an important property of red blood lysing. Additionally, they act as good antibiotic [44,46]. A hydrophobic ball-like structure of surfactin is formed in water and air [47,48]. Surfactin is produced from *Bacillus subtilis* ATCC21, 332 is used in the enhancement of the iron-remediation [49,50]. Anti-inflammatory activity is also reported from these surfactin. It also inhibits the expression of IFN- γ , IL-6, iNOS, and nitric oxide (NO) [51]. Cancer can be inhibited by the application of surfactin. Anticancer therapy can be improved by the optimization of nano-particle delivery system of surfactin [52–54]. The surfactin isolated from *Bacillus subtilis* HSO121 contribute maximum in daily life and industrial applications due to their diversified and effective characteristic features [55]. The structure of surfactin is given in Figure 4A–F and the types of surfactin is given in Figure 4G.

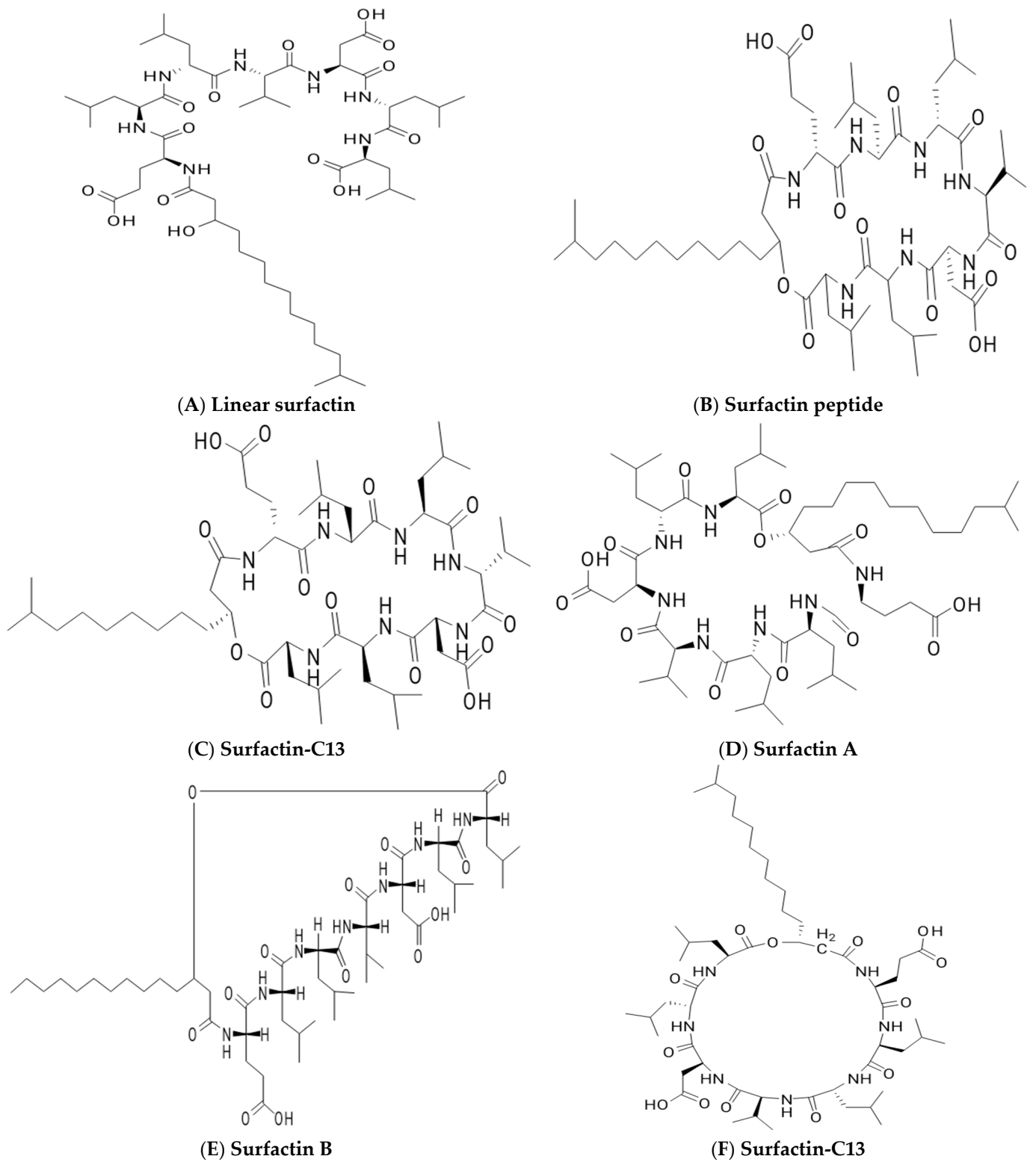


Figure 4. Cont.

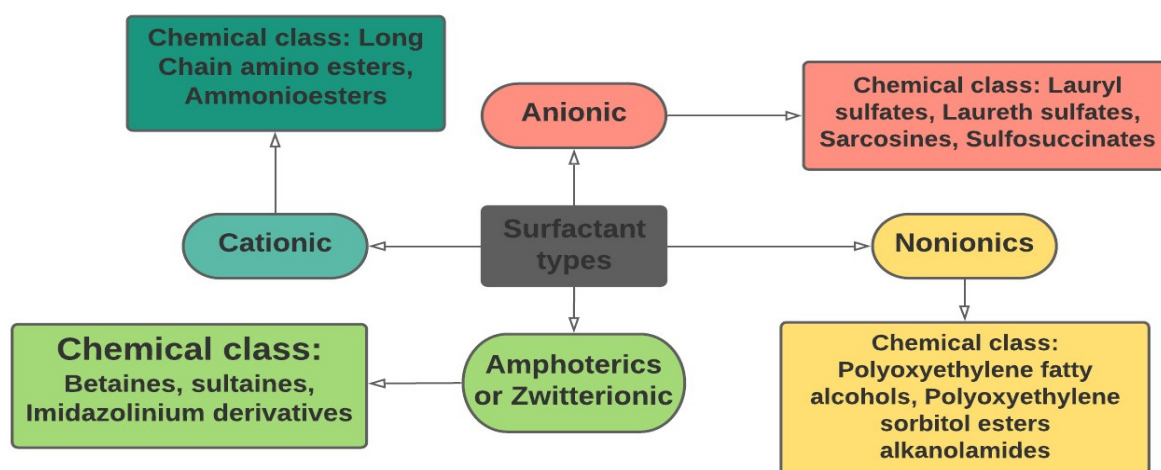


Figure 4. (A–G) The structure of surfactin; and (G) the types of surfactant.

2.3. Fatty Acids, Phospholipids, and Neutral Lipids

Several microorganisms such as bacteria and yeast produce fatty acid and phospholipid, which have received increasing demand in the current situation due to their highly diversified and effective properties as biosurfactants [8]. Microemulsions of alkanes were produced by these biosurfactants. [56] Lipoamino acid is produced by proteinogenic or non-proteinogenic amino acids. [57,58] Sulfur-reducing bacteria such as *Thiobacillus thiooxidans* produces this biosurfactant in higher amounts [8,9,59,60]. Phosphatidylethanolamine produced by *Rhodococcus erythropolis* reported to show a lowered interfacial tension against hexadecane to less than 1 mN/m and a CMC of 30 mg/L [8,61]. When grown on n-alkanes. *Acinetobacter* spp. produces phosphatidyl ethanolamine-rich vesicles and forms medically essential micro emulsions. According to Gautam and Tyagi, the major cause of respiratory-related problems is phospholipid protein complex deficiency. The gene from selected bacteria and yeast can be isolated and cloned, and by use of fermentative processes, surfactants can be produced. Biosurfactants produced by *P. putida* inhibited the growth of *C. albicans* [62,63]. Neutral lipids expected to be wax ester-like lipids produced by the marine hydrocarbonoclastic bacteria. Trehalose lipids synthesized from *Rhodococcus fascians* BD8 makes a greater contribution in the medical field due to their antimicrobial and other properties [64]. Biosurfactants produced by *Lactobacillus pentosus* (PEB) with antimicrobial and anti-adhesive activities showed greater inhibition effect towards the adverse effects and growth of the skin microflora, thus finding a greater place in the cosmetic industry [65]. The structures of fatty acids are given in Figure 5A–D.

2.4. Polymeric Biosurfactants

A complex mixture of biopolymers such as proteins, polysaccharides, lipopolysaccharides were the base components of the exocellular polymeric surfactants obtained from many bacterial species of different genera [8]. Through o-ester linkages the polysaccharides are covalently linked to fatty acids [9,66]. Emulsan, liposan, and mannoprotein are the best examples of polymeric biosurfactants [9,67,68].

2.4.1. Emulsan

Pure form of emulsan shows emulsifying activities under low concentrations. Emulsan produced by *Acinetobacter calcoaceticus* PTCC1318 showed crude oil degradation properties [69]. By coating the hydrophobic substrate, they are made readily available for microbial access. Mixtures of aliphatic and aromatic hydrocarbons can be emulsified efficiently in balanced proportions except their pure forms. Fatty acid components determine the emulsifying activities of emulsan [70,71]. *Acinetobacter calcoaceticus* BD4 emulsan showed the optimal emulsification activity when polysaccharides and proteins were mixed and when they are separate no activity is reported [72].

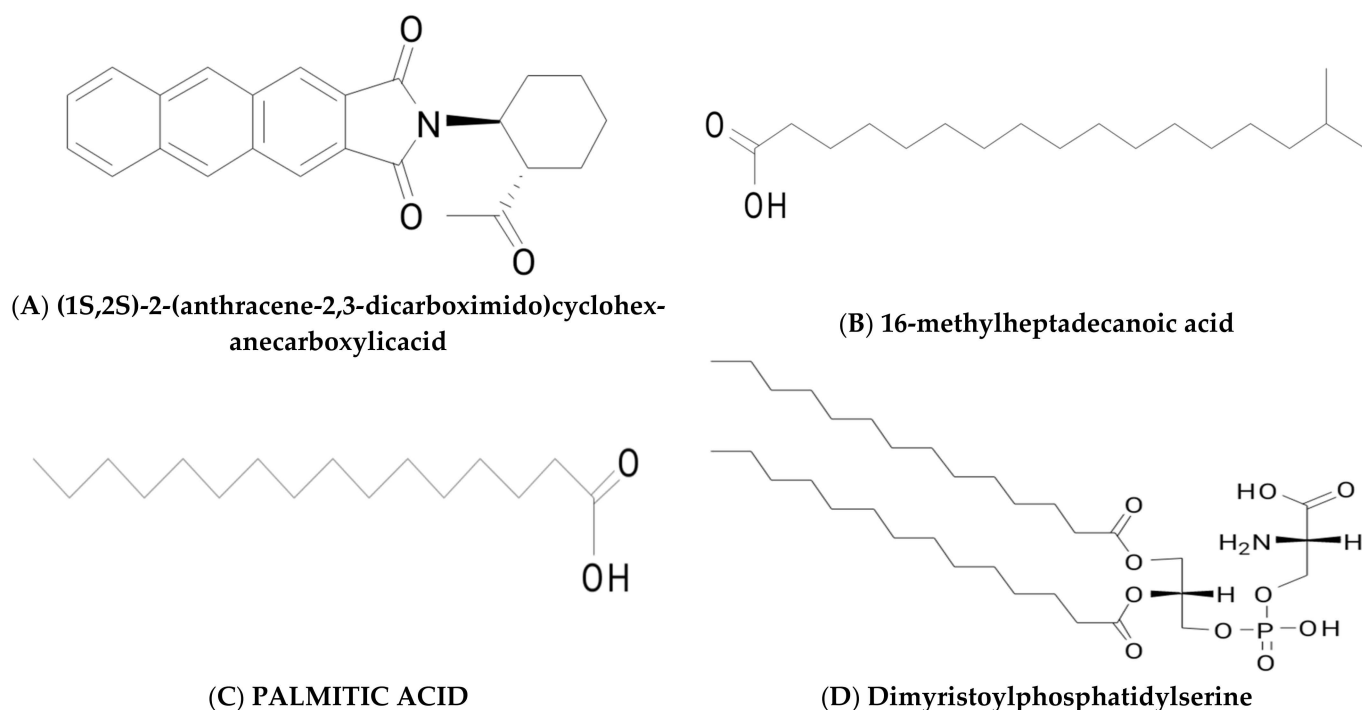


Figure 5. (A–D) shows the structures of fatty acids.

2.4.2. Liposan

This emulsifier is synthesized by *C. lipolytica* consisting of carbohydrates and proteins. It finds a prominent place in the food and cosmetic industries [73]. It forms stable emulsions with edible oils [74].

2.4.3. Mannoprotein

The major component of the cell wall of *Saccharomyces cerevisiae*, the mannoprotein is an effective bioemulsifier [75]. They were capable of forming stable emulsions with many hydrocarbons and other substances, thus suggesting their applications as cleaning agents. The structural and enzymatic classification of mannoproteins is dependent on its chemical composition and specific function. The most abundant ones are the structural mannoproteins, which are made up of mannopyranosyl attached to a small protein, but the enzymatic mannoproteins are the most effective emulsifiers and have more protein moieties. They activate the immune cells for the production of antibodies [76,77]. *Kluyveromyces marxianus* produced a mannoprotein that formed a stable emulsion in corn oil [78]. Mannoprotein bioemulsifier isolated from *Saccharomyces cerevisiae* 2031 showed similarity with the mannoprotein obtained from *Acinetobacter* sp. in 77% carbohydrate and 23% protein [79] 53% protein, 42% polysaccharide, and 2% lipid [80].

2.5. Particulate Biosurfactant

The vesicles and fimbriae synthesized by *Acinetobacter* sp. are the constituents of this surfactant. Protein, phospholipid, and lipopolysaccharide are the components of the purified vesicles. Microemulsion has been formed by these [9,81]. Hydrophilic and hydrophobic moieties of biosurfactants enable them in the accumulation in different phases of interfaces [82]. The biosurfactant consists of a simple polar head made up of simple or a mixture of compounds like amino acids, peptides, etc., whereas the nonpolar tail is made of fatty acids and rarely cyclic structures were also reported [8,83,84]. Spherical biosurfactant vesicles were formed at concentrations greater than their critical micelle concentration (CMC) [85,86]. The attachment of biofilm formers have been effectively

reduced by biosurfactant. The adherence of *L. monocytogenes* is strongly impeded by RHL. This shows the inhibition of bacterial populations during biofilm formation [87].

3. Biosurfactant Producing Mechanisms

Surfactants are a structurally varied and heterogeneous class of microorganism-produced surface-active chemicals. Surfactants are synthesized in both the synthetic and biological realms [88]. Biosurfactants (BSs) are surfactants obtained from biological entities, particularly bacteria and fungi. These compounds are synthesized as metabolic products or as a result of the surface chemistry of the cells. BSs are mostly created by aerophilic microorganisms in aqueous media using carbon source feedstocks such as hydrocarbon, polysaccharides, lipids, and oil derived from bacteria (*Pseudomonas*, *Bacillus*, and *Acinetobacter*), fungus (*Aspergillus* and *Fusarium*), and yeast (*Candida* and *Pseudozyma*). Rhamnolipids, surfactins, sophorolipids, emulsans, and mannosylerythritol lipids are the most often seen BSs [89]. These surface-active chemicals serve a physiologic purpose by allowing BSs generating microbes to grow on insoluble substrates, assure exponential biomass growth, demonstrate antimicrobial activity against potential predators, and enable them to withstand harsh environmental conditions, pathogenicity, and cell desorption. The mechanisms of biosurfactants vary according to the class to which they belong [88].

Mechanism of Action of Surfactants

When a decent number of surfactant molecules are accumulated in a solution, they mix to create micelles. As the micelle develops, the surfactant heads align themselves to be exposed to water, while the tails cluster together in the micelle's core, sheltered from water. Micelles cooperate together to reduce contaminants. The hydrophobic tails cling to and surround dirt, while the hydrophilic heads lift the clinging soils off the surface and into the cleaning solution. The micelles then reform, with the tails hanging the dirt in the structure's core [90,91].

4. Isolation of Biosurfactant

Microbes nearly usually exist in mixed populations in natural habitats, comprising a diverse range of strains and species. A pure culture is necessary to analyze the qualities of a specified organism isolated from such a mixed population. Apart from direct strain separation by dilution and plating, enrichment cultures using hydrophobic surfaces are very promising for the isolation of biosurfactant producing microorganisms [92]. Minimal salt medium is a widely used media to isolate microbes from hydrocarbon contaminated regions. Enrichment procedures were used to extract 130 oil-degrading isolates from hydrocarbon-polluted sites. The researchers used a mineral salts medium with crude oil as the only carbon source [93].

To get crude biosurfactant, cell-free supernatant was collected by centrifuging culture broth for 20 min at 10,000 rpm and 4°C. Then, 6 N HCl was added to the clear supernatant to adjust the pH to 2 [94]. Additionally, hydrophobic interaction chromatography and the replica plate technique are successful approaches [92]. To generalize, surveying polluted locations in combination with direct isolation or enrichment culture is a proven technique for finding novel biosurfactant producing bacteria. However, since the percentage of positives is just a few percent, many hundred isolates must be screened for each hit [94]. Isolation, screening and characterization of biosurfactants from natural habitat is shown in Figure 6.

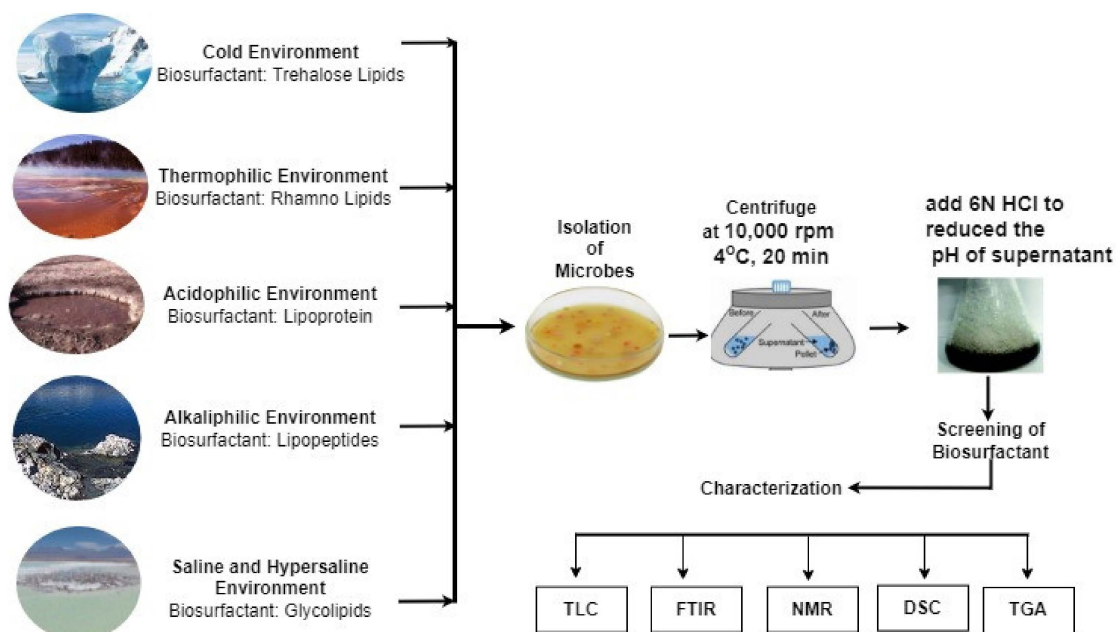


Figure 6. Isolation, screening and characterization of biosurfactants.

5. Biosurfactant Produced by Microorganisms

Various microorganisms contribute to the production of various biosurfactants. The detailed list is given in Table 2.

Table 2. Various biosurfactants produced by microorganisms.

Microorganism	Biosurfactant	Property	Technology/Application	References
<i>A. calcoaceticus</i> RAG-1 (<i>Arthrobacter</i> RAG)	Heteropolysaccharides	A very good bioemulsifier, which makes heavy crude oil less viscous.	Biostimulation	[95]
<i>Arthrobacter</i> . sp., <i>Rhodococcus aurantiacus</i>	Trehalose, sucrose, and fructose, lipids	Lower the interfacial tension and make hydrophobic compounds more “pseudosoluble.”	Biodegradation and Bioaugmentation	[96]
<i>Trehalose dimycolates</i>	<i>Mycobacterium</i> sp., <i>Nocardia</i> sp.	Used more in cosmetic industries because of its lower irritability.	Bioaugmentation	[97]
<i>Arthrobacter</i> MIS 38 <i>Bacillus atrophaeus</i> 5-2a <i>Pseudomonas fluorescense</i>	Lipopeptide	Low interphase surface tension due to emulsifying action.	Bioaugmentation and biostimulation	[51]
<i>Bacillus subtilis</i> ATCC 21332	Surfactin	Used in enhancement of the iron-remediation, anti-inflammatory activity.	Bioaugmentation and biodegradation	[49,50]
<i>Pseudomonas aeruginosa</i> L2-1, <i>Bacillus</i> sp. AB-2	Rhamnolipids	Playing a crucial role in the field of pharmaceuticals.	Biodegradation	[16]
<i>Candida bombicola</i> ATCC 22214	Sophorose lipids	Substantial % E24 against diverse hydrocarbons, including light and heavy crude oils, and high stability under salinity, pH, and extreme heat.	Bioaugmentation	[98]
<i>Candida tropicalis</i>	Mannan-fatty acid	Recognized as key antigenic determinants.	Biostimulation	[99]
<i>Candida lipolytica</i> Y-917, <i>Torulopsis bombicola</i>	Sophoros lipid	Produces hydrocarbon and oil emulsions in a liquid like water.	Biostimulation	[100]

Table 2. Cont.

Microorganism	Biosurfactant	Property	Technology/Application	References
<i>Clostridium pasteurianum</i> ; <i>Nocardia erythropolis</i>	Neutral lipids	Have novel organic pollutant catabolism pathways and potential soil bioremediation capabilities for hydrocarbons and aromatic chemicals.	Biodegradation	[101]
<i>Corynebacterium hydrocarbolastus</i> , <i>Corynebacterium lepus</i> Strain MM1, <i>Phaffia rhodozyma</i>	Protein-lipid-carbohydrate	Fluids viscosified with viscoelastic surfactants (VESs) used in hydrocarbon recovery procedures.	Biodegradation	[102]
<i>Myroides</i> sp., <i>Pseudomonas</i> sp., <i>Thiobacillus</i> sp., <i>Agrobacterium</i> sp., <i>Cluconobacter</i> sp.	Ornithine lipids	A potential bioremediation technique for contaminated sediments.	Bioaccumulation and biodegradation	[103]
<i>Corynebacterium insidiosum</i>	Phospholipids	Multiantibiotic resistant.	Biostimulation	[104]
<i>Ochrobactrum anthropi</i> HM-1 and <i>Citrobacter freundii</i> HM-2	Proteins	Reduces the viscosity of heavy oil, cleans oil storage tanks, and increases the flow of oil through pipelines.	Biostimulation	[104]
<i>Penicillium spiculisporum</i>	Spiculosporic acid	Used as a bioactive compound to remove heavy metal cations from water.	Biostimulation and bioaugmentation	[105]
<i>Rhodococcus erythropolis</i>	Trehalose-dicarynomycolate	A potential bioremediation technique for contaminated sediments.	Bioremediation and biotransformation	[106]
<i>Rhodococcus</i> sp. ST-5 <i>Rhodococcus</i> sp. H13-A <i>Rhodococcus</i> sp. 33	Glycolipid	Responsiveness to hazardous and refractory chemicals such chlorinated aliphatic and aromatic hydrocarbons, N- and S-heterocyclic compounds, and synthetic polymers.	Bioremediation and biotransformation	[106]

6. Properties of Biosurfactants

Biosurfactants such as rhamnolipid and greenzyme were attributed with a special property of reducing the surface tension of water and interfacial tension of n-hexadecane considerably [9,107,108]. Temperature stability, critical micelle concentration (CMC), and low interfacial tensions were the effective physicochemical properties of these biosurfactants. These properties enable the formation of microemulsions, which help in the solubilization of hydrocarbons in water [83] (Figure 7). Thus, micelle formation is one of the most important properties of a surfactant. With an increase in the surfactant concentrations, there is a gradual decrease in the surface tension [109]. At concentrations above CMC, surfactants such as rhamnolipids stabilize the micelle formation [110]. As a substitute for synthetic biosurfactants, the synergistic impact of antioxidants has led to the use of several biosurfactant extracts in the cosmetic sector. [111]. When different concentrations of biosurfactants were applied to dyed hair, the results revealed that the adsorption of dyed hair is high above the CMC of the biosurfactant, though maintaining the hair in a good state. This reveals their best application in the cosmetic industry [112]. A biosurfactant improves water loss, thus wetting the solid surfaces [107]. It is reported that many emulsifiers were attributed with only minimal surface tension reducing property [107]. Based on the concentration of the biosurfactant the clear zone varies [113]. Figure 8 shows types of biosurfactant used in various industries.

6.1. Surface and Interface Activity

Surfactin from *B. subtilis*, rhamnolipids from *P. aeruginosa*, and sophorolipids from *T. bombicola* were reported with high surface tension reduction and interfacial tension reduction properties of water and n-hexadecane, respectively. Comparatively, the biosurfactants are 10–40 times more efficient than the synthetic surfactants. The biosurfactant produced by the bacteria *Bacillus mojavensis* JF-2 was reported to reduce the interfacial tension of crude oil while lowering the surfactant concentrations [114].

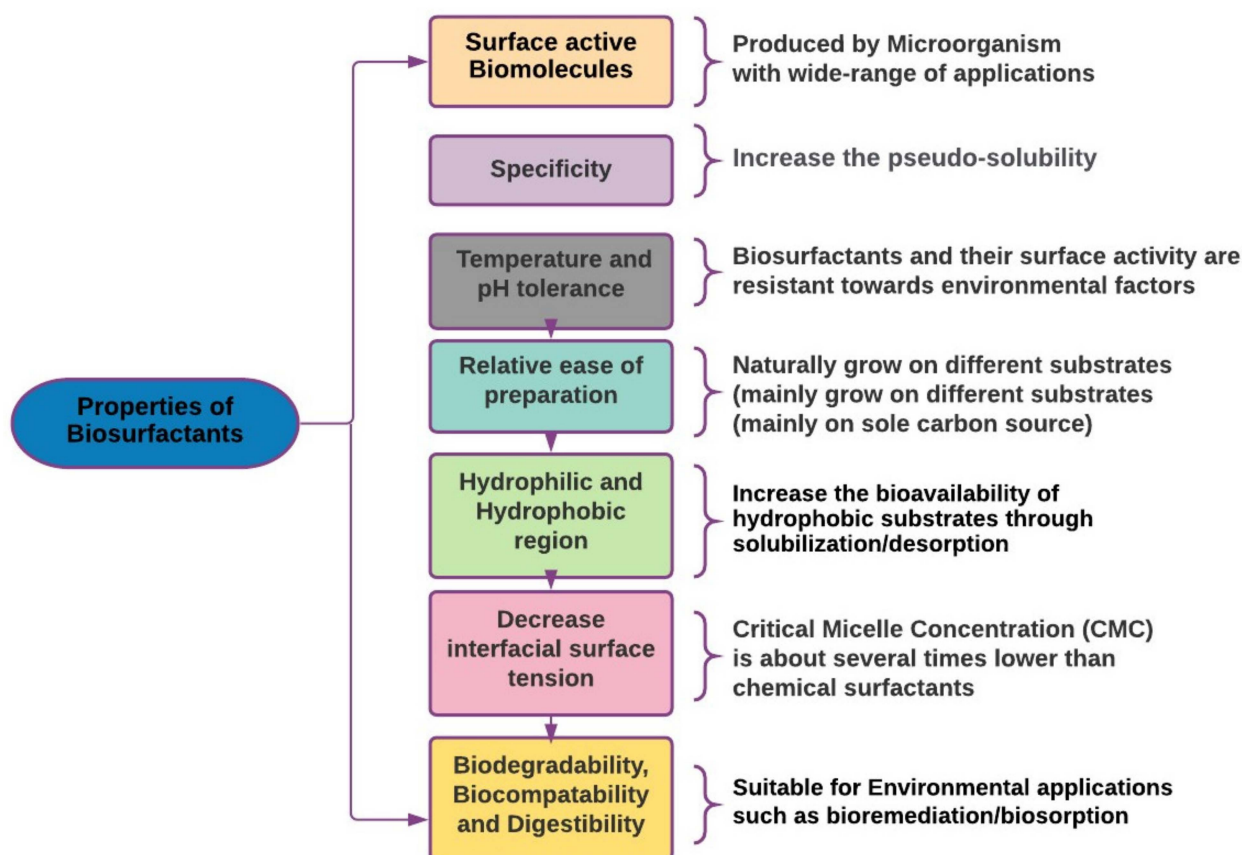


Figure 7. Properties of biosurfactant.

6.2. Temperature, PH, Ionic Strength Tolerance

Many biosurfactants and their surface activities remain unaffected by the environmental conditions like pH and temperature. The biosurfactant lichenysin from *B. licheniformis* JF-2 remains unaffected at temperatures up to 50 °C with pH range of 4.5 and 9.0. Similarly, a lipopeptide synthesized by *B. subtilis* LB5a sustained in a high temperature of 121 °C and at high salt conditions even for 6 months without losing its surface activities. At pH 4–10, high temperatures up to 120 °C, and a NaCl content of up to 10% (*w/v*), the biosurfactant maintains emulsification activity [115]. Even after high temperature treatment surfactants like surfactin and rhamnolipids showed anti-adhesive properties to various microorganisms such as *Staphylococcus aureus*, etc., thus protecting the surface from these pathogens [116]. Similar effects were produced by the biosurfactant synthesized by *C. lipolytica* UCP0988 but under extreme pH conditions. [117,118] The biosurfactants produced by *B. licheniformis* STK01, *B. subtilis* STK02, and *P. aeruginosa* STK03 showed emulsifying properties even after treating at temperatures up to 50 °C [119]. The surface activity and emulsification capacity is reported to be stable even at pH of 7–8 for the biosurfactant produced by *Bacillus subtilis* strain JA-1. The biosurfactant produced by *Pseudomonas aeruginosa* RS29 showed stable temperature stability, pH and saline stability. It was also attributed with high foaming and emulsifying activities even after these extreme conditions [120]. A similar condition has

been reported from the surfactant produced by *Virgibacillus salaries*, thus finding its efficient application in various industries, that to particularly in marine bioremediation [121]. Biosurfactant synthesized by *Streptomyces* sp. DPUA 1566 also showed stability with slight variations when exposed to various temperatures and pH [40]. The same conditions were also reported from the surfactants of *B. subtilis* and *B. tequilensis*.

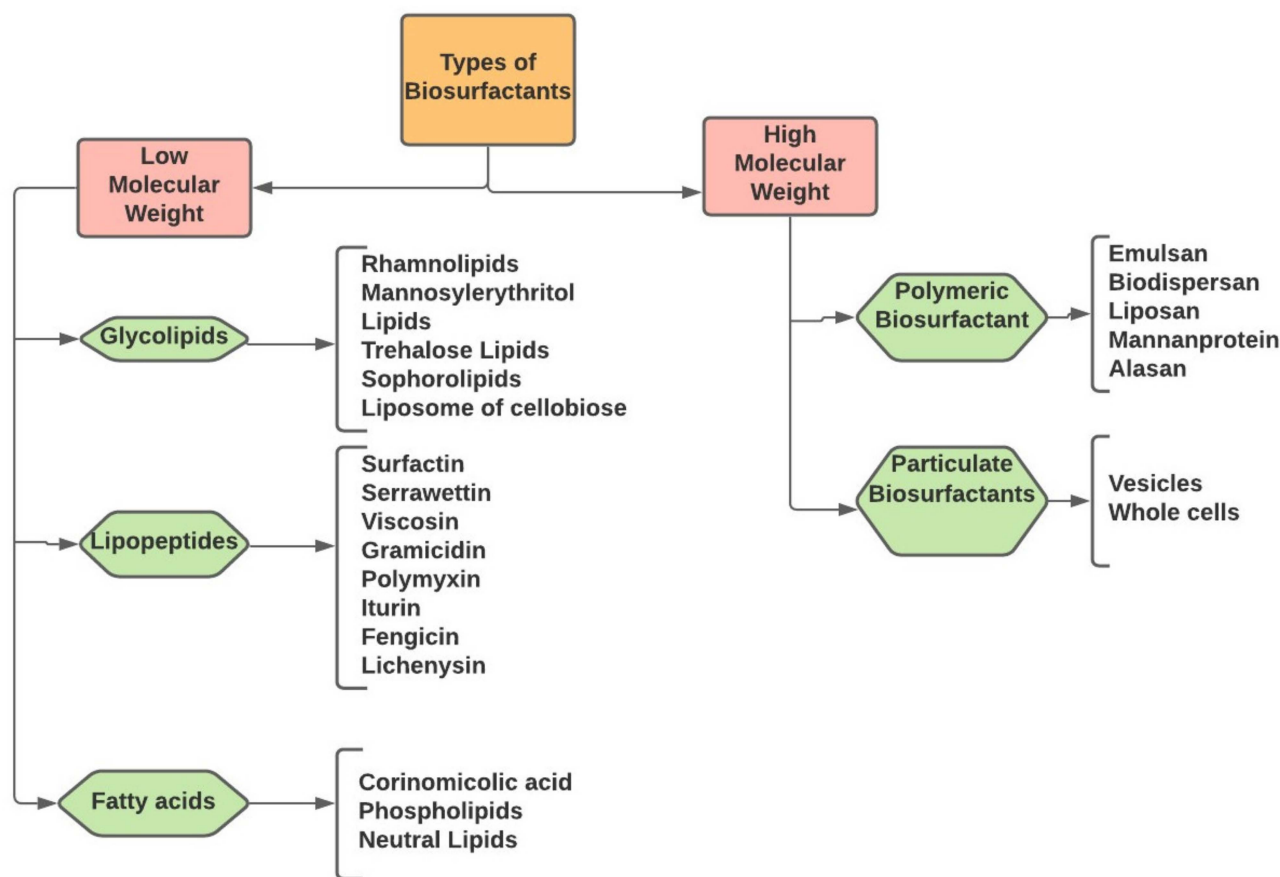


Figure 8. Types of biosurfactant used in various industries.

6.3. Biodegradability and Low Toxicity

Biosurfactants were the most appropriate tool for bioremediation without producing any harmful effects to the environment. They are highly eco-friendly and safer than the synthetic surfactants. Only a very few data have reported the harmful effects of biosurfactants. Rhamnolipids have been reported with a lethal concentration (LC) of 50% against *Photobacterium phosphoreum*, which is comparatively 10 times higher than that of a synthetic surfactant. On analyzing the toxicity of biosurfactants it has been reported that synthetic surfactant sucrose-stearate is similar in homology to biologically synthesized glycolipid, but it degraded faster than that of this biologically synthesized one. A comparative study between Marlon A-350, a synthetic surfactant and a biosurfactant synthesized by *P. aeruginosa* revealed that the synthetic surfactant was highly toxic in all the assays and its properties, whereas the biosurfactant was non-toxic [122]. An analysis revealed that the biosurfactant produced by *Candida sphaerica* reduced the surface tension when applied over the seeds of *Brassica oleracea*, *Cichorium intybus*, and *Solanum gilo* [102]. Rhamnolipid produced by *P. aeruginosa* PG1 showed a cytotoxic effect against the cell line L292, yet serves to be a promising class of biosurfactants with antimicrobial properties, thus being a very good option to be applied for the bioremediation of soil and crude oil processes [123]. When the application of surfactin produced by *Bacillus subtilis* HSO121

was analyzed, it revealed that it is a non-toxic, nonirritant compound, thus a safer one to be used in the detergent formulations [55].

6.4. Emulsion Forming and Emulsion Breaking

Produced emulsions have a life span of few months to years. Biosurfactants can either stabilize the emulsion as emulsifiers or de-stabilize the emulsion as de-emulsifiers. Comparatively, high molecular-mass biosurfactants are better emulsifiers than the low-molecular ones. Few surfactants might act as a good surface tension reducer, whereas they might not be good emulsifiers, whereas few others might not be good in reducing the surface tension, but they were good emulsifiers indeed. Polymeric surfactants coat the oil, thus making stable emulsions, thereby being applied in various cosmetic and food industries. When the agro waste *Beta vulgaris* (Beetroot) was used as a substrate, the bacterial isolates *B. licheniformis* STK01, *B. subtilis* STK02, and *P. aeruginosa* STK03 produced the biosurfactants, which showed very high emulsification rate for heavy hydrocarbons such as anthracene, and lubricant oil [119]. For the discovery of novel microbial emulsifiers, novel screening methods are required, due to the great potential that these molecules possess for green technology [124]. Few emulsifiers with higher emulsifying ratio have been reported from the species of *Saccharomyces cerevisiae* and *Acinetobacter* sp. [79,80]. Using the emulsification index E24 test, six isolates were identified which showed significant emulsification against heavy hydrocarbons without reducing the interfacial tension [125–127]. It was reported that exopolymers of *Bacillus subtilis* 28, *Alcaligenes faecalis* 212, and *Enterobacter* sp. 214 showed high emulsification activity [128]. Mannoproteins formed stable emulsions with almost all the compounds such as hydrocarbons, oils, etc., thus serves to be a promising class of biosurfactants to be applied in various industries [129]. Bioemulsifiers also serve to take a better place in the bioremediation of oil spills, heavy metal removal, food and agro industries, cosmetic industries, and pharmaceutical industries [127,130–133]. Thus, these serve to be better solutions in these industries rather than the application of toxic synthetic surfactants [134].

7. Application of Biosurfactants

In comparison to chemically synthesized surfactants, biosurfactants offer a number of advantages (Figure 9).

7.1. Agriculture

Agriculture productivity is a major challenge for all countries as it relates to meeting the expanding needs of the human population. It is now necessary to use green substances in order to achieve sustainable agriculture. This review demonstrates the widespread usage of caustic surfactants in agriculture and the agrochemical industry. Green surfactants may be biosurfactants generated by bacteria, yeasts, and fungus. Biosurfactants are used in agriculture to eliminate plant pathogens and increase nutrient bioavailability for beneficial plant microorganisms. Agricultural soil remediation with biosurfactants may greatly improve agricultural soil quality. Surfactants are employed in crop protection and pesticide formulations in an estimated 0.2 million tonnes per year [115].

Good wettability, inhibition of toxicants of pesticides and even distribution of fertilizers in the soil are achieved by the process of hydrophilization using biosurfactants [135]. Removal of plant pathogens and the advancement of the bioavailability of plant nutrients have been increased considerably upon the application of biosurfactants. In several ways, growth of rhizobacteria in the rhizosphere is increased, which helps in the plant growth promotion [136,137]. These biosurfactants improves the quality of soil, and increase the interaction between the plants and the beneficial microbes [138]. These have more advantages over the synthetic surfactants, which are currently used in the pesticides, as these biosurfactants are eco-friendly in nature, economically cheap and also increase the beneficial microbes in the soil [139–143].

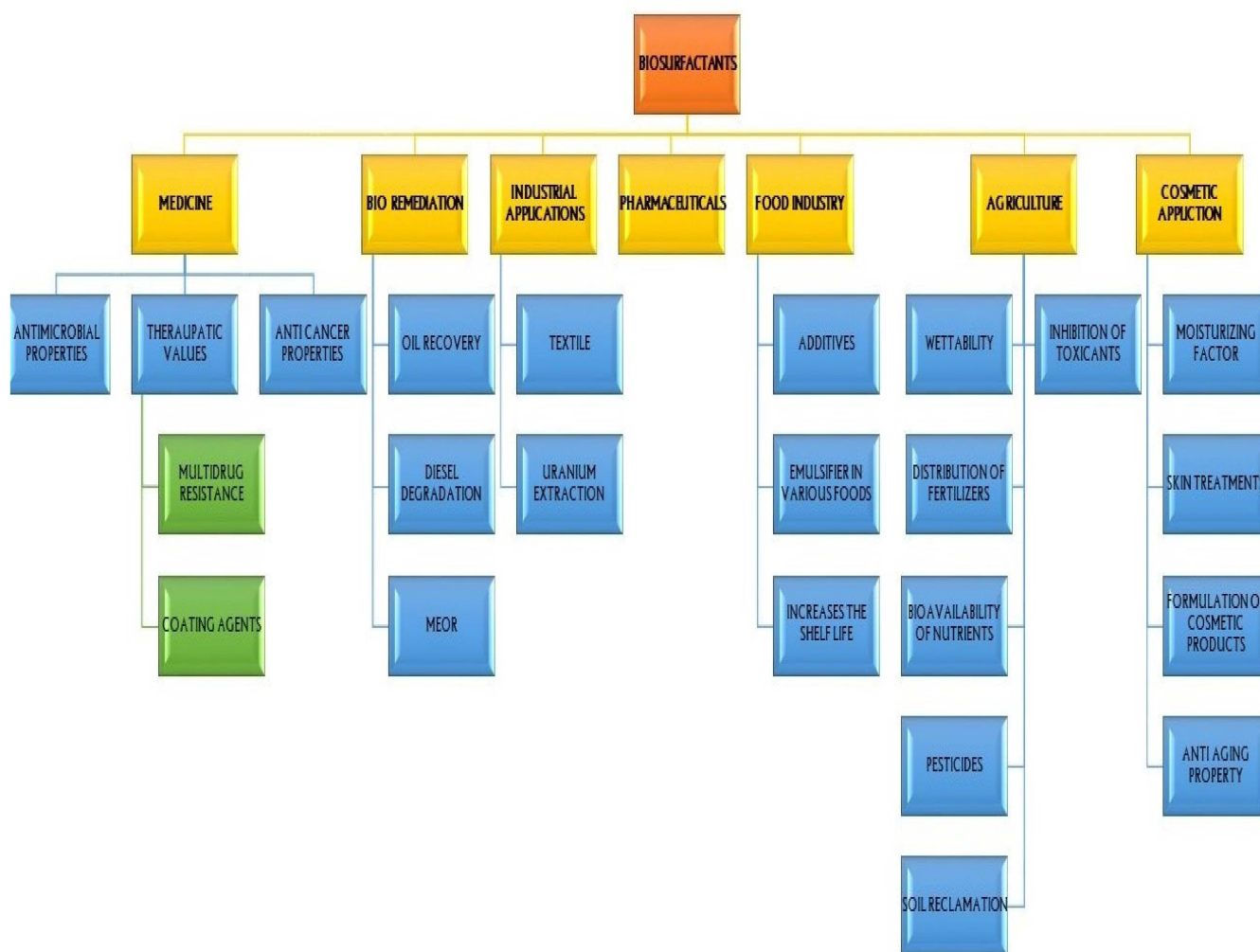


Figure 9. Application of biosurfactant in various fields.

7.2. Biosurfactants in Agriculture and Agrochemicals

A growing population demands more agricultural output in order to fulfil the needs of the world's growing population. It is imperative that green technology be used in agriculture to ensure long-term viability. It is possible to utilize biosurfactants as plant-protection measures in a variety of ways. It has the potential to increase the supply of nutrients for beneficial microorganisms associated with plants. Soil health may be improved by the use of biosurfactants, according to a variety of studies. For contemporary agriculture, biosurfactants are a necessary component. Biosurfactants derived from *Pseudomonas* sp. and *Burkholderia* sp. bacteria might be used as safe biopesticides. Cationic, anionic, anionic, and amphoteric surfactants may all be used to create these pesticides. Some microbe-derived biosurfactants exhibit anti-microbial efficacy against plant pathogens. They are a potential biocontrol agent for sustainable farming. A number of microbe-derived biosurfactants exhibit antibacterial properties against plant diseases, making it the preferred approach for biocontrol in agriculture. Antagonizing rhizobacterial bio surfactants. Pesticides, fungicides, and biosurfactants are often used in agriculture to suppress plant growth-promoting microorganisms. Agronomists utilize surfactants to boost bacteria's antimicrobial activity. Several in vitro and in situ investigations have shown the importance of surfactants in boosting insecticidal abilities [144].

7.3. Biosurfactant Mediated Plant Growth Promotion in Soils

When a microbial consortium is utilized for bioremediation, biosurfactants accelerate pesticide breakdown owing to the synergistic effect of microbial communities. Biosurfactants enhance soil quality, participate in PGP activity, and degrade/solubilize pesticides, according to reports. *Pseudomonas* sp. has been shown to have significant communication with their host plant, displaying a variety of plant growth promoting (PGP) characteristics, and is one of the most important biocontrol agents against pathogenic microorganisms. Rhamnolipids derived from *Pseudomonas aeruginosa* promote herbicide trifluralin and pesticide chlorpyrifos degradation in polluted soil [145]. PGP *Pseudomonas* sp. producing BS was recently identified from pesticide-contaminated fields and common reed roots with hydrocarbon-degrading capability [146]. Biosurfactant from *Pseudomonas cepacia* degrade hydrophobic herbicide 2,4,5-trichlorophenoxyacetic acid [147]. *Pseudomonas aeruginosa* possesses a biosurfactant that has the potential to breakdown huge amounts of quinalphos (a pesticide) in a shorter period of time [148]. Similarly, the glycolipid biosurfactant produced from *Pseudomonas* species has been shown to improve methyl parathion and endosulfan solubilization [145]. Moreover, while growing crops, biosurfactants may be used to increase the availability of micronutrients that are often present naturally or in wastes to plants. Additionally, it has been shown that rhamnolipids and lipopeptides enhance plant tolerance to phytopathogens by boosting plant immune systems. Additional rhamnolipid uses as biopesticides, fungicides, and anti-zoospore agents have been investigated in recent decades [149].

7.4. Food and Agricultural Waste as Substrates for Biosurfactant Production

Waste from the food and agricultural industry, as well as their related items, is a significant source of carbon. Numerous studies have demonstrated that agricultural waste lignocellulosic biomass can be converted into a variety of products [150], one of which is supplements for biosurfactant production [151]. As a result, this lignocellulose biomass offers microorganisms with a nutrient-rich habitat [152]. Sugar beets bagasse, banana, citrus, residual cooking oil, fried palm oil, moringa, and yam residues have all been studied for their propensity to create biosurfactants [152]. Glycolipids are extracted from low-cost raw materials such as distillery waste, fruit and vegetable byproducts, bagasse, rapeseed, sweet potato flour wastewater, bird's feather protein, and beverage effluent [153]. The type of biosurfactant generated is determined by the substrate utilized, since the content of the substrate aids microbial growth throughout the fermentation [153]. Emulsifiers are used in baking to enhance dough rheology, water retention, ingredient mixing and handling. They are good additives and preservatives in food manufacturing [154].

Prior to biosurfactant production, agricultural waste was hydrolyzed to yield hydrolysate sugar that could be utilized as a substrate for biosurfactant production [142]. The enzymes and live cells that can be used to hydrolyze agricultural waste [155]. It has been shown that *Penicillium citrinum* H9, a lignocellulolytic mold, is capable of hydrolyzing agricultural wastes, such as straw and hay [155,156]. *Penicillium citrinum* H9 hydrolyzed rice straw to produce as much as 209.25 g/mL sugar [155]. *Achromobacter* sp. BP(1)5, a hydrocarbonoclastic bacterium isolated from Balongan oil sludge, was shown to be more capable of making biosurfactant from rice straw hydrolysate [156]. *Achromobacter* sp. BP(1)5 can convert the hydrolysate sugar of rice straw and maize cobs by *Penicillium citrinum* H9. *Achromobacter* sp. BP(1)5 showed 98 percent query cover with *Achromobacter xylosoxidans*. The emulsifying efficiency of the crude extract of *Achromobacter* sp. BP(1)5 biosurfactant on kerosene was 27.22 percent and 36.84 percent, significantly [157].

The generation of biosurfactants by different Lactobacillus bacteria utilizing sugar beets molasses (cellulose fibers) and glycerol as substrate resulted in the formation of glycolipids and glycolipopetides as multi-component mixture. Rodrigues et al. reported same approach with minor modifications [158]. The fermentation was carried out in a laboratory-scale bioreactor using De Man, Rogosa and Sharpe medium supplemented with different quantities of lactose as substrate. The findings indicate that sugar beets

molasses and glycerol provide a higher yield than typical synthetic broth. The solvent approach might be used to extract the product from the broth [159]. Overall production was 2.43–3.03 g/L when sugar beets molasses was used as the substrate. The yield obtained in this research is much more than that obtained in earlier experiments, indicating that supplementing broth with yeast and peptone extracts improves the yield of biosurfactant synthesis [160].

7.5. Biosurfactant for Cosmetic Application

The natural moisturizing factor has been reported from the chemically modified sophorolipid synthesized by *Torulopsis bombicola*. The highest application in cosmetic industry has been reported from the sophorolipids, rhamnolipids, and mannosylerythritol lipids [32]. In the treatment of acne, dandruff, and body odors, sophorolipids play a crucial role due to their anti-microbial properties. Similarly, in the formulations of deodorants, nail care products, and toothpaste, and as an anti-wrinkle agent, rhamnolipids play a crucial role [161]. Mannosylerythritol lipids are used as anti-aging skincare products and are comparatively more effective than arbutin [162]. Application of lipopeptides as anti-wrinkle agents have also been reported [163]. Biosurfactants are highly eco-friendly and safer enough to be applied over the skin, and their non-toxic, non-irritant properties find them a best place in the cosmetic industry [112]. Biocompatible and low toxic alternative surfactant, bacterial biosurfactant are in increasing demand. The biosurfactant extracted from *Pseudomonas* sp. was used in the formulation of toothpaste due to their foaming, non-toxic and biodegradable properties thus of a great use in the cosmetics industry [151]. Mildness over the skin and superior interfacial activity of the newly synthesized biosurfactants phospholipid biosurfactants cytidine diphosphate, cytidine triphosphate proves to be a better solution in the cosmetic industry comparatively to the synthetic surfactants [164].

7.6. Environment and Bioremediation

The multi-biotech was used to market biosurfactants for the improved recovery process (a subsidiary of Geodyne Technology). The biosurfactant produced by *Bacillus licheniformis* JF-2 that seems highly helpful in the reduction of the viscosity of heavy crude oil has reported characteristics like anaerobic, halotolerant, and thermal tolerant. Biosurfactants were also attributed to the disappearance of crude oil from an oil tank [165]. Biosurfactants can be used best in every way possible in the pulp and paper, carbon, textiles, ceramics and uranium ore processing industries. Heteropolysaccharide was effectively used in the ceramics processing industry, which was isolated from different sources. Biosurfactants reduce surface tension between aquatic solutions and hydrocarbon mixtures. An analysis of the soil was carried out using 18 strains of actinomycetes and 13 bacterial strains, with high emulsification activity and an oil propagation property compared to the synthetic surfactants. Oil spreading properties were isolated [166]. The selective means to test the emulsifying activity of the isolates from mangrove hydrocarbon-contaminated sediment were sucrose and Arabian light oil. The results finally increased the production of biological agents for the time of incubation. This method is thus ideal in the country with low financial resources for the isolation and production of biosurfactants [167]. Fuel and other oil material leaks lead to severe environmental pollution. The study currently involves diesel degradation on Gram-negative microorganisms. A gravimetric analysis in DJLB isolate is used to detect 65.4–83.12 percent diesel degradation. With an increase in glucose consumption, the rate of diesel degradation and growth of the microbial cell have increased. Thus, both diesel degradation and emulsification activity were increased by glucose added. The increase in the production capacity for biofactants is announced in the diesel degradation property. In diesel degradation, efficiency of Gram-positive bacteria was identified from *Stenotrophomonas* sp. isolates DJLB, respectively [168]. An analysis of the bacteria strain B160 showed that the surface tension was extraordinarily reduced compared to other biostructure products, even under extreme pH conditions, temperature, etc. [169]. In general, the surface-active compounds during their growth phase were

shredded into the surrounding medium [170]. Polycyclic aromatic hydrocarbon (PAH) contamination addresses the serious threat to human beings and the environment and has thus been implemented to meet these requirements with efficient and cost-competitive remediation through the use of PAH. Thus, the bioavailability of PAHs is enhanced by the addition of bioreactors locally or ex situ [171]. The report was published. The production of biosurfactants increases the aqueous naphthalene concentration, thereby increasing the solubilities of the substrate through the production of biofactants [172]. In several industries, the process of the micelle formation of *Pseudomonas aeruginosa* synthesized rhamnolipid has been of greater relevance. Comparatively, less CMC was shown in the neutral dirhamnolipids than the negative. Centrifugation and dynamic light dispersion techniques demonstrate greater aggregate formation at concentrations above CMC [173]. When *Pseudomonas aeruginosa* biosurfactants were investigated, promising algicidal actions were shown against species such as *Heterosigma akashiwo* that causes severe harmful algal blooming (HAB). Other species causing HAB such as *Gymnodinium* sp. were also reported to inhibit *Prorocentrum dentatum* by rhamnolipids. During the prolonged application period of the rhamnolipids, serious algae ultrastructural damage was reported. The cell membrane is mainly integrated so that other cell organelles can be damaged [105]. A highly potential bioremediation product in several sectors was revealed by the biodegradation of diesel-contaminated water and the soil by rhamnolipids from *Pseudomonas aeruginosa* J4 and surfactin from *Bacillus subtilis*, ATCC21332. The highest amounts were achieved under glucose when bio synthesizing activity of bacteria was analyzed under different carbon sources, allowing a good insulation and analytical method for microorganisms in various industries [174,175]. When the biological activity of *Pseudomonas aeruginosa* LBI rhamnolipids is analyzed, exponential interface tension reduction properties, which were still far higher and more environmentally so than their synthetic equivalents, have thus become predominant in different industry sectors [143]. The results show that maximum biosurfactant efficiency was obtained from palm oil use as a carbon source when the biosurfactant synthesis of *Pseudomonas aeruginosa* A41 was investigated under various circumstances. The C18:2 were also reported to be a highly unsaturated fatty acid, the highest activity of oil displacement [176]. An analysis of the treatment of a biosurfactant for treatment of artificially contaminated pyrene soil has shown high surface activity [177]. The use of molecular biological tools has optimized hyper-manufacturing microbial strains for biosurfactant production [178]. In a few instances, there was no correlation between cell characteristics and the carbon sources used. [179]. The report was submitted. In some cases, however, the produced biosurfactant cannot be used during the bioremediation process due to different factors, like *Staphylococcus* sp. [180]. In some cases, however, microbes biosurfactants, isolated from a contaminated site, may use this to treat themselves [181]. After 15 days of *Lactobacillus pentosus* treatment with biosurfactant, 63% of the biodegreasal efficiency of octane by autochthonous microflora in soil is achieved. The application by various biosurfactants (e.g., rhamnolipids, sophorolipids) has shown an increase of 50% in halogenation of halogenated compounds, etc. [142,182]. Phenanthrene degradation of specific hexadecans and their production source has been reported. The Microbially Enhanced Oil Research (MEOR) [183] was a highly potential area of research. Seed germination stimulation, soil removal of motor oil, the biosurfactant produced by *S. marcescens* UCP 1549, and a non-toxic, environmentally friendly compound were also attributable [184].

7.7. Industries

Upon the analysis of the manipueira medium as a substrate for the production of biosurfactants of bacterial isolates, the results showed a significant reduction in the surface tension of the substrate, thus finding a place in various industrial applications as the most suitable alternative media [185]. The advancement and the qualitative analysis of the biosurfactant rhamnolipid over the synthetic surfactant sodium dodecyl sulfate have been reported using a new methodology known as the drop-collapse method. This methodology

proves to be more accurate, advantageous, efficient and cost effective, when compared to other methods for the same processes like du Nouy ring method [186]. Upon the analysis of the rhamnolipid biosurfactant synthesized by *Pseudomonas aeruginosa* LBM10, various significant surface properties have been reported, thus making a promising class of compounds in various industrial applications [187]. The production of biosurfactants by the PAH utilizing bacteria isolated from soil contaminated with petroleum wastes were analyzed. The results revealed the significant production of the biosurfactants under various substrates such as phenanthrene and naphthalene [188]. An anionic monorhamnolipid generated by *Pseudomonas aeruginosa* was tested for its capacity to eliminate residual hexadecane from sand columns. This research identified exploring the influence of low-concentration rhamnolipid on cell transport in a variety of natural soils and resolving the potential mechanisms [189]. Upon the experiments with *Pseudomonas aeruginosa* grown on nitrate and protease peptone media, a direct relation between the enhanced biosurfactant production and glutamine synthetase activity has been reported [14]. A recent analysis revealed the methods for the enhancement of the di-rhamnolipid production over the mono-rhamnolipid, synthesized by the strain *Pseudomonas aeruginosa* J16, thus for the implementation in various industries [190]. A 100% efficiency for the removal of heavy metals from soil and sludge by the biosurfactants has been reported [191]. The hazardous spent hydrodesulfurization (HDS) from the petroleum refineries is treated with various biosurfactants like rhamnolipids, etc., for the analysis of bioleaching, and significant results were obtained [192]. The heavy molecular biosurfactants were attributed with high emulsifying properties, whereas the low molecular biosurfactants were attributed with surface tension reducing properties. The combined effects of the biosurfactants synthesized by *Acidithiobacillus* sp. and *Meyerozyma quilliermondii* have been attributed with significant bioleaching properties against heavy metals such as copper, cadmium, etc. [192].

7.8. Pharmaceuticals

Biosurfactants were used as antibacterial, antifungal, antiviral, adhesive agents, immunomodulatory molecules, vaccines and also in-gene therapy. The adaptive nature by the formation of different surface forms of *Bacillus cereus* ATCC14579 has been analyzed, and its hemolytic activity and Gram-positive bacteria growth inhibition property have been reported [193]. Many characteristic features like anti-tumor properties; self-assembling potential into nanoparticles creates a great interest upon the application of this cyclic lipopeptide biosurfactant [194]. Due to their non-toxic and safe characteristics, biosurfactants were comparatively preferred than synthetic surfactants in the microemulsions drug delivery systems (MDDS). Out of all the biosurfactants, lipopeptide and glycolipids were the most preferable ones [195]. In the process of gene transfection, biosurfactant-based liposomes were highly efficient compared to synthetic liposomes. [32]. The problems in the drug delivery system are well addressed by the microspheres, nanoparticles, micelles, and liposomes [196–199]. In the future, the use of biosurfactants, either alone or in combination with other antibiotic or chemotherapeutic treatments, could provide a viable strategy for combating diseases, biofilm development, and microbial growth. Additionally, new discoveries about biosurfactant's ability to operate as an anticancer, immunomodulating, wound healing, and drug delivery agent have piqued researchers' interest in biosurfactant's ability to perform the aforementioned functions [159]. By utilizing a metagenomic approach, a new gene related to biosurfactant production and hydrocarbon degradation has been reported [200].

8. Critical Micelle Concentration of Various Biosurfactants

In recent years, microbial biosurfactants with low CMC, the potential to minimize surface and interfacial stress and excellent emulsifying activity, coupled with good biodegradability and low toxicity, have drawn extensive attention [201]. Surfactant as an amphiphilic compound may decrease the free interfacial energy to change the interface or surface area at low levels. Surfactant molecules may shape micelles at high concentrations, above

their CMC [202]. Using biosurfactants at concentrations greater than CMC to chemical surfactants helped to decrease the absorption ratio, improve solutions and accelerate PAH degradation [203]. At concentrations above the CMC, the biosurfactants increased the partition rate of fluorine, phenanthrene and pyrene [204]. Phenol can assist in improved solubilization of PAHs by biosurfactant. When phenol is applied to the solution, the biosurfactant CMC reduces due to the development of mixed micelles. The cause for the enhanced dissolution of PAHs could be the combined micelles [205]. A biodegradable, non-toxic biosurfactant, *Pseudomonas frederiksbergensis* has been successfully obtained from piggery waste water. This biosurfactant has been shown to improve the removal of ethylbenzene in BTF. The removal rate of ethylbenzene at the 1000 mg/m³ inlet and of EBRT of 30 s peaked at 87.2 percent with 0.1 CMC dosed biosurfactant. *P. aeruginosa* J4 generates rhamnolipids, which lower water surface tension to 31 mN/m at a critical micelle concentration of about 50 mg/L. For diesel and kerosene, the biosurfactant attained emulsion indexes of 70 and 78 percent, respectively, at about 300 mg/L [206]. Similar findings have been reported in swine waste water, suggesting that it might be an acceptable platform for the production of biosurfactants. At 25 °C, the CMC of the biosurfactant produced from *Bacillus stratosphericus* sp. A15 strain was 46.8 mg/L. In the near future, BS15 might be created as an alternative to antibiotics [207]. Biosurfactants have a molar mass of 500 to 1500 Da, and their effectivity is assessed by CMC values, which typically vary from 1 to 2000 mg. Polymeric micelles have a CMC in the range of 106–107 M, which is about a thousand times lower than the CMC of low molecular weight surfactants, making them thermodynamically very stable even at low concentrations [208]. The CMC level of biosurfactants is typically 1 to 200 mg L⁻¹ [209]. *B. Licheniformis* induces lipopeptide lichenysin. It displays the strongest surface and chelating action, indicating that antimicrobial activity is present in lichenysin. It has an improvement in surface tension in water of approx. 29 mN m⁻¹ and a lower CMC 15 mg L⁻¹ [210]. Similar analysis was reported in *B. Subtilis* RSL 2 strain which developed biosurfactant of 3.5 g/L at pH 4.0, 25°C and used in 7 days for 1 g/L crude petroleum as the only C-source, defined as critical micelle concentration (CMC) lipopeptides of 0.5 g/L. In comparison, the simultaneous feed of 0.5 CMC biosurfactant improved the biodegradation of oil by 72 percent [211]. Tensile CMC provided by *B. subtilis* ATCC21, 332 was recorded as 50 mg L⁻¹ by [206] incorporating CMC reduced from activated carbon to 10 mg L⁻¹. [212] recorded *B. salmalaya* 138SI biosurfactant CMC of 0.4%.

9. Recent Advances

9.1. Influence of Biosurfactant-Producing Bacteria on Compost

Composting is delineated as high microbial activity that results in the degradation of most environmentally friendly resources, mainly organic mixes, and the stability of organic residues. Microbes are extensively used in the degradation of organic matter, because of the extensive use of microbes in degradation in recent decades, biosurfactants were widely used in bioremediation and biodegradation; it is conceivable to use their benefits to increase the compost quality too. Therefore, it is understood that biosurfactants, which are amphipathic microbial compounds, may lower liquid–solid surface tension and increase organic matter bioremediation [213]. Furthermore, boosting bacterial growth in the presence of biosurfactant enhanced organic matter decomposition. The readily metabolized chemicals in the substrates, as well as the sudden rise in enzyme concentration and microbial biomass, contributed to the abrupt decomposition of organic matter. The impact of rhamnolipid and tween 80 on bacterial diversity were analyzed, where the rhamnolipid promotes microbial growth in composting. In the composting process, synergisms of *Bacillus* sp. and *Streptomyces* sp. resulted in a greater rate of breakdown of organic materials. The combination of biosurfactant generating bacteria consortium and biosurfactant containing cell suspension results in a rise in bacterial communities in composting; indicating that biosurfactants did not impede the development of bacteria in composting and even had a minor stimulatory impact on their growth. Biosurfactant producing bacteria can be cultivated in low cost

medium like whey, and the obtained wild bacterial strains will help in speeding up of decomposition of organic matter [214].

9.2. Effects of Biosurfactant on Green Waste Vermicomposting

Vermicomposting has recently gained popularity in green waste disposal. Along with biosurfactant, vermicomposting is proven as a promising technology, as well as an environmentally friendly method used in treating green waste disposal [215]. Microbial inoculation and rhamnolipid inclusion will have hastened the decomposition of organic matter into humic-like compounds, resulting in higher humic acid concentration in the additive treatments [216]. Rhamnolipid will enhance nitrogen concentration by boosting the activities of nitrogen-fixing microbes, as well as quicken the degradation of organic carbon, leading to an increase in combined total nitrogen contents [217]. The substantial and positive relationships between enzymatic interaction and bacteria populations show that increased enzyme activity in vermicomposts was related to increased microbial numbers. The impacts of utilizing the biosurfactant rhamnolipid, the lignolytic and cellulolytic fungus *Phanerochete chrysosporium*, and the free-living nitrogen-fixing bacteria *Azotobacter chroococcum* to vermicomposting green waste using *Eisenia fetida* revealed that the addition of rhamnolipid of a single microorganism or in consortium substantially increased the growth rate. The most efficient vermicomposting approach for green waste management was recommended to combine vermiwash with microbial consortia of *P. chrysosporium* and *A. chroococcum* [218].

9.3. Biosurfactants in Bionanotechnology

The combination of biosurfactants derived from microorganisms and nanoparticle is now regarded as the next generation of green chemistry or bioengineering nanocatalyst sources. The creation of nanoparticles using biosurfactant mediated synthesis has enormous promise for environmental remediation [219]. However, the nanoparticles generated using biosurfactant must be economically viable. Additionally, it must be energy efficient, have a high rate of toxicant elimination, and be environmentally friendly. Microbes in biosurfactants may stabilize and minimize nanoparticle production [205]. Microorganisms have a role in the synthesis of nanoparticles such as gold, silver, and titanium. Among many scientists and researchers, this innovative method of producing metallic nanoparticles utilizing a reducing agent of biosurfactants presents new possibilities. *Brevibacterium casei* MSA19 biosurfactants minimize nanoparticles and enable them to remain stable for around two months by lowering aggregate formation via electrostatic force of attraction, enhancing their use as an eco-friendly material for various products. Furthermore, research on biosurfactant stabilized nanoparticles is still in its infancy [220]. Therefore, additional study is urgently needed to stabilize nanoparticles utilizing biosurfactants before deploying them in nanotechnologies [91].

10. Future of Biosurfactants

Biosurfactants are diverse entities with vast applications of biodegradation and bioremediation to clean up toxic contaminants. They are also used in the dairy, medicinal and other sectors [220]. Such benefits and a wide variety of uses have culminated in continuous interest in biosurfactants. In particular, an increasing debate is taking place regarding environmental protection and the essential position of biosurfactants in the immediate future, for example, utilizing sustainable by-products as substrates, minimizing waste and eventually recycling treated waste. This has contributed to greater exposure being given to these microbial products in manufacturing. The whole surfactants give increased biodegradability and low toxicity to prevent the harmful effects of synthetic surfactants. Amphiphilic, surface active materials are common in nature and are formed by plants, animals and microorganisms, including bacteria and fungi [221]. In complex biofilm systems, biosurfactants are assumed to help the preservation of water and nutrient channels that

are important to the survival and efficient attachment of the colony to the host, so a long driven factor to handle the microbes for the production of synthetic surfactant.

The market performance of biosurfactants is currently constrained by high development costs. Biosurfactant has many benefits over synthetic surfactant (Figure 10). Optimized conditions of growth/production with green substrates that are economically viable and productive downstream will help produce a biosurfactant more sustainable and economically viable. As such, for certain microorganisms, biosurfactants are important for survival, promoting vital processes such as nutrient intake, associations with host surface attachment, and detachment and degradation of competing microbes [222]. Two forms of pollutants are produced when synthetic surfactants are used in industrial processes: by-products of industrial activities and by-products of surfactants. Both are harmful for humans and the atmosphere. In the environment, these toxins are persistent and they are difficult to biodegrade [223]. A significant usage of biosurfactants will need a different database, which would also allow researchers to operate very actively in the field of biosurfactants with regard to their maximum contribution to industry and the ecosystem.

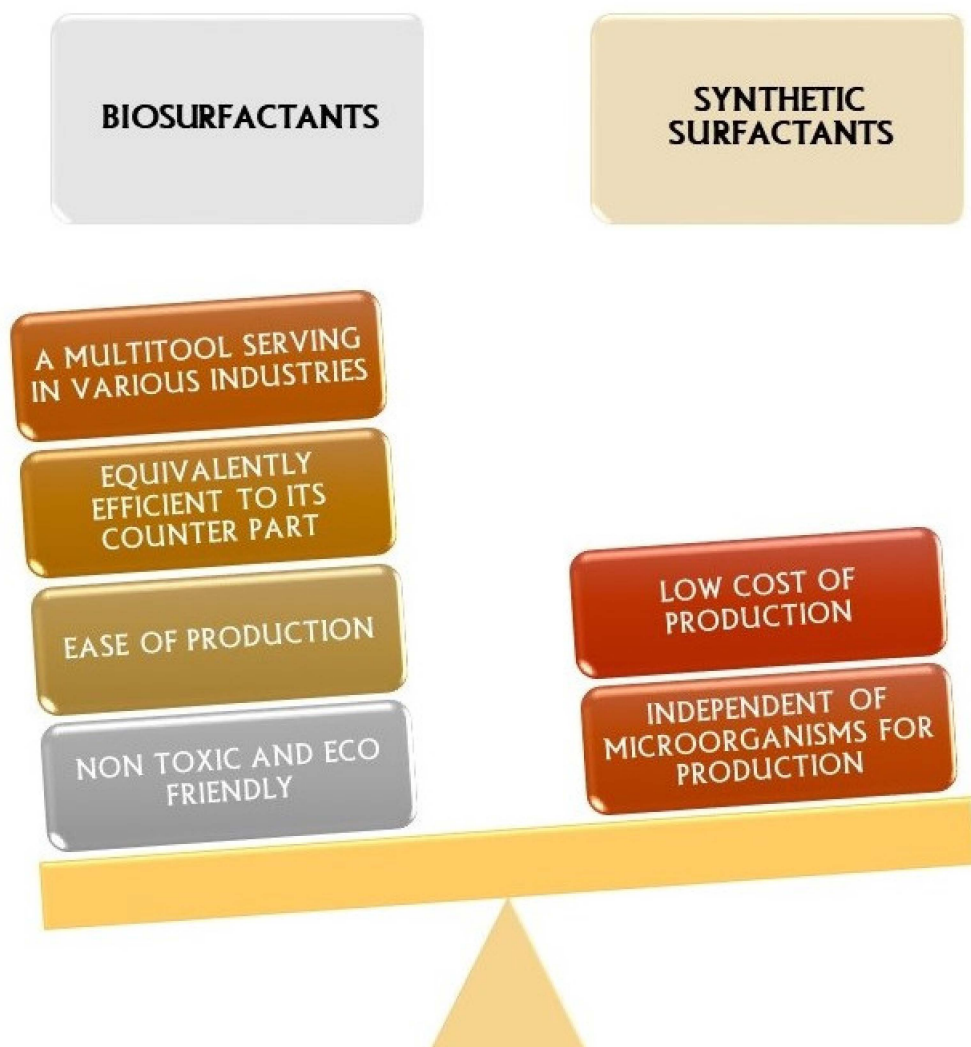


Figure 10. Comparison between biosurfactant and synthetic surfactant.

11. Prospective and Challenges of Biosurfactant Production and Application

- After many years of mediocre interest, biosurfactants have recently risen to the top of many corporations' agendas due to sustainability initiatives and green agendas.
- Biosurfactants may be customized for particular purposes and generated at a cost competitive with chemical surfactants.

- Several concerns must be addressed before large-scale exploitation may occur. The two issues facing rhamnolipids are safety and yield.
- Despite their immune system impacts and virulence factors, rhamnolipids are likely safe to use in many items, especially cleaning and laundry products.
- *P. aeruginosa*, being pathogenic is more challenging, although some companies have clearly overcome it, and the identification of potential new nonpathogenic producer organisms offers a potential solution, provided the products are suitable and yields are acceptable.
- The quorum sensing system controls rhamnolipid synthesis in *P. aeruginosa*, preventing hyperproducing strains from being produced through mutagenesis and selection or genetic manipulation. Inability to get large yields may prevent rhamnolipids from being used in many applications.
- Thus, these do not appear to be any significant barriers to the widespread use of biosurfactants in a variety of goods and applications over the next several years, and we may anticipate a growing range of home items containing at least sophorolipids and MELs on supermarket shelves.

12. Conclusions

The biosurfactant industry is a highly profitable and competitive industry that uses the biodegradation and development benefits of the medicinal, cosmetic, petroleum and food industry for renewable energy substrates. There has been a dramatic increase in the need for surfactants in the world; however, most of the surfactants currently available are chemical dependent. This study clarifies the potential benefits of biosurfactants for adapting their actions in a number of applications. Moreover, this comparison with synthetic surfactants helps to examine how physicochemical properties are affected by the composition of the surfactants and to make appropriate formulation choices. Therefore, we focus on these issues in order to provide a complete picture and perspectives for potential growth and practical applications, through the sources, processes and physicochemical properties of microbial biological factors. This is a description of the use of microbial biosurfactants in accordance with our best experience, because it is largely due to basic simplicity, low cost and widespread use. As the demand for biosurfactants is early on in growth, there are a variety of niche applications where biofactants are used. The fetters found in this sector are technical limitations, mainly their costs and disadvantages in terms of mixing technology. Since understanding of biosurfactant producing strains needs to be extended to include morphology, genetics and biochemistry, virulent strain screening and the advance of process technology will help minimize production costs. Heavy metal remediation by a biosurfactant happens either via complicated interaction with free metal residues or by buildup at a solid–liquid interface, resulting in direct contact between the metal and the biosurfactant. The biosurfactant metal complexes escape the soil surface and form micelles as a result of the desorption pathway. Further precipitation and separation of the biosurfactant from the metals is possible. Organic surfactants constitute a large part of the surfactant industry with stronger controls over greener practices and responses to enormous demand. Farmers are producing environmentally friendly surfactants from numerous natural and renewable sources as they quickly become an attractive option on the market. Using agro-industrial waste from both animal and plant origin to produce biosurfactants might reduce production costs and make biosurfactants economically viable and competitive with synthetic surfactants. Bio-based surfactants are meant to be used to treat heavy metals, polluted soils and water, to treat skin conditions, to enhance oil restoration, to preserve food and to eliminate plant disease. Recent research has shown that using a biosurfactant in the aerobic composting of municipal waste, yard waste, and crop residues enhances composting efficiency and product quality. Rhamnolipids are a kind of biosurfactant that is widely utilized and accessible commercially on vermicomposting of green manures. It is natural to use hereditary structure for the generation of mechanical

biosurfactants using inexhaustible substrates as raw material in the future that super dynamic microbial strains will be created.

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Nomenclature

El ₂₄	Emulsification Index (%)
CMC	Critical Micelle Concentration
Bs	Biosurfactant
BSs	Biosurfactants

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