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Advances in Microbial Biosurfactants: Sustainable Production, Diverse Microorganisms, and Eco-Friendly Substrates

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Abstract

Microbial biosurfactants are eco-friendly alternatives to synthetic surfactants, prized for their biodegradability, low toxicity, and versatile applications in industries like environmental remediation, pharmaceuticals, and food processing. This review explores the diversity of biosurfactant-producing microorganisms, emphasizing key genera such as Pseudomonas, Bacillus, and halophilic bacteria adapted to extreme conditions. It examines major biosurfactant classes—glycolipids, lipopeptides, and polymeric compounds—highlighting their structural and functional properties. The article delves into advanced fermentation strategies, optimization of culture conditions, and cutting-edge metabolic and genetic engineering approaches to boost production yields. Significant focus is placed on using agro-industrial residues and renewable by-products as cost-effective, sustainable substrates, with pretreatment methods like mechanical, chemical, and enzymatic hydrolysis enhancing substrate accessibility. This review underscores the dual benefits of biosurfactant production: promoting environmental sustainability through waste valorization and enabling scalable, green industrial processes. It advocates for integrated strategies combining process optimization, sustainable feedstocks, and life cycle assessments to address production challenges and unlock the transformative potential of biosurfactants for global environmental and industrial solutions.

What is "already known":

- Microbial biosurfactants are biodegradable, low-toxicity alternatives to synthetic surfactants with broad applications in pharmaceuticals, food, and cosmetics.
- *Pseudomonas*, *Bacillus,* and yeasts are key microbial sources producing diverse surfactants.
- Using agro-industrial and food wastes lowers costs and supports large-scale green production.
- Biosurfactants function effectively under extreme conditions (e.g., high salinity or pH), supporting their role in environmental remediation and oil recovery.

What this article adds:

- Comprehensive review of diverse microorganisms, including *Pseudomonas* and *Bacillus, Acinetobacter*, *Rhodococcus*, *Myroides*, *Lactobacillus*, halophilic Bacteria and yeasts for enhanced biosurfactant production.
- Emphasis on sustainable substrates from agro-industrial wastes (e.g., molasses, waste oils, dairy effluents) with detailed pretreatment methods to improve accessibility and yield.
- Overview of integrating advanced strategies such as metabolic/genetic engineering, bioreactor cultivation modes, and co-production with biofuels or enzymes to enable scalable and sustainable processes.
- Inclusion of life cycle assessment insights, highlighting reduced environmental impacts and economic viability through waste valorization and circular economy approaches.

1. Introduction

Biosurfactants are natural compounds with distinctive physicochemical properties and broad applications in industries such as environmental management, pharmaceuticals, and food production [1]. Biosurfactants, produced mainly by microorganisms, are characterized by their low toxicity, biodegradability, and structural diversity, making them sustainable and eco-friendly alternatives to synthetic surfactants [2]. Recent studies highlight their potential roles in drug delivery, antimicrobial formulations, and sustainable bioprocessing [3] and their increasing use in soil and water remediation, heavy metal removal, and circular economy-driven waste valorization [4]. Despite these advantages, industrial-scale production still faces challenges such as high costs and low yields; however, process optimization, metabolic engineering, and bioreactor scale-up strategies are showing significant promise. Moreover, biosurfactants are increasingly recognized as efficient catalysts for addressing emerging pollutants like Per- and Polyfluoroalkyl Substances (PFAS) and microplastics, positioning them at the forefront of sustainable remediation technologies. Their amphiphilic structure, with hydrophilic and hydrophobic domains, underlies key functions such as surface tension reduction, emulsification, and micelle formation, enabling a wide range of industrial and environmental applications [1].

The unique properties of biosurfactants, such as their ability to function effectively under extreme environmental conditions (e.g., high salinity, temperature, or pH), further enhance their appeal for industrial applications [5]. Unlike synthetic surfactants, which often persist in the environment and contribute to ecological harm, biosurfactants offer a biodegradable solution that aligns with global sustainability goals. Their production from renewable resources, such as agricultural waste and industrial by-products, supports the principles of a circular economy, reducing reliance on fossil-based raw materials [6]. Additionally, advancements in genetic engineering and synthetic biology are paving the way for the development of novel biosurfactant-producing microorganisms with enhanced yields and tailored functionalities, addressing some of the scalability challenges [7].

The growing interest in biosurfactants is also driven by their versatility in addressing contemporary environmental challenges. For instance, their ability to emulsify hydrophobic pollutants facilitates the biodegradation of complex contaminants, making them valuable tools in bioremediation strategies [8]. Furthermore, their low environmental footprint and compatibility with green chemistry principles position biosurfactants as key players in the transition toward sustainable industrial processes. As research progresses, interdisciplinary approaches combining microbiology, chemical engineering, and environmental science are expected to unlock new potentials for biosurfactants, expanding their applications and improving their economic viability [9].

This review focuses on microbial biosurfactants, exploring their production, properties, and applications in various industries, with an emphasis on their role in sustainable technologies. By synthesizing recent advancements and future prospects, this article aims to provide a comprehensive overview of the transformative potential of biosurfactants in addressing global environmental and industrial challenges.

2. Classification and Structure of Biosurfactants

Biosurfactants can be classified based on their microbial origin, chemical structure, molecular weight, and mode of secretion. These compounds include low-molecular-weight molecules such as lipopeptides, glycolipids, and phospholipids, as well as high-molecular-weight polymers or particulate forms. The chemical structure of biosurfactants generally consists of a hydrophilic moiety, which may include amino acids, peptides, monosaccharides, disaccharides, or polysaccharides, and a hydrophobic region composed of saturated, unsaturated, or hydroxy fatty acids. The polar region of the molecule can also carry an ionic charge, leading to classification as anionic, cationic, nonionic, or amphoteric [10, 11].

Biosurfactants can be classified based on the mode of secretion from microbial cells into intracellular, extracellular, or cell-bound types. Structurally, they include glycolipids, lipopeptides, phospholipids, lipoproteins, lipopolysaccharides, neutral lipids, or biopolymers. Among these, glycolipids and lipopeptides have gained the most attention due to their high yields and versatile applications; other significant types comprise rhamnolipids, trehalolipids, sophorolipids, fatty acids, and polymeric biosurfactants [12]. A comprehensive review emphasized the industrial applications and production potential of diverse classes such as mannosylerythritol lipids, trehalolipids, sophorolipids, rhamnolipids, and lipopeptides in detergents, soil and water remediation, enhanced oil recovery, agricultural biotechnology, and cosmetic formulations. Recent studies show that sophorolipids, particularly from *Starmerella bombicola*, reduce surface tension to 30–35 mN/m and exhibit thermal and pressure stability, alongside antimicrobial, antiviral, and anticancer properties, making them ideal for eco-friendly products [13].

3. Biosurfactant-Producing Microorganisms

Microorganisms producing biosurfactants are diverse, encompassing yeasts and bacteria with distinct metabolic pathways and functional properties. Their production is influenced by strain, growth conditions, and substrate availability, making microbial selection and cultivation strategies critical for optimizing yield and activity [14]. The main categories of the most common biosurfactants and the microorganisms that produce them are listed in Table 1.

Table 1. The main categories of the most common biosurfactants and the microorganisms that produce them

Class of biosurfactant	Type of biosurfactant	Microorganism	Reference
Glycolipids	Rhamnolipids	Pseudomonasaeruginosa	[15]
	Sophorolipids	Candidabombicola,Candidaantartica, Torulopsispetrophilum,Candida	[16, 17]
	Trehalolipids	Rhodococcuserythropolis,Arthrobacter sp.,Rhodococcusqingshengii,Nocardia erythropolis,Nocardiafarcinica, Crynebacteriumsp.,Mycobacteriumsp	[18, 19]
	Mannosylerythritol lipids	PseudozymaaphidisZJUDM34	[20]
	Cellobiose lipids	Ustilagomaydis,Sporisorium scitamineum	[21]
Lipopeptides	Surfactin	Bacillussubtilis,Bacilluspumilus, Bacillusamyloliquefaciens	[22, 23]
	Subtilisin	Bacillussubtilis,Amyloliquefaciens,B. licheniformis	[24]
Fatty acids, neutral lipids phospholipids	Phospholipids	Acinetobactersp	[25]
Polymeric surfactants	Emulsan	A.calcoaceticus	[26]
	Liposan	Candidalipolytica,C.tropicalis	[27]
	Alasan	A.radioresistens	[28]
Particulate surfactant	Wholecell	Cyanobacteria	[29]
	Vesicles and Fimbriae	Acinetobactercalcoacetius,P. marginilis,P.maltophilia	[29]

3.1. Pseudomonas Species

Pseudomonas species are among the most studied bacterial producers of biosurfactants, particularly due to their ability to synthesize diverse glycolipids and polymeric compounds. A marine *Pseudomonas* strain GU104 demonstrated large-scale biosurfactant production concomitant with quinoline degradation, indicating its dual functionality in pollutant bioremediation and biosurfactant synthesis [30]. *Pseudomonas nautica*, grown on hydrocarbons like heptadecane, produces bioemulsifiers from culture supernatants that effectively facilitate hydrocarbon uptake, highlighting strong extracellular emulsifying capabilities [31]. Reports note that certain marine *Pseudomonas* strains are capable of producing high-molecular-weight polymeric biosurfactants, reflecting their potential for bioemulsifier synthesis [32].

In an early study (1988), *Pseudomonas fluorescens*, a hydrocarbon-degrading bacterium, was found to produce protein-lipid dialkyl trehalose monoglyceride emulsifiers when cultivated on various hydrocarbons and carbon sources [29]. Other notable compounds include lipopeptides such as arthrofactin, viscosin, putisolvin, and amphisin, produced by strains like *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas* spp. DSS73. Rhamnolipids, a type of biosurfactant, are commonly secreted by *Pseudomonas* species and are amphiphilic glycolipid compounds exhibiting surface activity. Rhamnolipids remain the most prominent microbial biosurfactants studied today. Their unique physicochemical properties have made them attractive for applications in bioremediation, detergents, and even food-related formulations where environmentally friendly surfactants are required [12, 33]. Various *Pseudomonas* species generate rhamnolipid-type glycolipid biosurfactants that are recognized for their extensive industrial utility, particularly in processes such as microbial remediation of contaminants and facilitating enhanced oil extraction [34]. Recent optimization studies confirmed that *Pseudomonas aeruginosa* strains can effectively utilize low-cost agro-industrial wastes, significantly enhancing rhamnolipid yield under controlled fermentation strategies [35].

3.2. Bacillus Species

Bacillus species are recognized as major producers of lipopeptide biosurfactants, synthesizing molecules with strong amphiphilic properties and biological activity [36]. Surfactin, the most studied compound, is reported to lower water surface tension to nearly 27 mN/m, confirming its exceptional surfactant capability [37]. In addition to surfactin, other lipopeptides such as fengycin and iturin produced by *Bacillus* strains exhibit remarkable antimicrobial and antifungal activities [29]. Furthermore, the combined action of surfactin, iturin, and fengycin not only enhances antifungal efficacy but also induces systemic resistance in plants, highlighting their role in biocontrol strategies [4].

Bacillus subtilis C9 has been studied for biosurfactant production using carbohydrates as the primary carbon source, while hydrocarbon substrates can simultaneously support biosurfactant synthesis. Lipopeptide biosurfactants produced under these conditions are capable of emulsifying hydrocarbons, crude oils, and vegetable oils. Lipopeptides generated by *Bacillus licheniformis* mutants, obtained through accidental mutagenesis mediated by N-methyl-N,-N-nitrosoguanidine, have been investigated for their biosurfactant properties. Additionally, the production of lipopeptide biosurfactants by *Bacillus* strains has been enhanced using molasses under thermophilic conditions [12].

The biosurfactants synthesized by *Bacillus* species have attracted global interest, not only because of their structural diversity but also due to the Generally Recognized As Safe (GRAS) status of many *Bacillus* strains. This safety designation enhances their potential for applications in the food, pharmaceutical, and cosmetic industries, where non-toxic and biodegradable surfactants are highly desirable [29, 37].

3.3. *Acinetobacter* Species

Several *Acinetobacter* species are known to produce high-molecular-weight biosurfactants, such as emulsan and alasan. Emulsan RAG-1, synthesized by *Acinetobacter calcoaceticus*, is a protein-lipid–polysaccharide complex with remarkable emulsifying capacity. Its polysaccharide fraction, apoemulsan, contains diverse sugar residues including D-galactosaminuronic acid, D-galactosamine, and diamino-dideoxy glucosamine, while fatty acids (approximately 12% of the composition) contribute to its amphipathic character. The heptasaccharide repeating units of *A. calcoaceticus* emulsan BD4 are composed of L-rhamnose, D-glucuronic acid, D-glucose, and D-mannose in a molar ratio of 4:1:1:3 [38]. Alasan is an anionic heteropolysaccharide–protein complex rich in alanine. These biosurfactants are notable for their strong emulsification activity and structural stability, making them highly relevant for industrial processes such as petroleum recovery, wastewater treatment, and even applications in the food sector where robust emulsifiers are required [31, 33]. Certain *Acinetobacter* species produce these protein–lipid–polysaccharide complexes with intrinsic amphipathic properties, conferring excellent emulsifying activity suitable for environmental and industrial applications [39].

3.4. *Rhodococcus* Species

Members of the genus Rhodococcus are distinguished by their ability to synthesize glycolipid-type biosurfactants, most notably trehalose-based lipids. These compounds are structurally diverse and contribute significantly to the surface activity and emulsifying potential of the strains. For instance, the oil-degrading strain *Rhodococcus erythropolis* 3C9, isolated from coastal soil on Xiamen Island (Taiwan Strait), was shown to produce glycolipids, polysaccharides, free fatty acids, and trehalose dicorynomycolates [29].

The broad metabolic versatility of *Rhodococcus* species enables them to thrive in hydrocarbon-rich environments, making them valuable candidates for bioremediation and crude oil degradation. Furthermore, their trehalose lipids exhibit strong emulsifying activity and high environmental stability, which enhance their potential applications in petroleum recovery, wastewater treatment, and even environmentally friendly formulations in food and cosmetic industries [19, 40].

3.5. *Myroides* Species

Myroides species are rod-shaped, non-motile, aerobic, Gram-negative bacteria commonly found in marine environments. They produce extracellular bioemulsifiers composed of L-ornithine-derived lipids and a unique combination of iso-3-hydroxy and iso-fatty acids. These compounds exhibit strong surface activity, enabling efficient emulsification of aged crude oil. The bioemulsifiers from *Myroides* demonstrate high stability across a wide temperature range; however, their emulsifying capacity decreases under high salt concentrations and extreme pH conditions. By adhering to weathered crude oil, cell-associated surface-active compounds effectively enhance emulsification, making these bacteria valuable candidates for bioremediation in marine and oil-contaminated environments [29, 41]. *Myroides* sp. SM1, a marine bacterium isolated from oil-contaminated seawater, produces extracellular bioemulsifiers. These bioemulsifiers demonstrate effective emulsification of weathered crude oil and exhibit remarkable stability across a wide temperature range, from 30°C to 121°C [42].

3.6. *Lactobacillus* Strains

Several *Lactobacillus* strains have been screened for their ability to produce biosurfactants. These strains, maintained in standard culture collections and grown under controlled conditions, produce surface-active compounds that are non-toxic and GRAS, making them particularly suitable for food and pharmaceutical applications. *Lactobacillus* strains, commonly used as probiotics, can be isolated from various sources such as fermented foods and the gastrointestinal tracts of humans and animals [43, 44].

Biosurfactants from *Lactobacillus* demonstrate emulsifying properties and surface activity, contributing to their potential use as natural stabilizers, emulsifiers, and antimicrobial agents in food products. The strains are typically stored in De Man, Rogosa, and Sharpe (MRS) medium with glycerol at -80 °C and are precultured under controlled conditions to ensure optimal production. Their biosurfactants can serve as emulsifiers, natural stabilizers, and functional additives in various industrial formulations [45].

3.7. Halophilic Bacteria

Halophiles, or salt-loving microorganisms, require high concentrations of salt for growth and have evolved unique physiological and genetic mechanisms to survive in hypersaline ecosystems [12]. Based on salinity tolerance, they are categorized into mild, moderate, and extreme halophiles, with many species colonizing salt lakes, soils, and marine salterns [32]. Aerobic, anaerobic, and facultative anaerobic halophiles have been reported from environments such as the Dead Sea, Antarctic saline lakes, and Lake Magadi, reflecting their broad ecological distribution [41]. Members of the Halomonadaceae, especially *Halomonas*, are prolific extracellular polysaccharide (EPS) producers, and strains like *H. maura* S-30 and *H. rifensis* demonstrate diverse polymer structures with potential biosurfactant applications [46].

Halomonas species are primarily recognized for their ability to produce EPS with strong emulsifying properties. Certain strains, such as *Halomonas* ANT-3b, isolated from the sea-ice interface and seawater at Terra Nova Bay Station, Antarctica, also produce glycolipid-based bioemulsifiers. These bioemulsifiers, composed of proteins and uronic acids, exhibit remarkable surface activity and functional stability under low temperatures and high salinity conditions [29, 32].

The robust nature of *Halomonas* biosurfactants makes them particularly suitable for applications in extreme environments, including marine biotechnology, bioremediation of oil-contaminated cold regions, and as potential ingredients in environmentally friendly food and cosmetic formulations [32, 46].

3.8. Yeasts

In studies related to biosurfactants, yeasts have attracted more attention than bacteria. This preference is mainly due to the pathogenic potential of many bacteria, which limits the application of bacterial biosurfactants in the cosmetic, pharmaceutical, and food industries. Moreover, bacteria often produce biosurfactants at lower concentrations. Some yeasts are also classified as GRAS, meaning they pose minimal toxicity or pathogenicity, which enables broader industrial applications [47].

Several yeast species such as *Candida sphaerica*, *Starmerella bombicola*, and *Saccharomyces cerevisiae* produce biosurfactants with potent emulsifying, antimicrobial, and antioxidant properties [47]. Sophorolipids synthesized by *Starmerella bombicola* exhibit high emulsifying capacity and low toxicity, making them valuable for food, pharmaceutical, and cosmetic industries [48]. Recent studies highlight *Candida bombicola* as a promising yeast capable of large-scale biosurfactant production from low-cost substrates, with applications in bioremediation and industrial formulations [47]. Biosurfactants derived from *Lactobacillus* and yeast strains are increasingly explored as multifunctional agents, offering stability and bioactivity across diverse environmental and biomedical applications [13].

Yeasts can grow on water-insoluble substrates, such as vegetable oils and hydrocarbons, as well as water-soluble compounds like carbohydrates and glycerol. Culture conditions and medium composition directly influence the production and composition of biosurfactants. These factors also affect the survival and growth of the producing microorganisms and enhance the uptake of water-insoluble compounds. The biosurfactant produced by *Candida bombicola* exhibits attractive properties, including antimicrobial activity, inhibition of pathogenic microorganisms, and low cytotoxicity. Its production can be achieved by adding sugarcane molasses, frying oil, and corn alcohol to the culture medium, resulting in the synthesis of sophorolipids with satisfactory yields (~40 g/L) [47].

Yeasts are a heterogeneous group of unicellular fungi. Many yeasts are GRAS. The GRAS status allows the extensive use of a living organism or its metabolites in food or feed, thus enabling microbial biomass to serve as a valuable byproduct and reducing environmental impact. The baker's yeast *Saccharomyces cerevisiae* is a typical GRAS yeast approved by the U.S. Food and Drug Administration. *Saccharomyces* yeasts have also been extensively studied in the food sector. Research dating back to the 1980s reported the use of intracellular compounds with emulsifying activity extracted from *Saccharomyces* species, such as *S. cerevisiae*, enabling recovery of mannanoproteins from cell walls with good thermal stability. Studies have also described biosurfactants from *S. lipolytica*, capable of emulsifying and stabilizing oil-in-water emulsions in both vegetable and mineral oils. Among these yeasts, *S. cerevisiae* is one of the most widely used strains for ethanol production, fermented beverages, and baking, making it an industrially important yeast. Efforts have also focused on directing its cultures toward intracellular biosurfactant production to inhibit pathogenic microorganisms. Extracellular biosurfactants from this yeast have been shown to display similar properties when produced in media using olive oil as a carbon source [48].

When glucose and oil are used as carbon sources under optimized culture conditions, improvements in biomass yield and emulsifying activity are observed. Recent experiments with *S. cerevisiae* aimed to identify strategies for producing extracellular biosurfactants with maximum yield in the shortest time while facilitating scalability and reducing downstream processing costs, such as cell disruption. Culturing this yeast in media containing olive oil and ammonium nitrate, or solely on industrial residues and byproducts, produces biosurfactants with optimal surface tension and emulsifying properties, defined as glycolipids (~80% lipids and 20% carbohydrates) with a critical micelle concentration (CMC) of 0.8 g/L. These results were achieved when the yeast grew on residues [48].

The utilization of agro-industrial waste as a substrate for biosurfactant production by *Saccharomyces cerevisiae* URM 6670 offers economic benefits by lowering raw material expenses [49]. Cultivation of *Starmerella bombicola* on renewable feedstocks enables large-scale sophorolipid production with reduced environmental impact, supporting its GRAS designation and industrial relevance [50].

Additionally, several yeasts are considered emerging GRAS producers, including *S. bombicola*, which has a long history in food technology and poses no known threat to human health or the environment. Currently, *S. bombicola*, one of the best-known biosurfactant-producing yeasts, is assumed to possess all characteristics necessary for GRAS recognition. In contrast, many bacterial biosurfactant producers (e.g., *Pseudomonas aeruginosa*, *Escherichia coli*) represent potential hazards due to pathogenic strains in these species [48]. Exploring yeast strains as GRAS-certified producers broadens their applicability, with biosurfactants offering stability, emulsification, and bioactivity superior to those produced by pathogenic bacterial strains [11].

4. Microorganisms and growing media

According to the literature, diverse microorganisms including *Pseudomonas*, *Bacillus*, *Candida*, *Rhodococcus*, and *Corynebacterium* are employed for biosurfactant production. Among these, *Pseudomonas aeruginosa* was the most frequently used species for rhamnolipid synthesis in studies conducted between 2015 and 2021. Due to growing market demand, biotechnological strategies are increasingly applied to elucidate biosynthetic pathways and to develop hyperproducing or recombinant strains. For example, a mutant strain of *Starmerella bombicola* was shown to produce bolaform sophorolipids with enhanced stability at high pH, broadening their biomedical potential in drug delivery. Recent advances highlight that genetic engineering and systems biology approaches can significantly increase biosurfactant titers in laboratory and pilot-scale bioprocesses [43].
Microorganisms utilize a wide variety of carbon sources for biosurfactant synthesis, with glucose and glycerol being the most common. However, the use of glucose, a food-derived feedstock, raises production costs, while complex substrates may introduce impurities; for instance, fermentation with kerosene by *Serratia* sp. yielded non-cytotoxic biosurfactants but with high downstream impurities. Studies indicate that feedstock type and medium composition strongly influence not only yield but also the structural diversity of biosurfactants [50]. Production media are frequently supplemented with yeast extract or mineral salts (Na, Mg, K, Ca, Fe, etc.), with mineral-based formulations being predominant in ~40% of cases. To reduce costs, agro-industrial wastes and low-value byproducts have emerged as sustainable alternatives for sophorolipid and rhamnolipid production [51]. Recent studies have focused on enhancing production efficiency through optimization of growth conditions and substrate utilization. Optimization of such strategies is central to aligning biosurfactant production with industrial and environmental sustainability [52, 53].

5. Biosurfactant Production Pathways

The biosynthesis of biosurfactants can proceed through four main pathways: (a) concurrent synthesis of both carbohydrate and lipid components; (b) synthesis of the carbohydrate component, while lipid formation varies depending on the carbon-chain length of the substrate present in the medium; (c) synthesis of the lipid component, with carbohydrate synthesis determined by the substrate utilized; and (d) synthesis of both components, each being influenced by the type of substrate. As a result, the chemical structure of the carbon source employed during microbial fermentation plays a critical role in shaping biosurfactant production [6, 54, 55].
Different metabolic routes, governed by the primary carbon source in the cultivation medium, contribute to the synthesis of precursor molecules internally utilized for biosurfactant production [6, 55]. This substrate dependency is particularly relevant in industrial fermentation processes, including those applied in food and chemical engineering, where the selection of low-cost and renewable carbon sources can significantly influence yield and production efficiency [43].

6. Substrates for Biosurfactant Production

The use of various industrial by-products and wastes derived from agro-industrial or industrial processes for biosurfactant production represents a cost-effective strategy for both waste management and sustainable manufacturing. Industrial biosurfactant production can exploit resources from sectors such as the petroleum industry, starch from sugarcane or syrup processing, by-products of the sugar industry, fruit and vegetable processing, distilleries, and slaughterhouse animal fats. In this way, biosurfactants can be produced by utilizing industrial wastes. Biosurfactant yield can be further improved by employing alternative substrates such as agro-industrial by-products, peat hydrolysate, and urban waste. Transesterification of fatty acid methyl esters and reactions with various amines have also been applied to generate amide surfactants [12, 52, 56].

Over the past decade, the demand for low-cost materials that can serve as substrates for biosurfactant production has increased. Various wastes derived from renewable and inexpensive industries are being investigated to assess their potential as substrates for biosurfactant synthesis. These wastes include agro-industrial residues and food wastes. Utilizing waste as a substrate not only enhances the economic feasibility of the process but also contributes to the efficient management of rapidly generated waste. However, in addition to cost-effectiveness, other factors such as sustainability, type, and quantity of the material must also be considered when selecting an appropriate substrate for biosurfactant production [56].

Studies have shown that the integration of waste valorization with biosurfactant production reduces environmental burdens while providing added economic value [52]. Using such wastes not only supports circular bioeconomy approaches but also addresses the challenge of increasing waste generation. Recent reviews emphasize that the composition and pretreatment of agro-industrial residues significantly influence microbial growth and product yield [50]. Thorough evaluation of parameters like substrate type, cultivation strategy, and reactor design plays a key role in establishing biosurfactant production systems that are both efficient and sustainable [52, 56].

7. Pre-treatment of Biosurfactant Production Substrates

The use of biologically derived substrates for eco-friendly biosurfactant production requires careful pre-treatment to enhance microbial accessibility and substrate utilization. Pre-treatment typically begins with size reduction using mechanical devices such as hammer mills and drum mills, or physical methods like ultrasonication and liquid ammonia treatment. These steps increase surface area, porosity, and enzyme contact points, making substrates more amenable to microbial hydrolysis [50].

Following size reduction, substrates undergo hydrolysis, which can be chemical or enzymatic. Chemical hydrolysis involves acidic treatments using mineral acids (e.g., HCl, H_2SO_4) or alkaline treatments with hydroxides (e.g., NaOH, KOH), releasing fermentable sugars while controlling degradation. Enzymatic hydrolysis, using enzymes such as β-glucosidase, provides a milder alternative suitable for delicate substrates like bagasse. After hydrolysis, substrates are dried and incorporated into microbial growth media, serving as the primary sugar source to support microbial growth and subsequent biosurfactant production [50].

The specific pretreatment strategy, individual steps or sequential combination, depends on substrate type and aims to maximize monosaccharide availability while minimizing inhibitory compounds. Proper pre-treatment directly influences biosurfactant yield, process efficiency, and overall production cost. Pretreatment efficiency largely determines sugar release and has a direct impact on downstream biosurfactant productivity. Substrates are subsequently dried and incorporated into growth media to provide fermentable sugars. Among the available methods, enzymatic hydrolysis is preferred for its specificity and reduced generation of inhibitory by-products, though it requires higher costs for enzymes. Effective pretreatment is thus critical for enhancing yield, efficiency, and economic feasibility in biosurfactant production [50, 57, 58].

8. Types of Substrates Used for Biosurfactant Production

Environmental concerns and high costs have driven interest in using Agro-industrial residues, waste oils, and by-products as low-cost substrates for biosurfactant production. These sustainable feedstocks not only support cost-effective biosurfactant production but also contribute to circular bioeconomy strategies. Ideal substrates contain sufficient carbohydrates and lipids to support microbial growth and metabolite synthesis. Utilizing these wastes not only reduces production costs but also supports large-scale industrial biosurfactant synthesis through process optimization [43, 51, 59]. Figure 1 illustrates various types of substrates commonly utilized for the microbial production of biosurfactants.

Figure 1. Major categories of substrates employed in biosurfactant production [43, 51].

8.1 Agricultural Wastes

Agricultural residues such as sugarcane bagasse and corn cobs are sustainable substrates for biosurfactant production. Lignocellulosic residues like rice husk and wheat straw provide cellulose, hemicellulose, and lignin, serving as valuable feedstocks for microbial biosurfactant production. Using these residues promotes waste valorization, reduces environmental impact, and supports circular economy initiatives. Selection of appropriate waste types and microorganisms is crucial for high yields [51].

8.2 Fruit and Vegetable

Waste peels and residues from apple, banana, orange, carrot, and cashew apple provide cost-effective substrates. For example: Cashew apple juice supported rhamnolipid production by *Pseudomonas aeruginosa*, reducing surface tension by 29.5 mN/m. Banana peel was used for lipopeptide synthesis by Halobacteriaceae archaeon. Other residues like carrot, lemon, and orange peels have also successfully produced rhamnolipids [43].

8.3 Plant-Based Industry By-Products

Vegetable oils (e.g., olive, rapeseed, corn, sunflower, soybean) and refinery residues are rich in fatty acids, glycerides, and vitamins. Microorganisms like *Pseudomonas aeruginosa*, *Candida lipolytica*, and *Candida sphaerica* can convert these wastes into biosurfactants with high surface activity and stability [51].

8.4 Sugar Industry Wastes

Molasses and other sugar processing wastes (e.g., tapioca, corn, potato peel powder) are rich in sugars, vitamins, and minerals. Molasses reduces pre-treatment needs and supports biosurfactant synthesis by *Bacillus* and *Pseudomonas* species. These biosurfactants have applications in bioremediation, oil recovery, and pollutant degradation [51].

8.5. Dairy Industry Wastes

Buttermilk, and milk effluents are rich in lactose, proteins, and lipids, supporting microbial growth and biosurfactant production. Whey contains lactose, proteins, and vitamins, which makes it suitable for *Bacillus* and *Pseudomonas* species. Using these wastes reduces environmental pollution and provides a cost-effective substrate for industrial applications [43].

8.6. Olive Mill Effluent (OME)

OME contains nitrogen, sugars, residual oils, and polyphenols, making it a challenging waste for disposal but a promising substrate for rhamnolipid production by *Pseudomonas* species. Similar lipid-rich wastes like vegetable oils and soybean oil have been used for producing biosurfactants with antimicrobial and emulsifying properties [51].

8.7. Waste Cooking and Frying Oils (WCO)

Used oils from households and the food industry are rich in triglycerides and fatty acids, suitable for biosurfactant synthesis. *Pseudomonas aeruginosa*, *Candida lipolytica*, *Candida bombicola*, and other microbes can convert WCO into sophorolipids and rhamnolipids, supporting circular bioeconomy strategies [43].

8.8. Animal Fats

By-products like pork fat can serve as substrates for sophorolipid production by *Candida bombicola* and *Candida lipolytica*, often combined with nutrient-rich by-products like corn steep liquor [51].

8.9. Soapstock

Residues from oilseed refining can enhance rhamnolipid and heteropolysaccharide production when used with *Pseudomonas aeruginosa* or *Acinetobacter calcoaceticus*, providing additional bioactive compounds [43].

8.10. Starch-Rich Wastes

Starch-rich wastes from potato, cassava, and cereal processing can serve as renewable carbon sources for biosurfactant synthesis. Pretreatment may be required to improve microbial accessibility [43].

9. Process Parameters Involved in Microbial Fermentation

Microbial biosurfactants provide multiple advantages such as rapid production, multifunctionality, environmental compatibility, and scalability, making them more appealing than many chemical or biological surfactants. Although research on microbial biosurfactants began decades ago, their industrial importance has grown markedly in the past years, driven by increasing demand for eco-friendly alternatives. Various microorganisms, including bacteria, fungi, and yeasts, have been engineered for biosurfactant production [59].

A critical consideration is that during microbial culture growth, a range of internal and external dynamics, the type of substrate, media supplements, and microbial species all influence the quantity and quality of the produced biosurfactants. One popular technique for producing biosurfactants as metabolic by-products is microbial fermentation, which involves the growth of microorganisms in a controlled medium. The entire production process, from microorganism selection to product identification and scale-up, is carefully managed [59].

To prepare an inoculum, a small portion of a well-characterized stock culture is transferred to a fresh growth medium. Depending on the type of biosurfactant, the process begins with the selection of commonly used microbial strains such as *Pseudomonas* spp., *Bacillus* spp., and *Candida* spp. After proper monitoring and control, the inoculum is allowed to develop and adapt to the medium, followed by the preparation of the fermentation medium and subsequent extraction of the biosurfactant. When larger fermenters or bioreactors are required, the production process can be scaled up. For industrial applications, specific parameters and conditions of microbial fermentation vary depending on the microorganism and the type of biosurfactant produced. Achieving high yield and quality requires careful optimization. Furthermore, adherence to safety and regulatory requirements during the production process is of paramount importance [59]. Genetic engineering techniques enhance both yield and distinct properties of these compounds. Moreover, biosurfactants are among biotechnology's most adaptable products, capable of constitutive or inducible production. Their process economics improve significantly when renewable, low-cost raw materials such as agro-industrial waste are used for large-scale synthesis [59]. Submerged systems, especially stirred bioreactors, are preferred because they enable strict control of key parameters such as nutrient supply, oxygenation, pH, and temperature, thereby improving reproducibility and facilitating scale-up for industrial applications. Downstream processes include extraction, purification, and characterization of biosurfactants to ensure product stability and performance across different applications. For large-scale commercialization, optimization of culture strategies, careful scale-up in bioreactors, and compliance with regulatory and safety requirements remain essential [59].

10. Factors Affecting Biosurfactant Production

Biosurfactants are surface-active compounds produced by microorganisms which are gaining attention due to their eco-friendly properties and diverse industrial applications. The production of biosurfactants, especially rhamnolipids, is influenced by various environmental and nutritional factors, including nitrogen and carbon sources, aeration, agitation, salt concentration, temperature, pH, agitation speed, and oxygen availability [30, 57].

10.1. Nitrogen Sources

Nitrogen is essential for microbial growth and biosurfactant production. The type and concentration of nitrogen sources significantly impact the yield and composition of biosurfactants. Inorganic nitrogen sources such as sodium nitrate and ammonium sulfate are commonly used. Studies have shown that sodium nitrate enhances biosurfactant yields in *Pseudomonas aeruginosa*. Additionally, nitrogen limitation can trigger higher biosurfactant production by inducing the stationary phase, which favors secondary metabolite synthesis [35, 60].

10.2. Carbon Sources

Microbial growth and biosurfactant production primarily depend on carbon sources, which provide essential energy and carbon. Various substrates, such as glucose, glycerol, and hydrocarbons, have been utilized. Among these, glycerol is particularly effective for *Pseudomonas aeruginosa*, leading to significant biosurfactant yields. The concentration of carbon sources influences production: higher concentrations generally enhance yields up to a certain threshold, beyond which no further increase occurs [61, 62].

10.3. Aeration and Agitation

Adequate aeration and agitation are crucial to provide oxygen, which is essential for microbial metabolism and biosurfactant production. Optimal aeration rates and agitation speeds enhance oxygen transfer, leading to improved microbial growth and biosurfactant synthesis. Studies demonstrate that increased aeration and agitation significantly improve biosurfactant yields by promoting better nutrient mixing and oxygen availability in the culture medium [38]. Increasing agitation speed can enhance biosurfactant accumulation, as seen in *P. aeruginosa* UCP 0992 grown in glycerol medium. Similarly, *P. alcaligenes* cultured in palm oil showed that increased rotation speed led to a decrease in cell-free surface tension to 27.6 mN/m. However, agitation negatively affected surface tension reduction when using biosurfactants from *Serratia* sp. SVGG16 in hydrocarbon-based media [52].

10.4. Salt Concentration

Salt presence in the culture medium can influence biosurfactant production. Some biosurfactants are stable in saline environments, making them useful for marine and industrial applications. However, excessive salinity can inhibit microbial growth and reduce biosurfactant synthesis. Maintaining an optimal salt concentration is therefore crucial [30].

10.5. Temperature

Temperature affects enzymatic activity, metabolic rates, and overall microbial growth. Most biosurfactant-producing microorganisms, including *Pseudomonas aeruginosa*, are mesophilic, thriving at 25-37°C. Deviations from this range can reduce biosurfactant production due to decreased microbial activity or enzyme denaturation [57]. The most favorable temperature for biosurfactant production by various fungi is generally around 30 °C, observed for species such as *Candida* sp. SY16, *C. bombicola*, *C. batistae*, and *Trichosporon bombicola*. For C. *lipolytica*, 27 °C is reported as the optimal temperature [52].

10.6. pH

The pH of the culture medium also influences microbial metabolism and biosurfactant synthesis. *Pseudomonas aeruginosa* produces higher biosurfactant amounts at neutral to slightly alkaline pH. Extreme pH conditions can negatively affect growth and production, so maintaining an optimal pH is important for efficiency [60]. *Candida* species produce maximum biosurfactant yield across a wide range of pH values; for instance, pH 5.7 for *C. glabrata* UCP 1002, pH 7.8 for *Candida* sp. SY16, pH 5 for *C. lipolytica*, and pH 6 for *C. batistae*. Additionally, *Pichia anomala* and *Aspergillus ustus* achieve optimal biosurfactant yields at pH 5.5 and 7, respectively [52].

10.7. Incubation time

Incubation time also significantly impacts biosurfactant production, as microorganisms produce biosurfactants over different periods. Maximum biosurfactant production by *Aspergillus ustus* was observed after 5 days of incubation, while the incubation periods for *C. bombicola* were 7, 8, and 11 days [52].

11. Bioreactor Cultivation Strategies

Biosurfactant production in bioreactors involves selecting the optimal cultivation mode, such as batch, continuous, or semi-continuous, tailored to the microorganism and bioreactor design, with process parameters such as aeration and substrate concentration critically influencing yield. For instance, using a central composite design in *Aureobasidium pullulans* LB 83, the effects of aeration (0.1–1.1 vvm) and sucrose (20–80 g/L) were quantified, achieving peak oil-spreading activity and productivity under optimized conditions. Different bioreactor configurations beyond standard batch or continuous modes, such as membrane-based, immobilized-cell, and air-lift reactors, offer improvements in mass and heat transfer, lower shear stress, and help mitigate operational challenges like foaming and oxygen limitation [33]. By employing a semicontinuous bioreactor setup with molasses as the exclusive carbon source, the exponential growth period of *Bacillus subtilis* could be extended, leading to more efficient substrate consumption and higher biosurfactant yields [63].

12. Biosurfactant Extraction, Purification, and Characterization

Biosurfactants are commonly extracted from cell-free supernatants via solvent-based methods that include centrifugation, acidification, and organic solvent extraction, followed by purification steps such as chromatography and lyophilization. Structural and functional analysis typically employs Fourier Transform Infrared Spectroscopy (FT-IR) and (Nuclear Magnetic Resonance Spectroscopy) NMR to reveal chemical groups and molecular composition, with Folch's method widely used for lipid-based biosurfactants [12]. Chromatographic separation, particularly High Performance Liquid Chromatography (HPLC) coupled with mass spectrometry, enables accurate quantification and profiling of biosurfactant congeners, ensuring high analytical resolution [33]. Spectroscopic tools such as FT-IR, NMR, and Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) provide complementary insights, allowing precise identification of functional moieties and structural diversity of microbial biosurfactants [29]. The choice of purification technique strongly influences product recovery and downstream costs, with silica-based adsorption and preparative chromatography being particularly effective for rhamnolipid and sophorolipid isolation [43].

13. Co-Production with Other Products

In integrated biorefinery systems, biosurfactants can be co-produced with other high-value products such as biofuels, enzymes, polyhydroxyalkanoates (PHAs), or organic acids, thereby enhancing resource efficiency and contributing to the overall sustainability of the process. Recent research has shown that the co-production of biosurfactants with biofuels not only improves process economics but also reduces waste generation through the valorization of agro-industrial residues [43, 64].

14. Life Cycle Assessment (LCA) of Biosurfactants

Life cycle assessment (LCA) is a standardized and widely applied methodology for assessing the environmental impacts of products and processes across their full life cycle. Applying LCA to biosurfactants provides critical insights into cumulative energy demand (CED), greenhouse gas emissions, and other ecological indicators, thereby supporting the development of sustainable bioprocesses [65].

A recent prospective LCA (pLCA) on sophorolipid production from waste cooking oil combined with in situ separation strategies demonstrated a reduction in environmental impacts of up to 50% compared with conventional feedstock and batch fermentation routes [66]. Energy consumption was consistently identified as the dominant hotspot, contributing to more than 80% of total life cycle impacts, highlighting the urgent need for energy-efficient process designs [67].

Furthermore, comparative studies revealed that mannosylerythritol lipids, especially when derived from sugar industry residues, exhibit lower global warming potential (GWP) and reduced environmental burdens compared with rhamnolipids. This is attributed to favorable microbial performance and optimized fermentation processes [67].

Despite these advances, biosurfactants still present a relatively conservative environmental profile, as their GWP (3.7–17 kg CO_2-eq/kg) remains higher than that of conventional surfactants (0.9–1.6 kg CO_2-eq/kg). Variations in reported results are largely linked to feedstock selection, process scale, and methodological boundaries in LCA studies. Thus, large-scale industrial biosurfactant production still faces challenges and bottlenecks that must be addressed to ensure competitiveness and sustainability [67].

15. Conclusion

Microbial biosurfactants represent a transformative class of eco-friendly biomolecules, combining superior physicochemical properties—such as surface tension reduction, emulsification, and micelle formation—with low toxicity and biodegradability. Beyond well-established producers like *Bacillus* and *Pseudomonas*, emerging genera such as *Burkholderia, Serratia*, *Klebsiella*, *Pseudozyma*, and *Fusarium* offer novel biosurfactant production potential. Rhamnolipids and sophorolipids dominate industrial applications, with innovative compounds like aneurinifactin, ponctifactin, lichenysin-A, and friulimicin-B expanding their scope. These biosurfactants drive advancements in petroleum, healthcare, cosmetics, detergents, agriculture, environmental management, and food industries. Despite their promise, high production costs remain a barrier to replacing synthetic surfactants on a large scale. However, leveraging novel microbial strains, cost-effective substrates like agro-industrial residues, and optimized bioreactor systems offers significant potential for scalability. Integrated strategies combining advanced fermentation, genetic engineering, and life cycle assessments are critical to overcoming economic and technical challenges, positioning biosurfactants as sustainable solutions for a greener future.

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