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# **BIOSURFACTANTS: THE POTENTIAL GREEN SURFACTANTS IN THE 21ST CENTURY**

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### **ABSTRACT**

Biosurfactants are amphiphilic microbial molecules with hydrophilic and hydrophobic moieties that partition at liquid/liquid, liquid/gas or liquid/solid interfaces. These unique characteristics allow these biomolecules to play a key role in emulsification, foam formation, detergency and dispersal, which makes them ideal for use in different industrial sectors. The production of biosurfactants is considered to be the prime development in industrial technology in the  $21<sup>st</sup>$ century. Coupled with this feature, increasing public awareness of environmental pollution promotes the search and development of emerging technologies that help in cleaning up of organic and inorganic contaminants. Biosurfactants and biosurfactant producing microorganisms provides an alternative and eco-friendly method of remediation technology of environment contamination with these pollutants. Thus, biosurfactant production, possibility of their application as renewable resources and "green" products has now been the subject of extensive research in recent years. This review deals with the accumulated knowledge regarding biosurfactants gained over the years and possibility of their applications in various industrial fields, in oil recovery, in keeping the environments green, in medicinal sectors. The economic issue regarding the production of biosurfactants on an industrial basis in this regard also has been critically dealt with.

**Keywords:** Biosurfactants, Functional properties, Sources and producers, Interaction mechanism, Industrial application, Green surfactant*.*

#### **1. INTRODUCTION**

Biosurfactants, having excellent surface activating ability, capable of reducing the surface and interfacial tensions to a greater extent, are a structurally varying group of surface-active substances. They are amphiphilic compounds which structure is composed of hydrophilic and hydrophobic moieties in the molecular structure [1, 2]. The hydrophilic group consists of mono-, oligoor polysaccharides, peptides or proteins and the hydrophobic moiety usually contains saturated, unsaturated and hydroxylated fatty acids or fatty alcohols [3]. The hydrophobic and hydrophilic moieties partition preferentially at the interface between fluid phases that have different degrees of polarity and hydrogen bonding, such as oil-water or air-water interfaces. This property explains their potential use in several environmental applications, medicinal field and oil industry [4, 5]. They are mostly derived from plants and microorganisms, although production from microorganisms has several advantages over plant based, due to multifunctional properties, rapid production, high biodegradability, high temperature stability, scaleup capacity [6, 7] of the former. The term 'biosurfactants' and 'bio emulsifiers' are not identical from the physical point of view: those surfactants, derived from microorganisms, reduce the surface tension at the interface are biosurfactants, whereas, those, that lead to emulsification are bioemulsifier. The former very often possess emulsifying capacity but the latter do not necessarily reduce surface tension [7, 8]. On the basis of chemical structure, biosurfactants can be classified into five groups: (I) Lipopeptides and Lipoproteins (II) Glycolipids (III) Fatty acids and phospholipids (IV) polymeric surfactant (V) particulate [9]. Among these, lipopeptides and glycolipids are the scientifically well-known biosurfactants [10]. Although the maker living being solely controls the amount as well as effectiveness of the created microbial surfactants, factors like nitrogen, carbon, air circulation, humidity controls their production [2]. On the other hand, all the commercial surfactants are derived from non-sustainable oil based commodities. They are costly and a threat to the environment because of their toxic properties. In comparison to chemically derived

surfactants, biosurfactants have several advantages: they are environmentally friendly, biodegradable, less toxic and non hazardous [11]. Coupled with these factors, the effectiveness at extreme temperatures, low to moderate pH, salinity as well as the possibility of production from industrial wastes and by products arose the interest regarding biosurfactants among the scientific and industrial community, chiefly because of their environmental friendly nature, suitability for large scale production, selectivity and efficacy of performance under critical circumstances [12]. Due to their amphiphilic structure, biosurfactants increase the surface area of hydrophobic water soluble substance and change the properties of bacterial cell surface. Biosurfactants are effective replacements for oil based surfactants in several industrial processes, such as lubrication, wetting, softening, fixing dyes, making emulsions, stabilizing dispersions, foaming as well as in agriculture, food, fertilizer, biomedical and pharmaceutical industry and bioremediation of organicor inorganic-contaminated sites [13, 14]. Many of the versatile applications that have been reported for biosurfactants solely depend on whether they can be produced economically and this topic has now the subject of extensive research so far as the process optimization is concerned. Production of biosurfactants from inexpensive waste substrates which decreases their production cost [15] has been reported. In recent times, with the implication of strict regulations concerning environmental pollution by industrial venture and health regulations will markedly affect the possibility of dominance of the biodegradable biosurfactants replacing their chemical counterparts [12].

The review aims to present the classification, properties and mechanism of interaction of selected class of biosurfactants in detail. The surface activity, biological activity, antimicrobial activity, antiadhesive activity also has been presented. The application of biosurfactants in biomedical and therapeutic field, as a potential green anticorrosive agent, in the field of bioelectrochemistry, in oil industry, in different aspects of environmental biotechnology, in food industry and cosmetics has been explored. The possibility of producing cost effective biosurfactants in large scale, keeping the green approach in mind, in near future has been discussed.

#### **2. MECHANISM OF INTERACTION**

Biosurfactants, being microbial amphiphilic molecules,

interact with the interface between two phases in a heterogenous system, In an aqueous environment, incorporation of biosurfactants results in the formation of emulsions as well as desorption of hydrophobic compounds [16], resulting in reduction of interfacial tension between immiscible liquids and increase the solubility of the hydrocarbons. The reduction of interfacial tension leads to increased penetration of porous materials (soil and sediments) through the aqueous phase [17, 18]. It is a well-known fact that surfactants, in general, in the amounts greater than the critical micellar concentration (CMC) can form micelles in aqueous solution. In aqueous phase, above CMC, surfactant micelles have a hydrophobic core and therefore, they can accumulate hydrophobic hydrocarbons, resulting in increasing the aqueous hydrocarbons' solubility [19], which may cause an easier transport of pollutant particles to the microbial cells in an aqueous solution [20], causing increase in bioavailability [21, 22]. Another possibility is that, the hydrocarbons can be directly taken by microbial cells from micelles [23]. It is a general principle, that, organic molecules from the aqueous phase tend to immobilize towards the interface, for all interfacial systems, where they form a conditioning film that may change the surface properties of the original surface [24]. In the same analogy, biosurfactants may interact with the interfaces, affect the adhesion, leading to detachment of bacteria. Besides hydrophobic interactions, a series of interactions are involved as a result of adsorption of charged biosurfactants to the interface. Therefore, ionic conditions, electrostatic forces as well as pH are the key factors to investigate the nature of interactions of charged biosurfactants with the interface [25]. Thus, for charged biosurfactants, using the surface-active approach [26], it is possible to predict the theoretical locations as well as the orientations of the particular biosurfactant. For neutral systems, the situation is far more complex.

## **3. GENERAL PROPERTIES OF BIOSURFACTANTS**

Excellent surface activity makes biosurfactants superior emulsifier, foaming and dispersing agents, when compared to their chemically synthesised equivalent. They display various properties such as high biodegradability, non-toxicity, effective critical micellar concentration, remarkable surface activity, emulsification /deemulsification characteristics, antimicrobial action in addition to their inertness towards various environmental factors such as pH range, high salt concentration, extreme temperature etc [27].

# **3.1. Surface activity**

The characteristic of biosurfactant adsorption on the surface and/or interface has attracted greater attention. This is particularly due to the fact that biosurfactants are more effective and efficient and their CMC in general, is about several times lower than chemical surfactants, i.e. for maximum decrease on surface tension, less surfactant is necessary. Surfactin produced by *B. subtiliscan reduce surface tension of water to 25 mN m<sup>-1</sup>* and interfacial tension for water/hexadecane system to less than  $1 \text{ mN m}^{-1}$  [28]. The rapid adsorption and the close alignment of surfactin on the surface or interface may be contributed to its specific amphiphilic structure with a hydrophobic moiety, consisting of long chain fatty acid along with some lipophilic groups and a hydrophilic moiety with the backbone. The characteristic reduction of surface tension of aqueous solution has great implications for adsorption of hydrophobic molecules, biological assimilation and transport. The longer the fatty acid chain, the more intense is the repulsion force between water and fatty acid chain, results in fast adsorption of surfactin to the surface/interface. Similarly, the rhamnolipids produced by *P. aeruginosa* decreased surface tension of water to 26  $mN$  m<sup>-1</sup> and interfacial tension of water/hexadecane to value less than  $1 \text{mN m}^{-1}$  [29].

# **3.2. pH and thermal activity**

The surface activities of a considerable number of biosurfactants are stable towards natural factors, for example, temperature and pH. In case of surfactin, as pH decreases, it becomes less soluble in water because of the protonation of the carboxyl group [30], whereas, in neutral and basic media, since the carboxyl group is in the ionic form, both the solubility and emulsifying ability increases in aqueous solution [31]. Moro et al. studied the influence of pH on the stability of surfactants produced by *B. subtilis, B. gibsonii* and *B. amyloliquefaciens* [32]. In strongly acidic conditions, the emulsifying activity significantly decreased for both *B. Subtilis*  ODW02 and *B.* subtilis ODW15. As pH increased from 7 to 12, the stability of the surfactant produced by *B. Subtilis* ODW12 decreased even further, but the one by *B. Subtilis* ODW15 remained stable. *B. amyloliquefaciens* showed significant increase in emulsifying activity in both acidic and basic conditions. The dissimilarity in the behaviour towards variation of pH for different biosurfactants enhances their chance of application in different industrial field [33]. On the other hand, the biosurfactants, in general, shows remarkable thermal stability.

# **3.3. Antiadhesive Property**

Biofilm formation is a process whereby microorganisms irreversibly attach to and grow on a surface and produce extracellular polymers that facilitate attachment and matrix formation, resulting in an alteration in the phenotype of the organisms with respect to growth rate and gene formation. Microorganisms that form biofilms include bacteria, fungi and protists and biofilm formation over a particular surface is affected by several factors, including type of microorganisms, hydrophobicity and electrical charges of the surface and environmental conditions [34]. Biosurfactants have gained considerable interest particularly in clinical and hygienic sectors due to their potential to disperse microbial biofilm, which is found to be superior in comparison to traditional inhibitory agents against bacteria and yeast biofilms. This makes biosurfactants potential for use in new generations as microbial dispersal agents. It has been reported that prior adhesion of biosurfactants to solid surfaces might constitute a new and effective method to fight against immigration of pathogenic microorganisms [35]. Pre-coating vinyl catheters by running the surfactin solution through them before inoculation with media resulted in a decrease in the amount of the biofilm formed by *Salmonella typhimurium, Salmonella enterica, E-coli* and *Proteus mirabilis* [36]. In addition, the use of lactobacilli as a probiotic for the prevention of urogenital infections has been widely studied [37]. A surfactant from *Streptococcus thermophilus* slows down the colonisation of other thermophilic strains of streptococcus over the steel which are responsible for fouling [38]. The results have great potential applications particularly in the field of medicine and industry [34].

# **3.4. Biodegradability**

The most significant feature of microorganisms derived compounds are their ease of degradation as compared to their synthetic analogue and thus are suitable for environmental applications such as bioremediation, anticorrosive agents, biosorption [39, 40]. Therefore, biosurfactants tends to serve as an alternative in view of expanding ecological concern, as synthetic chemical surfactants impose serious environmental threat because of their non-biodegradable nature. Biodegradability, expressed in the form of BOD/TOD (Biochemical

oxygen demand to total oxygen demand ratio) for sophorolipids after 8 days of cultivation has reached the level of 61% [41]. Similar is the behaviour of surfactin and arthrofactin, as compared to synthetic surfactants, which shows no biodegradability after 8 days [41]. Mohan et al., [42] has reported that rhamnolipid biosurfactants are biodegradable under both aerobic and anaerobic conditions within stipulated time limits, whereas, Triton X-100 is non-biodegradable under anaerobic conditions and only partially biodegradable under aerobic conditions.

## **3.5. Low Toxicity**

Although little work so far has been published regarding the toxicity of biosurfactants, it is a well-accepted feature that biosurfactants are generally considered low or non-toxic products which enhances their possibility to be used in pharmaceutical, cosmetic and food industry. The literature reported data [43, 44] shows that biosurfactants in comparison to synthetic surfactants pose haemolytic activity to human erythrocyte lower than cationic surfactants (CTAB, TTAB) and anionic SDS. They do not pose adverse effect to heart, lung, liver and kidney and interfere in blood coagulation in normal clotting time. Flasz et al., [45] compared the toxicity and mutagenic profile of biosurfactant from *Pseudomonas aeruginosa* and chemically derived surfactants and indicated the biosurfactant as non-toxic and non-mutagenic.

## **3.6. Emulsifying ability**

The inherent property of biosurfactants is that, they may act as emulsifiers. By definition, an emulsion is a heterogenous system, consisting of at least one immiscible liquid dispersed in another in the form of droplets. Such systems have minimal stability, which can be increased by surfactant additives that work by reducing the interfacial tension, decreasing the surface energy between the two phases. The activity of biosurfactants as emulsifier for the processing of raw materials, in bakery products, influencing the rheological characteristics of flour, meat products in the emulsification of fat has been reported [46, 47].

### **3.7. Antioxidant activity**

Utilising the antioxidant property, particularly in the food sector, the potential of natural compounds is of growing interest in recent years [48]. Biosurfactants have proved sufficient to replace existing synthetic antioxidants because they have significant antioxidant

activity, since the generation of toxic compounds, development of rancidity and undesirable flavours are negative balances of lipid self-oxidation in addition to the fall in food security [49].

#### **3.8. Antimicrobial activity**

The antimicrobial activity of several biosurfactants has received considerable attention in recent years [38, 50]. Reid et al., [51, 52] described a possible probiotic role for the biosurfactant producing lactobacilli in the restoration and maintenance of healthy urogenital and intestinal tracts, protection of skin and gastrointestinal (GI) systems [53], conferring protection against pathogens and suggested a reliable alternative treatment and preventive regimen to antibiotics in the near future. The key role of biosurfactants is to control the microbiota through the quorum sensing systems [50] and the microbial activity. These features protect them and consequently, the human body principally from microbial and fungal pathogens, which makes the biosurfactants as promising bioactive molecules. In some recent studies, it has been pointed out that, members of Lactobacillus genus are the most versatile lactic acid bacteria, producing biosurfactantscomposed principally of protein, polysaccharides and phosphates in different ratios, have significant antimicrobial effect against several common potential pathogenic bacteria such as *Neisseria gonorrhoeae* [54], *Escherichia coli, Enterobacteraerogenes* and antifungal activity against *Candida albicans* [55]. The study conducted by Giri et al., [56] provided new diverse antimicrobial biosurfactants deriving from different bacteria with possible applications in the biomedical field.

### **4. FACTORS AFFECTING BIOSURFACTANT PRODUCTION**

Producer strain plays the key role in determining the type and emulsifying activity of a particular biosurfactant. On the other hand, the nature of carbon source, the nitrogen source, the C:N ratio, nutritional limitations as well as some environmental factors, e.g., pH, air-circulation, salinity affects largely the type, activity and amount of production of biosurfactants.

#### **4.1. Carbon source**

The quality as well as quantity of biosurfactant production is influenced by the nature of the carbon substrate [57]. Diesel and crude oil are identified to be good sources of carbon for biosurfactant production by

organisms [58]. Other water-soluble compounds such as glucose, sucrose and glycerol are significant carbon sources for biosurfactant production. Hydrophobic substrates like corn oil, lard (rich in unsaturated and saturated fat) and long chain alcohols maximized biosurfactant production; on the contrary, hydrophilic substances like glucose and succinate delivered poor yields. Robert et al., [59] have reported that *Pseudomonas aeruginosa* can be produced from a variety of carbon sources such as C11 and C12 alkanes, succinate, pyruvate, citrate, fructose, glycerol, olive oil, glucose and mannitol.

# **4.2. Nitrogen Source**

Since protein and enzyme synthesis inevitably depend on nitrogen, this element plays a key role for microbial growth and thus nitrogen source is essential for biosurfactant production. Different nitrogen source such as yeast extract, urea peptone, ammonium sulphate, ammonium nitrate, sodium nitrate, meat extract as well as malt extract are used for this purpose. Ammonium salts and ureas are preferred nitrogen sources for biosurfactant production by *Arthrobacterparaffineus*, whereas, ammonium nitrate supports maximum surfactant production in *P. aeruginosa* [2]. Although yeast extract is the most efficient nitrogen source for biosurfactant production, its usage largely depends on concentration in organism and culture medium.

## **4.3. Environmental factors**

As the cellular growth and activity of microorganisms depend on environmental factors such as pH, temperature, oxygen availability, these factors affect the ease and extent of biosurfactant productions. Medium pH plays an important role in sophorolipid production by *T. bombicola*. Rhamnolipid production, with *Pseudomonas* sp., on the other hand, was its maximum at a pH range 6 to 6.5 and decreases sharply above pH 7. Most biosurfactant production are accounted for to be performed in a temperature scope of 25-300ºC and thus it may be considered, in general, biosurfactant production shows a moderate thermal stability. Agitation and oxygen availability also effect biosurfactant production through their effects on cellular growth and activity, as both encourage the oxygen exchange from the gas stage to the fluid stage. It has been suggested that oxygen availability affects the physiological function of biomeulsifier which, in turn, enhance the solubilization of water insoluble

substrates and facilitates nutrient transfer to microorganisms. Most biosurfactants, however, are not affected by variation of salt concentration up to a limit of 10%, although slight reduction in their CMC values may occur.

## **5. RAW MATERIALS FOR BIOSURFACTANT PRODUCTION**

Use of industrial waste, particularly agro-industry waste is one of the preferred routes towards the implementation of feasible biosurfactant production on an industrial scale, which obviously requires the optimisation of different variables, keeping in mind on the recovery, recycling and reuse to minimise the expenditure, in the commercial domain. In this connection, a number of waste products are employed in biosurfactant production as described in the literature. At this point, it is pertinent to mention that the selected waste products should ensure the proper balance of nutrients to allow microbial growth and consequent biosurfactant production. In this regard, industrial waste with a high content of carbohydrate or lipids is ideal for this purpose. A number of waste products are described in the literature in this connection. These are vegetable oils, oily affluents [60], starchy effluents [61], animal fat [62, 63], vegetable fat [64], soapstock [65], molasses [66], dairy industry waste (whey) [67], corn steep liquor [68] and oil distillery waste [69].

## **6. CLASSIFICATION OF BIOSURFACTANTS AND THEIR PRODUCERS**

The classification of chemically synthesised surfactants is based on their dissociation pattern in water. On the contrary, biosurfactants are categorized on the basis of their chemical composition, molecular weight, physicochemical properties, mode of action and microbial origin. On the low molecular mass category, there are glycolipids, phospholipids and lipopeptides; whereas, in the high molecular mass category, biosurfactants /bioemulsifiers containing amphipathic polysaccharides, proteins, lipopolysaccharides, lipoproteins or complex mixtures of the biopolymers. The low molecular mass biosurfactants are effective in lowering the surface and interfacial tensions. The high molecular mass biosurfactants are more efficient in stabilizing oil-in-water emulsions [70, 71]. The classification of biosurfactants along with their sources are depicted in the following table.



### **7. APPLICATIONS OF BIOSURFACTANTS**

Besides manifold applications in the field of environmental biotechnology and medicinal field, biosurfactants have widespread applications in petroleum, foods, beverages, cosmetics, detergents, textiles, paints, mining and nanotechnology [87].

### **7.1. Petroleum recovery**

For enhancement of oil recovery, heat, tensioactive agents and gas injection is the primary requirement to recover the significant portion of retained residue oil, which remains unextracted during the extraction process. The high cost of chemical tensioactive agents is the main obstacle in the process of oil recovery, so far as the economy of the extraction process is concerned. Biosurfactants have been employed effectively to reduce the interfacial tension between oil/water as well as oil/rock, which leads to a reduction in the capillary forces between the interface and also form an emulsion in the oil/water interface, thereby, facilitates the extraction of residual oil [88].

### **7.2. Bioremediation**

Transportation of different vehicles lead to splitting of oils and this in turn heavily affect both marine and terrestrial ecosystems. In this context, bioremediation is the most effective natural degradation of these toxic compounds, carried out by plants and microorganisms. These results into either partial conversion of generated contaminants into less toxic compound or complete conversion into carbon di oxide and water. Biosurfactants, in this context, appears as a safe alternative, for improving the solubility of hydrophobic compounds by allowing the desorption and solubilisation of hydrocarbons, thus facilitating the assimilation of these toxic compounds by microbial cells [89].

### **7.3. Removal of heavy metals**

Heavy metals mainly absorb to the surface of soil in the form of ions or metal compounds. They may be removed by surface associated complexations [90] and/or ion exchange [91]. Thus surfactant-enhanced bio-extraction can be applied to the remediation of soils contaminated with heavy metals. The efficiency of surfactin, Rhamnolipid and sorpholipids in this regard has been established in recent years [92, 93].

### **7.4. Food industry**

Emulsification plays the key role in the formation of consistency and texture in foods as well as the

solubilisation 0f aromas [94]. Biosurfactants can be used as emulsifiers in the processing of raw materials, the stabilisation of aerated systems and to maintain the consistency of fat-based products. However, the food industry has not yet permitted the use of biosurfactants in large scale.

### **7.5. Nanotechnology**

The fact that biosurfactants have been used in nanotechnology and nanoparticle synthesis is now an emerging part of green chemistry [95]. A biosurfactant produced by *P. aeruginosa* grown in a low cast medium has been employed to stabilise silver nano particles in the liquid phase [96].

### **7.6. Cosmetic industry**

Biosurfactants are amphiphilic molecules that can be included in cosmetic formulations because of their surface properties and biological activity. The current trend among consumers is the pursuit for natural ingredients in cosmetic products, as many of these products exhibit equal, better or additional benefits in comparison with chemical based products. In this sense, biosurfactants are natural compounds with great potential in the formulation of cosmetic products, by acting as wetting agents, cleansers, foaming agents, detergents and emulsion-forming agents [97].

## **7.7. Commercial laundry industry**

Surfactants play a very important role in laundry and household cleaning products ingredients. In a recent study [98], on the application of lipopeptide biosurfactant, produced by *Bacillus subtilis* SPB1, during the formulation of washing powder, has revealed that the biosurfactant acts additively with a commercial detergent and enhances their performance to a considerable extent. Because of low toxicity and high biodegradability, microbial biosurfactants play a promising role as laundry additives in near future.

### **8. CONCLUSION AND FUTURE POSSIBILITIES**

The biosurfactant industries has accelerated remarkable growth in recent years, although the production on large-scale of these biomolecules remains a challenge from the economic point of view, which is largely due to remarkable difference between the financial investment and industrial output. Also, the lack of sufficient knowledge, about the possible toxic effect on human systems, restricts the use of biosurfactants mainly in the cosmetic, food industry and in medicinal sector. Again, the creation of biosurfactant at a plant scale remains a testing issue as the arrangement of conclusive item is heavily influenced by the supplement, micronutrient and natural compound.

From the forgoing discussions, it seems clear that biosurfactants do not economically compete with chemically synthesised surfactants. But the strong dominance of biosurfactants over synthetic surfactants in terms of higher biodegradability, better environmental compatibility, high selectivity, specificity, inertness at high temperature, pH and salinity, will encourage chemists, environmentalists, scientists in various industrial sectors to carry out further research to sort out the correct route for economic production, process and technology of biosurfactants and we definitely step towards the much awaited green revolution in near future.

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#### *Conflict of interest*

None declared

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