**Biosurfactants for sustainable soil management**

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**Abstract**

Biosurfactants, a diverse group of biologically-derived surface-active molecules, have garnered significant attention due to their unique properties and wide-ranging applications. Produced by microorganisms, biosurfactants exhibit exceptional surface tension reduction, emulsification, and foaming abilities, surpassing their synthetic counterparts in several aspects. This abstract provides an overview of biosurfactants, their production and their diverse applications.

**Keywords:** Biosurfactants, Microorganism

**Introduction**

Bio-surfactants are amphiphilic molecules which are mainly derived from plants and microorganisms. However, microbially produced bio-surfactants have advantages over plant-based surfactants due to scale-up capacity, rapid production, and multifunctional properties. Plant-based bio-surfactants have excellent emulsification properties, although they are expensive to produce on an industrial scale. Moreover, plant-based surfactants have other issues such as solubility and hydrophobicity. Bio-surfactants significantly affect the bioavailability and biodegradation kinetics of hydrophobic compounds (Ahmad *et al*., 2016).

Normally they increase the bioavailability of hydrophobic compounds by reducing their water repellence. Based on the chemical characteristics of the bio-surfactants, the pollutants, and the physiology of the microorganisms, both stimulating and inhibitory effects of bio-surfactants have been reported. Bio-surfactants play a physiological role in increasing the solubility and bioavailability of hydrophobic compounds and are involved in promoting the swarming motility of microorganism and in cellular physiological processes of signalling and differentiation. These bio-surfactants are also involved in biofilm formation and can also interact with various microbial proteins. In this way, they can change the enzyme conformation structure, thus, altering the enzyme specificity, activity, and functions and consequently improve the degradation of chemical contaminants. Biosurfactants may also contribute to agricultural sustainability by acting as antimicrobial agents for disease control. In this chapter, we specifically discuss the use and application of bio-surfactants for sustainable soil management with the view that bio-surfactants and bio emulsifiers can potentially play a more prominent role in the future.

**Historical perspective and current status**

Bio-surfactants are commonly known as surface active agents of biological origin and are of great benefit because of their unique properties and environment friendly nature. First a biosurfactant named “surfactant,” produced by Bacillus subtilis, was purified and characterized by Arima *et al.* (1968). Since then, several workers have investigated the bio-surfactants worldwide (Ahmad *et al.,* 2016). However, there are many aspects that are still to be understood about their functionality. Despite the fact that several types of bio-surfactants have been isolated and characterized (Banat, 1995), there are five major categories of bio-surfactants including glycolipids, phospholipids and fatty acids, lipopeptides and lipoproteins, polymeric biosurfactants, and particulate bio-surfactants having application in agriculture, pharmaceutical, food, cosmetics, and detergent industry. Bio-surfactant can meet the needs of the modern market in natural products, especially new generation surface active substances. Despite the high demand in the organic market, their production on a commercial level cannot be easily achieved due to their high cost and fermentation processes. According to one estimate, the cost of raw materials in most of the biotechnological processes including bio-surfactant production accounts for almost 30 per cent of the total production cost. Hence, there is a growing interest in utilizing low-cost renewable raw materials for such a process to make them economically feasible. Process optimization is one of the ways to make this technology economical feasible. Biosurfactants are generally ecologically safe for the environment and sustainable for use in agriculture. The relative importance of surfactant and bio-surfactants is generally indicated by the size of the markets for these materials and the rate of market growth.

**Sources of bio-surfactants**

Bio-surfactants are mainly produced by microorganisms and plants. However, microbially produced bio-surfactantsare receiving more attention due to ease in culturing, lower production cost, and greater functional properties.

1. **Plant-Based Bio-surfactants**

**Table 1: Plant based biosurfactants**

|  |  |  |
| --- | --- | --- |
| **Biosurfactant**  | **Source**  | **References**  |
| Lecithin  | Soybean oil seed, root mucilage of maize, lupin and wheat  | Read *et al.* (2003)  |
| Saponin  | Tea seed  | Wang *et al.* (2016) |
| Soybeans, broad beans, peanuts, kidney beans  | Xu *et al.* (2011)  |
| Chinese soapberry  | Zhou *et al.* (2013)  |
| Phospholipid  | Maize roots and lupin  | Read *et al.* (2003)  |
| Humic acid-like substance  | Soapnut plant  | Mukhopadhyay *et al.* (2013)  |

The most common plant-based bio-surfactants are saponins, lecithin, soy protein, and cyclodextrins (Table 1). Soybean is the source of three natural bio-surfactants: lecithin, soy protein, and soy saponin (Xu *et al.,* 2011). Among the plant-based bio-surfactants, lecithin is the most widely used and is predominantly manufactured from soybean oil seed. Read *et al.* (2003) also detected lecithin in the root mucilage of maize, lupin, and wheat. This bio-surfactant has been showing to modify the physical and chemical properties of soil. Vecino *et al*. (2014) evaluated the surface tension activity of corn steep liquor originating from the corn milling industry and extracted the bio-surfactant contained in this residue with organic solvents. Furthermore, extraction of bio-surfactants from this liquor was optimized using chloroform as a potential organic solvent. Later on, this lipopeptide surfactant was envisaged as a good remediation alternative in soil because it had a great potential to facilitate the treatment of sewage sludge contaminated with polycyclic aromatic hydrocarbon.

1. **Microbially Produced Bio-surfactants**

Microorganisms such as yeasts, bacteria, and some filamentous fungi are capable of producing bio-surfactants with different molecular structures and surface activities. Microorganisms use different carbon sources to obtain energy for growth through the oxidation process. The combination of carbon sources with insoluble substrates facilitates the intracellular diffusion and production of different substances. Recently, there has been an increased interest in the isolation of microorganisms that produce tension active molecules with good surfactant characteristics, including low critical micelle concentration, low toxicity and high emulsifying activity. The literature describes bacteria of the genera Pseudomonas and Bacillus as great biosurfactant producers; however, many other genera have also been reported for this function. For example, extracted the biosurfactant bound to the cells of *Lactobacillus pentosus* grown on hemi cellulosic hydrolysates from Grape Marc.

**Table 2: Bio-surfactant producing bacteria**

|  |  |  |
| --- | --- | --- |
| **Sl. No.**  | **Microorganism**  | **Biosurfactant**  |
| 1.  | *Pseudomonas sp*  | Ornithine lipids  |
| 2.  | *Pseudomonas fluorescens*  | Viscosin  |
| 3.  | *Pseudomonas aeruginosa*  | Rhamnolipids  |

**Table 3: Bio-surfactant producing fungi**

|  |  |  |
| --- | --- | --- |
| **Sl. No.**  | **Microorganism**  | **Biosurfactant**  |
| 1.  | *Candida antarctica*  | Mannoserthritol lipid  |
| 2.  | *Candida bombicola*  | Sophorous lipids  |
| 3.  | *Penicillium chrysogenum*  | Polyketide derivative  |
| 4.  | *Yarrowia lipolytica*  | Carbohydrate complex  |

**Table 4: Bio-surfactant producing yeast**

|  |  |  |
| --- | --- | --- |
| **SL. No.**  | **Microorganism**  | **Biosurfactant**  |
| 1  | *Debaryomyces polymorphus*  | Carbohydrate complex  |
| 2.  | *Saccharomyces cerevisiae*  | Mannanoprotein  |
| 3.  | *Pseudozyma aphidis*  | Mannoserthritol lipids  |

**Classification of Bio-surfactants**

**Based on molecular weight**

1. **Low molecular weight biosurfactants:** Examples-Glycolipids, phospholipids and lipopeptides

Low-molecular-mass biosurfactants are efficient in lowering surface and interfacial tensions, whereas high-molecular-mass biosurfactants are more effective at stabilizing oil-in-water emulsions. Low molecular weight biosurfactants have their molar mass ranging between 0.5 and 1.5 kDa, while bio-emulsifiers can reach up to 500 kDa

1. **High molecular weight biosurfactants:** Example-Polysaccharides, proteins, lipopolysaccharides, lipoproteins

**Based on charge/ nature of their polar groups**

1. **Anionic biosurfactant**: Sulfopon sodium coco sulfates
2. **Cationic biosurfactant**: Protelan AGL sodium Nα-cocoyl glutamate
3. **Amphoteric biosurfactant**: Lecithins
4. **Nonionic biosurfactant**: Mono- and diglyceride mixtures

**Biosurfactant production**

Bushnell Haas broth is used as production medium and inoculated with 24-48h old bacterial culture prepared in Nutrient broth medium or 144-168h old fungal culture prepared in potato dextrose and broth medium placed at room temperature in a shaking condition. Allow the inoculated culture to grow under optimum condition for 7-10 days. Centrifuge the culture broth at 10000 rpm for 15 min to remove the cells in order to obtain clear sterile supernatant.

**Biosurfactant recovery**

1. **Cold acetone precipitation method**

In the process three volumes of chilled acetone added to the crude biosurfactant solution and allowed to stand for 10 h at 4°C. Precipitate were collected by centrifugation at 10,000 rpm for 20 min and the resulting pellet is served as partially purified biosurfactant which is further evaporated to dryness to remove residual acetone after that dissolved it in sterile water.

1. **Acid precipitation method**

Biosurfactant can also be precipitated by adjusting pH of the cell-free broth culture to 2.0 pH using 6 N HCl and keeping it at 4°C for overnight. Pellet thus precipitated are collected by centrifugation (8000 rpm for 15 min at 20°C) and dissolved in sterile distilled water. After that pH is being adjusted at 8.0 by using 1 N NaOH for further use.

1. **Ammonium sulphate precipitation**

Ammonium sulphate precipitation is used for precipitation of high-molecular weight biosurfactants such as emulsan, bio-dispersion (protein rich compounds). As per the type of biosurfactant, a different concentration of ammonium sulphate is being used. In case of ammonium sulphate precipitation, the rhamnolipid is precipitated by salting out process and the product is further purified by a dialysis procedure and lyophilized.

**Screening of Isolates for Biosurfactant Production**

1. **Drop collapse test**

The drop collapse test is an easy and fast procedure to asses a microbe for biosurfactant production. This test does not require any special equipment, only a small volume of microbial sample is required. Drops of oil placed on the slide and then add 10μl of the microbial sample by piercing the drop using micropipette without disturbing the dome shaped of the oil. If the drop collapsed within 1 min is considered to be positive for the drop collapse test.

1. **Blood agar haemolysis**

This is used for preliminary screening of microorganism for the ability to produce biosurfactants. Blood agar plate containing 5 per cent sheep blood is used for test of haemolytic activity. Positive strains will cause lysis of the blood cells and exhibit a colourless, transparent ring around the colonies. Therefore, those microorganisms which show positive blood haemolysis are considered as biosurfactant producers. The basic principal approach of the followed screening that biosurfactants cause lysis of erythrocytes. The assay also predicts about the surface activity of biosurfactant producing microorganisms. As per recommendation the blood agar method as a preliminary screening method which should be supported by other techniques based on surface activity measurements.

1. **Oil displacement activity**

This is rapid and easy method to carry out requires a small volume of sample. Due to its high precision, it is applied when the activity and quantity of biosurfactant is low. The biosurfactant producing organism displaces the oil (increase in diameter) and spread in the water. The increase in the diameter of oil is measured due to activity caused by surfactant. If biosurfactant is present in the supernatant, the oil is displaced and a clearing zone is developed. The diameter of this clearing zone on the oil surface correlates to surfactant activity. This technique is also known as oil spreading technique.

1. **CTAB agar plate method**

The CTAB (Cethyl trimethyl ammonium bromide) agar plate method was developed by Siegmund and Wagner (1991). It is a semi-quantitative assay for the detection of extra cellular glycolipids or other anionic surfactants. The CTAB agar assay is a comfortable screening method, specific for anionic biosurfactants. The disadvantage of this method is that CTAB is harmful as it inhibits the growth of some microbes. Cethyl trimethyl ammonium bromide contains the cationic surfactant. In the process microbes growing on the plate secrets anionic surfactants which form a dark blue, insoluble ion that pair with cethyl trimethyl ammonium bromide and methylene blue (basic dye). Thus, productive colonies are surrounded by dark blue halos.

**Applications of bio-surfactants**

* Bioremediation of contaminated agricultural soil.
* Plant growth promotion by elimination of phytopathogens.
* Application in pesticide industries.

**Advantages of bio-surfactants**

* Biodegradability, Low Toxicity
* Biocompatibility
* They can be produced from cheap raw materials that are easily available in large quantities
* It exhibits emulsification capacity
* Specificity
* Tolerance to temperature, pH and ionic strength
* They are ecologically accepted due to their property of maintaining sustainability
* Low critical micelle concentration

**Disadvantages of bio-surfactants**

* Expensive large-scale production
* Difficulty in obtaining pure substances
* Strong foam formation

**Conclusion**

In conclusion, biosurfactants represent a remarkable class of natural molecules with immense potential and versatility. These biologically-derived surface-active compounds offer a wide array of benefits over their synthetic counterparts, making them attractive for various industrial applications.

One of the most significant advantages of biosurfactants is their eco-friendly nature and low toxicity, which aligns with the growing global focus on sustainability and environmental protection. Their production by microorganisms, such as bacteria and fungi, offers a sustainable alternative to chemical surfactants, reducing the environmental burden associated with conventional surfactant manufacturing. In agriculture, biosurfactants can enhance nutrient availability and soil remediation, promoting sustainable and efficient farming practices. Their potential to support microbial interactions and biofilm formation enhances biodegradation processes, contributing to environmental cleanup and pollution mitigation.

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