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# Optimization Strategies Towards Enhanced Production of Microbial Surfactants for Diverse Industrial Applications: A Futuristic Approach

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**Abstract**

*Chemical or synthetic surfactants being employed in numerous industries pose significant environmental hazards due to their persistence in the environment and potential toxicity to aquatic life. Biosurfactants are low-molecular-weight surface-active compounds that offer a promising alternative to surfactants owing to their less toxicity, biodegradability and biocompatibility nature. They are derived from a variety of natural sources, including bacteria, fungi, and plants. In recent years, there has been a surge in interest in producing biosurfactants using alternative substrates, such as different types of organic waste including food processing waste, animal fats and oils and agricultural residues. This emerging trend of utilizing alternative substrates for biosurfactant production has not only broadened their potential applications but has also sparked significant research interest across various sectors. The exploration of new sources for biosurfactants has led to innovative solutions in industries such as bioremediation, food processing, pharmaceuticals, and detergents, highlighting the versatile nature and growing importance of biosurfactants in sustainable development. In bioremediation, they enhance the solubility of pollutants, aiding in their removal from soil and water. In food processing, biosurfactants act as emulsifiers and foaming agents, improving the texture and stability of food products. Additionally, their antimicrobial properties make them valuable as therapeutic agents, while in the detergent industry, they serve as effective cleaning agents with low environmental impact. The present review undertakes a discussion on methods for isolating and characterizing microbial biosurfactants, optimizing microenvironment conditions for enhanced production, and exploring their applicability in various industries. By further researching and implementing biosurfactants, industries can reduce their reliance on harmful chemicals, leading to a more sustainable and environmentally friendly approach to surfactant use.*

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## INTRODUCTION

Surfactants are compounds synthesized from petrochemicals that minimize surface tension or serve as emulsifiers have flourishing commercial value in numerous products including laundry detergents, as additives of health care products, food industry bioremediation and clinical setups [1]. Their presence in water can harm microorganisms and disrupt marine food chains [2]. Long-term use of chemical surfactants in detergents can also cause skin irritation and other health issues [3]. To address these concerns, there's a growing need for environmentally sustainable alternatives to chemical surfactants and biosurfactants act as promising replacement candidates. Biosurfactants are active surface compounds produced primarily from plants and microorganisms. They are amphiphilic comprising polar molecules (hydrophilic moiety) involving mono, oligo or poly-saccharides, proteins or peptides and non-polar that is a hydrophobic moiety comprising saturated, unsaturated and fatty alcholates or hydroxylated fatty acid [4]. Surfactants have low surface tension (attraction between molecules) which creates an adhesive force between the molecules of a liquid in the interaction between oil and water. Biosurfactants offer the benefit of low toxicity, biodegradabilty and activity at severe temperatures and pH over chemical surfactants [5, 6].

Plants produce biosurfactants as secondary metabolites, such as the saponins found in Acacia concinna (Shikakai herb), used in traditional shampoo [7, 8]. However, microbial biosurfactants production is preferred due to easier optimization in controlled environments, unaffected by seasonal variations. Microorganisms like bacteria, fungi, and yeast can synthesize diverse surface-active compounds, making them valuable substitutes in industries such as detergents, oils, cosmetics, and water treatment [9].

## CLASSIFICATION OF MICROBIAL SURFACTANT

Biosurfactants from microbial sources are gaining attention for their unique properties, classified based on molecular weight, chemical structure, and microbial origin [10, 11]. They typically range from 0.5 to 1500 KDa, with low-mass biosurfactants like phospholipids, lipopeptides, and glycolipids reducing surface tension, while high-mass ones like particulate surfactants and polymers excel in emulsification [12–14]. Biosurfactants can be anionic (with sulphonate or sulphur groups), cationic (with quaternary ammonium groups), non-ionic, or amphoteric (containing both ionic and non-ionic groups). Glycolipids, a common type, consist of sugar molecules (e.g., rhamnose, mannose) linked to aliphatic acids or hydroxyl aliphatic acids via ether or ester groups [15]. Sophorolipids, trehalolipids and rhamnolipids are well known glycolipids [16]. Rhamnolipids is produced by species like *Pseudomonas sp., Burkholderiasp. (Chrzanowski). Pseudomonas aeruginosa*-derived rhamnolipids have been well studied and structurally consists of either mono or di-rhamnose sugar molecules linked with one or two molecules of hydroxyl decanoic acid [17, 18].

Sophorolipids are the glycolipids produced by yeasts mainly *Torulopsis bombicola*, *T. Apícola* [19]. They consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid by ß - glycosidic linkage. Sophorolipids, generally are a mixture of six to nine different hydrophobic sophorolipids and lactones [20]. Two forms of sophorolipids exists depending on their functional group first one is acidic(non-lactonic) having free carboxylic acid group, while lactonic sophorolipids form a macrocyclic lactone ring with intramolecular esterification of the 4'-hydroxyl sophorose group [21].

Trehalolipids are another group of glycolipids are the trehalolipids, which comprised of disaccharide trehalose connected to the sixth-position carbons in the benzene ring. They are produced by *Rhodococcus erythropolis, Mycobacterium sp., Arthrobactersp, Corynebacterium, Nocardia, Dietzia, Gordonia, Williamsi,and Tsukamurella sp. Trehalolipids* derived from *Arthrobacter* and *Rhodococcus* have excellent capacity in reducing interfacial and surface tension up to a large extend [22, 23].

Several biosurfacants integrated from *Bacillus licheniformis* synergize with each other and show exceptional susceptibility to temperature, salt and pH. They are also similar to surfactin in their auxiliary properties [24].

Some of the microbes such as yeast and bacteria like *Corynebacterium lepus, Nocardiaerythropolis* and *Thiobacillus thiooxidans* produce large quantities of phospholipid, fatty acids and neutral lipids while utilizing different n-alkanes.

Lipoproteins and Lipopeptides are produced by *Bacillus sp* (*Bacillus licheniformis, Bacillus subtilis, Bacillus brevis, Bacillus polymyxia,), Pseudomonas fluorescens* and *Serratia marcenscens* consisting of ring structure of amino acid linked to chain of fatty acid with the help of lactone bond [25].

## ADVANTAGES OF BIOSURFACTANTS

Biosurfactants offer numerous advantages over conventional surfactants, including low toxicity, enhanced foaming activity, environmental safety, biodegradability, and effectiveness over a wide range of pH and temperature [26]. They are safe for use in the food, pharmaceutical, and cosmetics industries. Unlike synthetic surfactants, biosurfactants are biodegradable and easily degraded by microorganisms, making them suitable for bioremediation processes [27]. Their complex structure with multiple functional groups allows them to detoxify contaminants, act as de-emulsifying or emulsifying agents. They remain active even at high temperatures and in the presence of high salt concentrations, making them versatile and easy to produce from raw materials or waste products [28].

## DIFFERENT METHODS FOR EXTRACTION AND PURIFICATION OF BIOSURFACTANTS

There are many approaches for biosurfactant recovery and purification from different fermentative medium are described as follows:

a. *Acetone precipitation:* Cell free supernatant is blended with chilled acetone to precipitate biosurfactants, then mixed with phosphate buffer solution (PBS). The mixture is then incubated at 4°C for 14- 18 hours to precipitate the biosurfactants [29, 30].
b. *Ethanol precipitation:* Ethanol is a common solvent used for the biosurfactant extraction from the culture supernatant of various micro-organisms. Cell free broth (supernatant) is obtained by centrifuging microbial culture at 10000g for 20 minutes to remove cells, then it is precipitated using cold ethanol [31].
c. *Ammonium sulphate precipitation:* Thirty percent of Ammonium sulphate $(NH4)_2SO_4$ is directly added to the fermentation broth without removing cells and allowed to stand overnight., The obtained precipitate is further suspended in 3% saturated ammonium sulphate and then centrifuged. Additional $(NH_4)_2SO_4$ is added to achieve the 40 percent final concentration then extracted with ether and centrifuged. Pellets are further re-suspended in $(NH_4)_2SO_4$ after overnight cooling. For the elimination of residues, the pellet collected after centrifugation is dissolved in water and removed with same volume of hexane which is further purified and lyophilized [32].
d. *Acid precipitation:* This is a simple, inexpensive and readily available method to recover crude biosurfactants from cell free supernatant by minimizing its pH to 2 by utilizing concentrated HCl and placed at 4°C overnight, resulting in precipitation of biosurfactants and these precipitates is collected by centrifugation or extracted with different organic solvents which later on can be evaporated by rotary evaporator. Mainly biosurfactants like surfactin, glycolipids, lipopeptides *etc* recovered by this method [33–35].
e. *Ion exchange chromatography:* Those biosurfactants that attain negative charge at higher pH environments can be eluted with buffer containing 10% (v/v) ethanol after being attached to ion exchange resins. Highly purified rhamnolipids from *Pseudomonas sp.* has been purified by this method [36].
f. *Centrifugation*: After overnight acidification of cell free broth it is then centrifuged at 11,000 rpm for 20 min at 4°C to collect biosurfactant precipitates and can dry and extracted with multiple solvents [37].
g. *Crystallization*: Extracted biosurfactant precipitate is re-dissolved in an organic solvent and reaction is coupled with reduction in temperature resulting in crystallization of biosurfactants. Pure crystals of rhamnolipids Rha-Rha-C10-C10 is obtained by this method [38].
h. *Isoelectric focusing:* It is one of the novel approaches used for purification of biosurfactant according to their respective isoelectric point. This procedure requires 10–12 h at 400 V and a current of 1.5 A. Columns help to separate fractions with the changes in pH. Once total

separation occurs, electro-focusing is discontinued and the activity of purified biosurfactant is compared with the crude form [39].

i. *Solvent extraction:* Biosurfactants are extracted with the aid of different solvents like hexane, acetic acid, chloroform, methanol, di-chloromethane, butanol, di-ethyl ether, isopropanol *etc*. Rhamnolipid, liposan, trehalose lipids, sophorolipids, *etc* are isolated by these solvents [40].
j. *Ultrafiltration:* It is a low pressure driven mechanical process carried out using amicon filter paper, filters of 0.22 μ, 0.45 μ pore sizes. Biosurfactants are recovered by ultrafiltration low-pressure which permits passing of small molecules from a fermentation broth such as salts, amino acids, organic acids, alcohols and other metabolites whereas, macromolecules viz., extracellular proteins with nominal molecular diameter higher than that molecular weight cut off of the membrane and are concentrated [41].

## PRELIMINARY CHARACTERIZATION OF BIOSURFACTANTS

There are various methods which are used for the characterization of biosurfactants from diverse microorganisms.

a. *Thin layer chromatography:* By utilizing this method biosurfactant biochemical nature can be determined simply by spotting crude biosurfactant samples and separated using the solvent. Ninhydrin reagent is used to detect lipopeptide and Anthrone reagent for glycolipid biosurfactant detection.
b. *High pressure liquid chromatography (HPLC):* It separates different components according to their relative affinities towards stationary phase and separated components structure can be studied after purification. HPLC is very useful for separation of biosurfactant like lipopeptide [42].
c. *Infra-red (IR) spectroscopy:* The functional groups present in biosurfactant samples can be determined by IR spectroscopy. It is very useful in the elucidation of structure and identification of compounds [43].
d. *Nuclear magnetic resonance (NMR):* The functional groups as well as bond position information present in biomolecules like lipids and carbohydrates can be studied with the help of NMR. Biosurfactant samples which have to be analysed must be prepared using solvents that contain deuterium in place of hydrogen [44].
e. *Fast atom bombardment- mass spectroscopy (FAB-MS):* For analysis of biosurfactant sample by Fast atom bombardment- mass spectroscopy biosurfactant, the sample is first dissolved in methanol and then mixed with matrix (m-nitrobenzyl alcohol) which dissolves the sample and helps for desorption, ionization. *Psuedomonas* derived rhamnolipids were identified by Manso Pajarron *et al* (1993) by this technique [45].

## FACTORS INFLUENCING BIOSURFACTANTS PRODUCTION

The synthesis of biosurfactants rather than relying solely on the generating micro-organism often depend on considerations including the quality of the supply of carbon, the source of nitrogen and also on carbon and nitrogen ratio(C:N) and nutritional limitations. Physical parameters like temperature, pH, aeration, divalent cations, and salinity level affect the biosurfactant yield too.

### Carbon Sources

Microorganisms utilize diverse organic compounds as sources of carbon and energy for their metabolism and the production of their metabolites. Biosurfactants are produced using specific carbon sources, differing from species to species. Biosurfactant yield as well as its quality is affected by the type of carbon source used in the culture media [46, 47]. Diesel, raw petroleum, crude oil, glucose, sucrose, glycerol and mustard oil have been reported to be good sources of biosurfactant carbon substrates [48]. As reported by Casas *et al* even greater sophorolipid yields (120 g / L) have been produced from *Candida bombicola* within eight days of using sugar and oil as carbon sources [49, 50]. Glucose was better utilized by *P. aeruginosa* MTCC 7815 than any other forms of carbon like glycerol, fructose and starch for production of higher concentrations of biosurfactant, and with better emulsification index.

 

**Nitrogen Source**

Nitrogen source is a significant contributer to biosurfactant yield and quality. It not only plays important role in micro-organism growth but also serves as a regulator of pH. To enhance the production of biosurfactants, different nitrogen sources are utilized such as organic nitrogen sources (extracts of yeast and meat, tryptone, peptone) and inorganic nitrogen sources (ammonium sulphate, ammonium nitrate, sodium nitrate). Yeast extract is better source of nitrogen for biosurfactant growth. Mulligan and Gibbs reported that *Pseudomonas aeruginosa* utilizes nitrates, ammonium as a source of nitrogen for the production of biosurfactant [51]. High yields of sophorose lipids derived from fungi *Torulopsis bombicola* and *Candida Bombicola*, have been achieved by utilizing a combination of yeast extract and urea as the nitrogen source. Ammonium nitrate and yeast extract nitrogen source increase the yield of mannosylerythritol lipid by *Candida sp* [52].

**Metal Ions**

In the synthesis of biosurfactants, metal ions play a significant role. Macronutrient minerals such as potassium (K) serve as an energy source and calcium (Ca) ions mediate cell signalling, magnesium (Mg) ions coupled with ATP and iron (Fe) act as a cofactor in the micro-organism metabolism reaction. In regulating osmotic pressure and controlling the membrane potential of the cell, both potassium and calcium ions play a pivotal role in preventing the lysis of the cell in the medium [53, 54]. As stated by, iron, manganese, and magnesium be enzyme cofactors involved in biosurfactants synthesis of surfactin from *Bacillus subtilis*. The presence of low concentrations of iron, magnesium, manganese, phosphorus and sulphur can influence the composition, yield and consequently the chemistry of the biosurfactants [55, 56].

**Physical Factors Affecting Biosurfactant Production**

***Temperature and PH***

Physical factors like temperature and pH, are critical for an increase in the biosurfactant yield. To maximize biosurfactants yield it is essential to optimize temperature and pH required for the maximum microbial growth. According to Zinjarde and Pant (2002) the biosurfactant production increases when the pH is 8.0 [57].

***Aeration and Agitation***

The rate of agitation affects the efficiency of the mass transfer of both oxygen given and medium components effectively utilized by microbes. It is considered crucial for the growth of microbial cells and biosurfactant production. With an increase in agitation from 50-200 rpm there is an increase in approximately 0.12-0.55 mg/L level of dissolved oxygen in batch culture, thus there is an increase in both the growth of cells as well production of biosurfactants [58].

**APPLICATIONS OF BIOSURFACTANT**

Biosurfactants, peculiar properties make them safer relative to synthetic surfactants which are mostly non-biodegradable in nature and thus remain toxic to the environment, accumulate and pose hazards to the environment. Biosurfactants have a wide range of commercial applications encompassing those in cosmetics, pharmaceutical, food, petroleum, wastewater treatment and in textile industry. Subsequently, these biomolecules also act as stabilizing, wetting, antimicrobial, moisturising, emulsifying, and anti-adhesive agents, hence categorizing them as multifunctional agents [59].

**Commercial Laundry Detergent**

The key constituent of laundry detergents is chemical-based surfactants that not only affect the cloth but are also hazardous to the living species of fresh water. Public awareness about the environmental pollution through chemical surfactants is increasing, so there is a need to utilize natural substitutes instead of synthetic surfactants. Cyclic Lipopeptide (CLP) derived from *Bacillus subtilis* withstand high temperatures and wide pH range (7.0-12.0) without losing surface property. They have good emulsion properties which makes them suitable laundry detergent [60]. *Pseudomonas* derived rhamnolipid biosurfactants are also used as a constituent in detergent as they exhibit good foaming activity [61].

**Application in food Processing Industry**

Biosurfactants are employed as food formulation additives to facilitate emulsion formation and stabilization because of their unique property of minimizing surface tension and interfacial stress. They are employed in regulating fat globules's agglomeration, improve the texture, stabilise starch-containing products, their shelf life and improve the quality and consistency of fat-based products. Environmental Protection Agency allowed the use of *P. aeruginosa* derived rhamnolipids in food and pharmaceuticals industry [62]. *Lactobacillus sp* derived biosurfactants have anti-adhesive properties which are beneficial in the elimination of food borne pathogens.

**Cosmetic Industry**

Surfactants are utilized in cosmetic industry but the use of synthetic surfactants is limited because they cause irritation and also induces skin allergy. Hence microbial biosurfactants are being explored for cosmetic products owing to their properties of foaming, emulsification, spreading affecting the viscosity and product consistency. They act as emulsifying, foaming agents, cleansers, solubilizers, antimicrobial agents, wetting agents. Biosurfactants are present in various cosmetic products like baby products, toothpaste, contact lens solutions, mascara, lipsticks, facial makeup, creams, and hair products and dentine cleansers [63, 64]. Sophorolipids derived from *C. bomicola* has been primarily used as active component in the cosmetics industry as they have exceptional properties like skin compatibility and excellent moisturizing properties. Skin care formulations containing mannosylerythritol lipids and rhamnolipids have been employed as anti-wrinkle and anti-ageing products because of their skin compatibility and relatively low skin irritation [65].

**Application of Biosurfactant in Oil Recovery Enhanced by Microbes**

Biosurfactants are novel molecules that can be utilized as biocatalysts in oil reserves for petroleum upgrading, biofouling degradation, and hydrocarbon biocorrosion [66, 67].

Chemically synthesized surfactants used in the petroleum industry for enhanced oil recovery are costly and environmentally harmful, polluting underground water and soil [68, 69, 70]. Microbial biosurfactants offer a safer alternative, as some microorganisms can utilize crude oil and hydrocarbons, aiding in oil spill cleanup and reducing interfacial tension [71, 72] Microbial enhanced oil recovery (MEOR) involves using microorganisms like *Bacillus subtilis* and *Pseudomonas aeruginosa* to reduce oil viscosity and interfacial stress, promoting oil mobilization and improving recovery [73]. MEOR can be applied in-situ or ex-situ, with ex-situ applications involving biosurfactant production in bioreactors and injection into oil reservoirs, while in-situ applications introduce microbes and nutrients directly into the reservoir to produce biosurfactants and enhance oil recovery [74, 75].

**Agriculture**

Biosurfactants produced from micro-organisms such as *Bacillus sp. Acinetobacter sp* and *Pseudomonas sp*. are helpful in the elimination of heavy metals like Mn, Cu, Zn, Cd, Li, Ba, Mg, Ni and Ca from polluted soils and to biodegrade toxins such as hydrophobic organic contaminants (HOC) to improve soil quality [76]. Some biosurfactants exhibit antimicrobial activity against different plant pathogens. They have also gained popularity as an alternative pesticide against pesticide-resistant insect populations. Lipopeptides have insecticide activity which has been studied against the fruit fly *Drosophila melanogaster* [77]. In agriculture, biosurfactants are helpful in elimination of plant diseases and improving nutritional bioavailability for beneficial microbes associated with plants. Biosurfactants from *Pseudomonas* and *Bacillus* have shown inhibition of soft rot caused by *Pectobacterium sp.* Rhamnolipids have shown the inhibition of zoospore-forming plant pathogens which exhibited resistance to commercial chemical pesticides [78].

 

**Medicine**

The role of biosurfactants in the field of medicine has been explored day by day over the last decade. Due to their antifungal, antibacterial, insecticidal, anti-mycoplasma, and anti-viral effects, biosurfactants have diverse uses in the medicinal field and can also be used as anti-adhesive agents [79–81]. *Bacillus circulans-derived* Lipopeptides show inhibitory activity against wide range of bacteria. The occurrence of disease-causing microorganism in the gastro-intestinal tract is hindered by lipopeptides synthesized by some *Bacillus species* [82]. *Pseudomonas aeruginosa* derived rhamnolipids demonstrate antibacterial and antifungal properties towards various fungi [83]. *Candida bombicola*-derived sophorolipids are effective against different micro-organisms like *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa,*respectively. Urethral catheters coated with surfactin solution derived from *Bacillus subtilis* reduces biofilm produced by different micro-organism like *Salmonella typhimurium, Salmonella enteric and Proteus mirabilis* [84]. Some lipopeptides exhibit antifungal activity by affecting yeast cell membrane as well its morphology [85]. Daptomycin isolated from *Streptomyces roseosporus* is a commercially available, branched cyclic lipopeptide antibiotic used for the treatment of skin infections [86]. A biosurfactant obtained from *Pseudomonas fluorescens* prevents adherence of *Listeria monocytogenes* which contribute to the formation of biofilms in the food industry [87]. Isolated Sophorolipids from *Candida Bombicola* have Spermicidal activity, cytotoxic properties and anti-HIV action. Some studies indicate that surfactin has the ability in in-vitro tests to inactivate herpes and retroviruses [88].


**Figure 1.** Depicts the Biosurfactant Applications in Different Fields.

## DIFFICULTIES TO SCALE UP BIOSURFACTANTS FOR INDUSTRIAL USE

Biosurfactants present a potential alternative to synthetic surfactants. However there are certain difficulties associated with product recovery, low concentration of biosurfactants, the cost of their production is high of the same as compared to the synthetic surfactants, and its downstream method involves several consecutive phases, which makes its production very difficult at large scale, hence limiting their commercial use. Through the application of low-cost green substrates, several studies have made attempts to cut down the cost of production of biosurfactants.

A wide range of substitute raw materials are now being served as nutrients for industrial fermentations, including different agricultural and industrial waste [89].

Some of these examples are as follows:
Agriculture based wastes like straw of wheat and rice, molasses derived from sugarcane and beet, sugarcane bagasse, soy hull, flour and waste-water derived from rice processing, steep maize liquor, high starch, carbohydrates and lipid content of pulses and cereals are rich in nitrogen and carbon and act as suitable medium for synthesis of biosurfactants [90, 91].

Animal fat constituents can be readily available from the meat processing industry in vast amounts and can be used as a source for micro-organisms producing biosurfactants. Animal fat media serve as a good source for the growth of yeast like *C.Bombicola* which produces sophorolipids. This also increases the growth of *C. lipolytica UCP* 0988 which maximizes the production of glycolipid [92].

Different frying oils and edible fats are known to be good carbon sources for biosurfactants production. Vegetable oils consist primarily of different chains of saturated or unsaturated fatty acids. Different oils, like soybean oil, canola oil seeds, sunflower and olive oils, serve as effective sources for the biosurfactants synthesis. The strains of *P.aeruginosa* produce biosurfactants from residues of corn, soybean and canola oil plants [93, 94].

Dairy industry wastes can be beneficial alternative media for biosurfactant production as waste milk products, such as whey waste, cheese whey and curd whey, all of which contain abundant amounts of biomolecules like lactose, proteins, vitamins and organic acids that can serve as good source for the production of biosurfactants [95–97].

## CONCLUSION

This review paper intends to explore isolation techniques and characterization of microbial biosurfactants. Manipulation of optimization conditions to enhance biosurfactant yield has also been discussed. Further, prospective industrial applications of these natural surfactants have been reviewed. Owing to the poor recovery and the high isolation cost of these biosurfactants, certain agricultural and industrial residues have been suggested as future raw materials for these surface active compounds.

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