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A Review on Production of Microbial Surfactants and their Potential applications

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Introduction

Surfactants are amphiphilic surface active agents possessing both hydrophilic and hydrophobic moieties that reduce surface and interfacial tensions by accumulating at the interface between two immiscible fluids like oil and water. Surfactants are key ingredients used in detergents, shampoos, toothpaste, oil additives and a number of other consumer and industrial products. They constitute an important class of industrial chemicals widely used in almost every sector of modern industry. They are of synthetic or biological origin. Due to their interesting properties such as lower toxicity, higher degree of biodegradability, higher foaming capacity and optimal activity at extreme conditions of temperatures, pH levels and salinity, these have been increasingly attracting the attention of the scientific and industrial community (Kosaric, 1992).

The total surfactant production has exceeded 2.5 million tons in 2010 for many purposes such as polymers, lubricants and solvents. From the total surfactants output, about 54% of them is consumed as household or laundry detergents, with only 32% destined for industrial use. Almost all surfactants currently in use are chemically derived from petroleum. Synthetic surfactants exhibit a low rate of biodegradation and a high potential to aquatic toxicity. Majority of surfactants produced today is of petrochemical origin beside of the renewable resources like fats and oils. Petroleum-related industries have been identified as one of the major source of pollution in our environment. For these reasons, biosurfactants are seen to be the promising alternative for many purposes.

Surface-active compounds

Surface-active compounds produced by microorganisms are of two main types

i. those that reduce surface tension at the air-water interface (**biosurfactants**) and

ii. those that reduce the interfacial tension between immiscible liquids, or at the solid-liquid interface (**bioemulsifiers**).

Biosurfactant

Biosurfactant usually refers to surfactants of microbial origin. Most of the bio-surfactants produced by microbes are synthesized extracellular and many microbes are known to produce biosurfactants in large relative quantities. Biosurfactants are polymers, totally or partially extracellular, amphipathic molecules containing polar and non polar moieties which allow them to form micelles that accumulate at interphase between liquids of different polarities such as water and oil thereby reducing surface tension and facilitating hydrocarbon uptake and emulsification.

These amphiphilic compounds have functional properties like surface and interface activity, emulsification, wetting, foaming, detergency, phase dispersing, solubilization and density reduction of heavy hydrophobic compounds and find wide applications in industries (Walter, 2010).

Interest in microbial surfactants has been progressively escalating in recent years due to their diversity, ecofriendly nature, possibility of large-scale production, selectivity, performance under intense circumstances and their impending applications in environmental fortification.

At present, biosurfactants plays an important application in petroleum-related industries which is use in enhanced oil recovery, cleaning oil spills, oil-contaminated tanker cleanup, viscosity control, oil emulsification and removal of crude oil from sludge.

Properties of biosurfactants

The properties of interest are changing surface active phenomena, such as lowering of surface and interfacial tensions, wetting and penetrating actions, Spreading hydrophylicity and hydrophobicity actions, microbial growth enhancement metal sequestration and Anti-microbial action.

Sources of Biosurfactants

Many of the biosurfactant producing microorganisms are found to be hydrocarbon degraders. However in the past decades, many studies have showed the effects of microbial produced surfactants not only on bioremediation but also on enhanced oil recovery.

Bacterial Biosurfactants

Microorganisms make use of a wide range of organic compounds as a source of carbon and energy for their growth. When the carbon source is in an insoluble form like a hydrocarbon, microorganisms make possible their diffusion into the cell by producing a variety of substances, the biosurfactants. Some of the bacteria and yeasts excrete ionic surfactants which emulsify the CxHy substance in the growth medium. A few examples of this group of biosurfactant are rhamnolipids that are produced by different *Pseudomonas spp*. or sophorolipids that are produced by several *Torulopsis spp*. Some other microorganisms are able to change the structure of their cell wall which is achieved by them by producing nonionic or lipopolysaccharides surfactants in their cell wall. Some examples of this group are: *Rhodococcus erythropolis* and various *Mycobacterium spp*. and *Arthrobacter spp*. which produce nonionic trehalose corynomycolates. There are lipopolysaccharides, such as emulsan, produced by *Acinetobacter spp*. and lipoproteins such as surfactin and subtilisin, which are produced by *Bacillus subtilis*.

Fungal Biosurfactants

Among fungi, *Candida bombicola*, *Candida lipolytica* , *Candida ishiwadae Candida batistae* , *Aspergillus ustus* and *Trichosporon ashii* are the explored ones. Many of these are known to produce biosurfactant on low cost raw materials.

The major type of biosurfactants produced by these strains is sophorolipids (glycolipids). *Candida lipolytica* produces cell wall-bound lipopolysaccharides when it is growing on n-alkanes.

The biosurfactants produced from microbial sources were listed in Table 1.

Organisms	**Biosurfactants**	**References**
	BACTERIA	
Serratia marcescens	Serrawettin	Lai *et al*. (2009)
Rhodotorula glutinis, R. graminis	Polyol lipids	Amaral *et al*. (2006)
Rhodococcus erythropolis, Arthrobacter spp.,	Trehalose lipids	Muthusamy *et al*. (2008)
Nocardia erythropolis	Ornithine lipids	Desai and Banat(1997)
Corynebacterium spp.		
Mycobacterium spp.		
Pseudomonas spp.,		
Thiobacillus thiooxidans		
Agrobacterium spp.		
P. fluorescens,	Viscosin	Banat *et al*. (2010)
Leuconostoc mesenteroids		
P. aeruginosa,	Rhamnolipids	Jadhav *et al*.(2011)
P. chlororaphis,		
Serratiarubidea		
P. fluorescens, Debaryomyces polmorphus	Carbohydrate-lipid	Nerurkar *et al*. (2009)
P.aeruginosa	Protein PA	Hisatsuka *et al*.(1971)
Lactobacillus fermentum	Diglycosyl diglycerides	Mulligan *et al*.(2001)

	FUNGI	
Torulopsis bombicola	Sophorose lipid	Kim *et al*. (1997)
Candida bombicola	Sophorolipid	Casas *et al*. (1997)
Candida lipolytica	Protein-lipidpolysaccharide complex	Sarubbo *et al*. (2007)
Candida lipolytica	Protein-lipidcarbohydrate complex	Rufino *et al*. (2007)
Candida ishiwadae	Glycolipid	Thanomsub *et al*. (2004)
Candida batistae	Sophorolipid	Konishi *et al*. (2008)
Aspergillus ustus	Glycolipoprotein	Alejandro *et al*. (2011)
Trichosporon ashii	Sophorolipid	Chandran and Das (2010)

Classification of biosurfactants

Biosurfactants are classified into two categories by Rosenberg and Ron (1999) on the basis of their chemical structure and microbial origin (Rosenberg, E. and Ron, E.Z., 1999). The major classes of biosurfactant include

- Glycolipids
- Lipopeptides and lipoproteins
- Phospholipids and fatty acids
- Polymeric surfactants and
- Particulate surfactants.

Microbial surfactants can also be divided into two major classes according to their molecular-mass. The low molecular-mass biosurfactants include glycolipids such as rhamnolipids and sophorolipids or lipopeptides like surfactin and polymyxin, has a function in lowering the surface and interfacial tensions, whereas the high molecular-mass biosurfactants such as lipoproteins, lipopolisaccharides and amphipathic polysaccharides are more effective at stabilizing oil-in-water emulsions.

These categories are:

I. High-mass surfactants

i) polymeric and
ii) particulate surfactants

II. Low-mass surfactants

i) glycolipids
ii) lipopeptides and
iii) phospholipids

High-mass surfactants

i. polymeric biosurfactants

Polymeric biosurfactants are high molecular weight biopolymers, which exhibit properties like high viscosity, tensile strength and resistance to shear. The following are the examples of different classes of polymeric biosurfactants. The best studied polymeric biosurfactants are compiled from several well-known components such as emulsan, liposan, manno-protein, and other polysaccharide-protein complexes (Shoeb *et al*., 2013).

ii. Particulate biosurfactants

Extracellular membrane vesicles partition hydrocarbons to form a microemulsion which plays an important role in alkane uptake by microbial cells (Monterio *et al*., 2007 and Mukherjee *et al*., 2006).Vesicles of *Acinetobacter sp*. with a diameter of 20 to 50 nm and a buoyant density of 1.158 g/cm3 are composed of protein, phospholipid and lipopolysaccharide (Kappeli *et al*., 1976).

II.Low mass surfactants

i. Glycolipids

These are the most common carbohydrate in combination with long chain aliphatic acid of hydroxyl aliphatic acid reported by Singh, V.,2008 The glycolipid can be categorised as:

- **Rhamnolipids -** widely studied biosurfactant commonly produced by *Pseudomonas aeruginosa* (Edward and Hayashi, 2003).

- **Trehalolipids** - Ristau and Wagner (1983) isolated Trehalose lipids from ***Rhodococcus erythropolis*** . Singh.V, 2008 in his studies reported that Trehalolipids are commonly associated with ***Actinomycetes***, ***Mycobacterium***, ***Nocardia*** and ***Corynebacterium***.

- **Sophorolipids** - Cooper and Paddock., 1984 reported that Sophorolipids were produced by different strains of Yeast and ***Torulopis bombicola*** and ***T. petrophilum***.

ii. Lipopeptides and Lipoprotein

Singh.V reported that a large number of cyclic lipopeptides linked to a fatty acid including Decapeptide antibiotics (gramicidins) and lipopeptide antibiotics (polymyxins) possess remarkable surface-active properties. Several bacteria are known to produce these antibiotic-like molecules particularly the cyclic lipopeptide surfactin. It is produced by *Bacillus subtilis* and is one of the most powerful and active biosurfactants It also possesses anti-bacterial, antiviral, antifungal, antimycoplasma and hemolytic activities.

iii. Fatty Acids, Phospholipids, and Neutral Lipids

Cooper, D.G., *et al*.,1978 reported that large quantities of fatty acid and phospholipid surfactants are produced by several bacteria and yeast during growth on n- alkanes. Fatty acid and phospholipid produced during growth on n-alkanes by several bacteria and yeast, has received considerable attention as surfactants. These biosurfactants are able to produce optically clear micro emulsions of alkalaines in water. Examples of microorganisms that produce these types of biosurfactant are sulphur-reducing bacteria.

Advantages of biosurfactants

There are many advantages of biosurfactants as compared to the chemically synthesized surfactants. Some of those are

i. Biodegradability
ii. Generally low toxicity
iii. Biocompatibility and Digestibility

It allows their application in cosmetics, pharmaceuticals and as functional food additives.

i. Availability of raw materials

Biosurfactants can be produced from cheap raw materials which are available in large quantities; the carbon source may come from hydrocarbons, carbohydrates and/or lipids, which may be used separately or in combination with each other.

ii. Acceptable Production Economics

Depending upon application, biosurfactants can also be produced from industrial wastes and by-products and this is of particular interest for bulk production (e.g. for use in petroleum- related technologies).

Use in environmental control

Biosurfactants can be efficiently used in handling industrial emulsions, control of oil spills, biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil.

iii. Specificity

Biosurfactants are complex organic molecules with specific functional groups and are often specific in their action (this would be of particular interest in detoxification of specific pollutants): de-emulsification of industrial emulsions, specific cosmetic, pharmaceutical, and food applications.

iv. Effectiveness

At extreme temperatures, pH and salinity. Most of the biosurfactants are high molecular-weight lipid complexes, which are normally produced under aerobic conditions. This is achievable in their ex situ production in aerated bioreactors.

Significance and role of biosurfactants to microbes

The significance and role of biosurfactants to the microorganisms, which produce them could be unique to the physiology and ecology of the producing microorganisms and it is impossible to draw any universal generalizations or to identify one or more functions that are evidently common to all microbial surfactants. Some of the properties of biosurfactants that are of some significance for microbes are as follows:

Advantages of Biosurfactants for Microbes

Adhesion

Hua *et al.*, 2003 reported that growth of the microbes on certain surfaces is influenced by the biosurfactant, which forms a conditioning film on an interface, thereby stimulating certain microorganisms to attach to the interface, while inhibiting the attachment of others. Rosenberg, 1999 studied that microorganisms can use their biosurfactants to regulate their cell surface properties in order to attach or detach from surfaces according to need.

Emulsification

Many hydrocarbon degrading microorganisms produce extracellular emulsifying agents, the inference being that emulsification plays a role in growth on water immiscible substrates. There is correlation between emulsifier production and growth on hydrocarbons. The majority of *Acinetobacter* strains produce high-molecular-mass bioemulsifiers. Barkay *et* al.,1999 described that for the growth of microbe on hydrocarbons, the interfacial surface area between water and oil can be a limiting factor and the evidence that emulsification is a natural process brought about by extracellular agents.

Bioavailability and Desorption

Biosurfactants can enhance growth on bound substrates by desorbing them from surfaces or by increasing their apparent water solubility. Surfactants that have lower interfacial tension are particularly effective in mobilizing bound hydrophobic molecules and making them available for biodegradation. Recently, it has been demonstrated that Alasan (a polymeric biosurfactant) increases the apparent solubility's of PAHs 5 to 20-fold and thus significantly increases their rate of biodegradation which was reported by Rosenberg, (1999) and Jordan *et al*.(1999).

In addition to adhesion, desorption also plays an important part in the natural growth of the microorganisms. After a certain period of growth, conditions become unfavourable for further development of microorganism e.g., toxin accumulation and impaired transport of necessary nutrients in crowded conditions. Desorption is advantageous at this stage for the cells and need arises for a new habitat. In fact mechanisms for detachment seem to be essential for all attached microorganisms in order to facilitate dispersal and colonization of new surfaces. *Puchkov et* al., 2002 reported that one of the natural roles of an emulsifier/bio- surfactant may be in regulating desorption of the producing strain from hydrophobic surfaces.

Defence Strategy

According to Puchkov., (2002) apart from two main natural roles suggested for surface-active compounds (increasing availability of hydrophobic substrates and regulating attachment and de- tachment to and from surfaces) the biosurfactants could be an evolutionary defence strategy of microbe as evidenced by high mycocidal activity of the MC secreted by *C. humicola*. Similar analogy can be made for the lipopeptides biosurfactant producing strains of *B. subtilis*. The lipopeptide (antibiotic) would have strong influence on the survival of *B. subtilis* in its natural habitat, the soil and the rhizosphere.

Potential applications of biosurfactants for mankind

Surfactants are the most important class of industrial chemicals which are used widely in almost every sector of modern industry. The following are the potential applications of Biosurfactants.

Biosurfactants and Marine Bioremediation

Microorganisms capable of hydrocarbon degradation have often been isolated from marine environments. An emulsifier produced by *Pseudomonas aeruginosa* SB30 was capable of dispersing oil into fine droplets and inferred that it may be useful in removing oil from contaminated beaches.

Biosurfactants and Soil Bioremediation

Bioremediation of soil contaminated with organic chemicals is a viable alternative method for clean-up and remedy of hazardous waste sites. The main objective in this approach is to convert the parent toxicant product into a readily biodegradable one, which is harmless to human health and/or the environment.

The biological remediation process can be performed

i. In situ;
ii. In a prepared bed; and
iii. In a slurry reactor system.

In general, biodegradation of the hydrocarbons at any given site will depend upon:

- indigenous soil microbial population,
- hydrocarbon variety and concentration,
- soil structure,
- nutrient availability,
- Oxygen availability.

Soil microorganisms reported to degrade hydrocarbons under favourable conditions include *Pseudomonas, Flavobacterium, Achromobacter, Arthrobacter*, *Micrococcus* and *Acinetobacter*. Hydrocarbons with less than 10 carbon atoms tend to be relatively easy to degrade as long as the concentration is not too high to be toxic to the organisms. Benzene, xylene and toluene are examples of gasoline components that are easily degraded. Complex molecular structures, such as branched paraffins, olefins, or cyclic alkanes, are much more resistant to biodegradation. Soil structure, which is the form of assembly of the soil particles, determines the ability of that soil to transmit air, water, and nutrients to the zone of bioactivity.

Biosurfactants in Bioremediation of Oil Pollutants

Oil pollution is an environmental problem of increasing importance. Hydrocarbon-degrading microorganisms, adapted to grow and thrive in oil-containing environments, have an important role in the biological treatment of this pollution. The rhamnolipid biosurfactant produced by *P. aeruginosa* stimulates the uptake of hydrophobic compounds finally leading to its degradation. Study has shown that the bacteria are efficient biosurfactant producers in petroleum oil-contaminated soil which offers the advantage of a continuous supply of natural, nontoxic and biodegradable biosurfactants by bacteria at low cost for solubilizing the hydrophobic oil hydrocarbons prior to biodegradation.

Application of Biosurfactant in Petroleum Industry

Indigenous or injected biosurfactant-producing microorganisms are exploited in oil recovery in oil-producing wells. Microbial enhanced oil recovery (MEOR) is often implemented by direct injection of nutrients with microbes that are able to produce desired products for mobilization of oil, by injection of a consortium or specific microorganisms or by injection of the purified microbial products (e.g., biosurfactants). Oil recovery was shown to be increased by 30-200 % with injection of biosurfactants, bacteria (e.g., *P. aeruginosa*, *X. campestris, B. licheniformis*).

Biosurfactants and oil storage tank cleaning:

Biosurfactants are also used for oil storage tank cleaning. Surfactants are used for reducing the viscosity of heavy oils which facilitates recovery, transportation and pipelining . A glycolipid surfactant reduces the viscosity of heavy crude oil by 50%. These cleaning processes are economical and less hazardous to persons involved in the process compared to conventional processes. This leads to less disposal of oily sludge in the natural environment and is an environmentally sound technology.

Biosurfactants in Medicinal and Therapeutic Industry

Biosurfactants also have some medicinal and therapeutic applications. Some of these applications are as follows:

III. **Surfactin** is one of the earliest known biosurfactants having various pharmacological applications such as inhibiting fibrin clot formation and haemolysis. It has also been reported to have an antitumor activity and anti-fungal properties. Itokawa *et al*., (1994) have provided the details about the use of surfactin against human immunodeficiency virus 1 (HIV-1).

IV. Thimon *et al*., (1995) described **Iturin** as an anti-fungal biosurfactant. It is a lipopeptide produced by ***B. subtilis***, which affects the external structure and membrane structure of yeast cells.

V. Naruse *et al*., (1990) provided the detail about the inhibitory effect of **pumilacidin** (surfactin analog) on herpes simplex virus 1 (HSV-1). They also reported the defence against gastric ulcers in vivo.

VI. Isoda *et al*., (1997) investigated the biological activities of microbial glycolipids of C. Antarctica T-34 and reported an induction of cell differentiation in the human promyelocytic leukemia cell line HL60.

VII. Kosaric (1996) describes possible applications as emulsifying aids for drug transport to the infection site, for supplementing pulmonary surfactant and as adjuvants for vaccines. Respiration failure in premature infants is caused by a deficiency in pulmonary surfactant.

VIII. The succinoyl-trehalose lipid of *Rhodococcus erythropolis* has been reported to inhibit HSV and influenza virus) was reported by Stanghellini *et al*., (1996).

Biosurfactants for agricultural use

The global concerns about pesticide pollution have encouraged the efforts to find alternative biological control technologies. Biosurfactants have potential for the biological control of zoosporic plant pathogens studies was reported by Kleckner *et al*., (1993). For the hydrophilization of heavy soils to obtain good wetting ability and also to achieve equal distribution of fertilizers and pesticides in the soils Surface active agents are needed.

Biosurfactants use in mining

For the dispersion of inorganic minerals in mining and manufacturing processes biosurfactants may be used. Also for the stabilization of coal slurries to aid the transportation of coal Kao Chemical Corporation (Japan) used *Pseudomonas*, *Corynebacterium*, *Nocardia*, *Arthrobacter*, *Bacillus* and *Alcaligenes* to produce biosurfactants.

Biosurfactants in the Cosmetic Industry

Multifunctional biosurfactants have several cosmetic applications because of their exceptional surface properties such as detergency, wetting, and emulsifying, solubilizing, dispersing and foaming effects were reported by Brown,(1991) and Piljac,(1999). The most widely used biosurfactant glycolipids in cosmetics are sophorolipids, rhamnolipids and mannosylerythritol lipids. Sophorolipids have good skin compatibility and excellent moisturizing properties, rhamnolipids are natural surfactants and emulsifiers that can replace petrochemical based surfactants used in most of the cosmetic products. Bloomberg. G, (1991) described that biosurfactants have been used in acne pads, anti-dandruff, anti-wrinkle and anti-ageing products, deodorants, nail care products and toothpastes in several different formulations, because of their high surface and emulsifying activities. Mannosylerythritol lipids are generally used in skin care formulations as the active ingredient to prevent skin roughness.

Biosurfactants use in the food industry

Biosurfactants are used as emulsifiers for the processing of raw materials in the food industry.

In food industries worldwide, Lecithin and its derivatives are currently in use as emulsifier.

- Busscher *et al*.,(1996) established that a biosurfactant produced by thermophilic dairy *Streptococcus spp* could be used for fouling control of heat exchanger plates in pasteurizers, as they retard the colonization of *S. thermophilus* responsible for fouling was reported by Van Haesendonck, I.P.H. et al., (2004).
- Biosurfactants are also utilized as fat stabilizer and anti-spattering agents during cooking of oil and fats, improve the texture and shelf-life of starch-containing prod- ucts, modify rheological properties and stability of wheat dough and act as controlling consistency in bakery and ice cream formulation.

Biosurfactant in Commercial Laundry Detergents

Almost all surfactants, an important component used in modern day commercial laundry detergents, are chemically synthesized and exert toxicity to fresh water living organisms. Furthermore, these components often produce undesirable effects. Therefore, growing public unrest about the environmental hazards and risks associated with chemical surfactants has stimulated the search for eco-friendly, natural substitutes of chemical surfactants in laundry detergents. Mulligan, C. and Cooper D.G., (1985) reported the use of Crude CLP biosurfactants showed good emulsion formation capability with vegetable oils and demonstrated excellent compatibility and stability with commercial laundry detergents favouring their inclusion in laundry detergents formulations.

Other Applications of Biosurfactants

Other important commercial and industrial applications of microbial surfactants includes using biosurfactants in paper industry, textiles and ceramics industries and paint industries due to their enhanced mixing properties. These are also used as dewatering agents in pressing peat.

Conclusion

During the recent years there is an increasing environmental awareness and therefore, it might be reasonable to assume that microbial surfactants have a promising role to play in the years to come. Considering the importance of biosurfactants, there is an urgent need to gain a greater understanding of the physiology, genetics and biochemistry of biosurfactant- producing strains and to improve the process technology to reduce production costs for commercial level production of biosurfactants. Therefore, an extensive cooperation among different science disciplines is needed in order to fully characterize the biochemical properties of biosurfactant and exploration of their potential applications in different industrial sectors. With increased efforts on developing improved application technologies, strain improvement and production processes, biosurfactants are expected to be among the most used and produced chemicals in the near future.

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