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**FACTORS AFFECTING BIOSURFACTANT AND THEIR APPLICATIONS**

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**ABSTRACT**

Biosurfactants are surface active compounds that lower interfacial tension between liquids and solids. Their special properties, such as nontoxicity, biodegradability, environmental friendliness, and high stability, as well as a wide range of industrial applications, make them a very useful group of chemical compounds. Biosurfactants are made by a wide range of microorganisms. The aim of this study was to isolate and characterise biosurfactant-producing bacteria from oil-contaminated soil, as well as to investigate their growth kinetics at various temperatures and pH levels. The isolation and growth analysis was done in MSM composition with kerosene oil as the sole carbon source for the bacteria. Gram positive bacteria can produce lipopeptides form biosurfactant, and the isolated strains B1 and B2 were found to be Gram positive. Bacilli strains were isolated. pH 7 and temperature 30°C were found to be the best conditions for bacterial growth for strain B1 and pH 8 and temperature 30°C for strain B2.

**Keywords: Biosurfactant, Biodegradable, Microorganism**

**INTRODUCTION**

Surfactants are surface active compounds that lower interfacial tension between liquids and solids. Surfactants are organic compounds that contain both hydrophobic

and hydrophilic moieties (the head and tail of the surfactant). As a result, surfactant comprises both water insoluble (water repellent) and water soluble (water loving) groups. Biosurfactants, like chemical surfactants, are surface active compounds, but unlike chemical surfactants, biosurfactants are produced by microbes such as bacteria, fungi, and yeast. Biosurfactants are non-toxic and biodegradable and have the properties of lowering surface tension, stabilising emulsions, and facilitating foaming. Biosurfactants have recently gained popularity due to their versatility, operational flexibility, and environmental friendliness [1, 2]. Furthermore, the ability to produce them on a wide scale, selectivity, success under extreme conditions, and potential applications in environmental fortification have all piqued the interest of scientists. These molecules could be used in cosmetics, pharmaceuticals, humectants, food preservatives, and detergents, among other industries (1). However, due to the high cost of synthetic media for microbial development, industrial production of biosurfactant remains a challenge. Biosurfactants are categorised according to their structural diversity and microbial origin. Biosurfactants produced by a variety of microorganisms, primarily bacteria, fungi, and yeasts, vary in chemical composition and nature, and the amount produced

depends on the type of microbes. As a result, a broad range of biosurfactants can be generated to meet demand and applications.

### **Biosurfactant and microorganism involved:**

Biosurfactant	Microorganism
Glycolipid	<i>Pseudomonas aeruginosa</i>
Rhamnolipids	<i>Rhodococcus erythropolis</i>
Trehalose lipids	<i>Arthobacter sp.</i>
Sophorolipids	<i>Candida bombicola</i> , <i>C.apicola</i>
Mannosylerythritol lipids	<i>C.antartica</i>

### **Classification of biosurfactant:**

The ionic charge in the polar part of the molecule is used to classify synthetic surfactants. An electrical charge's presence or absence Surfactants are classified as anionic, cationic, non-ionic, or amphoteric in Biodegradation of Vegetable Waste for Cellulase Production [5]. The majority of biosurfactants are anionic or neutral, with just a few exceptions, such as those containing amine groups, being cationic. Longchain fatty acids make up the hydrophobic moiety, while the hydrophilic moiety may be a starch, amino acid, cyclic peptide, phosphate, carboxyl acid, or alcohol [6].

Biosurfactants are generally categorised based on their biochemical composition or the microbial species that create them. These compounds are divided into five classes based on their composition [7].

- Glycolipids – polarity is determined by the hydrocarbons used as a substrate;

examples include rhamnolipids produced by *Pseudomonas aeruginosa* and sophorolipids produced by *Candida* organisms.

- Lipopolysaccharides – molecules with a high molecular mass that are soluble in water; for example, emulsan, an extracellular emulsifier formed by the bacteria *Acinetobacter calcoaceti* from hydrocarbons.

Lipopeptides: Surfactin produced by *Bacillus subtilis* (one of the most potent biosurfactants published in the literature) is an example of lipopeptides.

- Phospholipids – structures found in several microorganisms; for example, *Corynebacterium lepus* biosurfactant; Fatty acids, neutral lipids (some of which are glycolipids), and hydrophobic proteins are all examples.

### Properties of biosurfactant

Biosurfactants are amphiphilic compounds with hydrophilic and hydrophobic portions, which are referred to as the head and tail, respectively. Hydrophobic groups are referred to as lyophobic in aqueous media, whereas hydrophilic groups are referred to as lyophilic [8]. Low toxicity, bioavailability, biodegradability, high foaming, environment-friendly, low cost in terms of feedstock availability in nature, pH, and salinity are only a few of the advantages they have over synthetic chemical-derived surfactants. As a result,

they are a safer and more effective alternative to chemical surfactants in a variety of applications, including food, pharmaceuticals, cosmetics, and edible oils [9]. In terms of toxicity and mutagenic properties, a biosurfactant from *P.aeruginosa* was compared to a synthetic surfactant commonly used in the industry. The chemically derived surfactant was found to have a higher degree of toxicity and mutagenic effect in both assays, while the biosurfactant was found to be marginally non-toxic and non-mutagenic. Surface movement, resilience to pH, temperature, and ionic quality, biodegradability, low poisonous quality, emulsifying and demulsifying capability, and antimicrobial action are all characteristics of microbial surfactants [10].

### Industrial application of biosurfactant

Biosurfactants are used in a variety of industries, including cosmetics, pharmaceuticals, food, petroleum, wastewater, irrigation, textiles, painting, and a variety of others. Furthermore, these compounds are well-known as multifunctional agents [11], with properties such as stabilising, wetting, antimicrobial, moisturising, emulsifying, and anti-adhesive properties.

### Application of biosurfactants in different industries

Body washes, hair care, lotions, eye shadow, acne cream, lip colour, deodorants, anti-wrinkle products, skin smoothing, antimicrobial agent, antifungal agent, anti-adhesive agents, anti-cancer agent, antiviral agent, foaming agents [12]. Antimicrobial agent, antifungal agent, anti-adhesive agents, anti-cancer agent, antiviral agent, foaming agents

- Food industry: Emulsifying agents, anti-adhesive agents, anti-microbial agents, and food preservation agents are all used in the food industry [13].
- petroleum industry[ : Emulsifiers, demulsifiers, oil recovery enhancers, and transportation aids are all used in the petroleum industry [13].
- Wastewater treatment: biocomposite agent, bio-adsorbent for heavy metal removal [13].
- Textiles: lubricant, scouring agent, levelling agent, and bleaching aid [14].

#### **Applications of biosurfactants in contaminated soils**

Because of their complex structure, enhanced physicochemical properties, environmentally friendly features, and suitability for a number of purposes, including the remediation of hydrophobic organic compounds (HOCs) from soil and

the removal of heavy metals from polluted soil, most recent studies have centred on the environmental applications of biosurfactants. Heavy metal is contributing to major environmental issues. Lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), zinc (Zn), copper (Cu), and nickel (Ni) are the most common heavy metals found in polluted soils, and they can cause a range of health problems for humans, livestock, and plants [13]. The sorption of biosurfactant molecules at the interfaces between sludge (wet soil) and metal in aqueous solution separates heavy metals adsorbed on the surface of soil particles. Biosurfactants absorb the metal, which is then trapped inside the micelle by electrostatic interactions.

#### **Biosynthesis and Regulation**

The hydrophobic and hydrophilic moieties are synthesised using the main metabolic pathways of hydrocarbons and carbohydrates, respectively [14]. These two classes of precursors have different metabolic pathways that use different enzymes. The first enzymes in many cases are regulatory enzymes. As a result, despite their variety, these precursors are synthesised and controlled using a number of common principles. In the literature, metabolic pathways have been extensively studied and documented [15]. List the following options for the synthesis and

control of a biosurfactant's two moieties [16].

- a) The hydrophilic and hydrophobic moieties are synthesised by de novo synthesis by two separate pathways;
- b) the hydrophilic moiety is synthesised by de novo synthesis while the hydrophobic moiety is induced by the substrate;
- c) the hydrophobic moiety is synthesised by de novo synthesis while the hydrophilic moiety is based on the substrate.
- d) both moieties' synthesis is interdependent.

#### **Factors affecting biosurfactant production:**

The factors that influence biosurfactant development can be divided into two categories: environmental and nutritional. Microbe development, as well as the quality and quantity of their products, are influenced by pH temperature and oxygen availability [17]. There are numerous reports suggesting that the structure and development of this secondary metabolite is affected by microbial growth.

• **Environmental factors:** Since they have been shown to influence cellular growth and function, environmental factors such as pH, temperature, agitation, and oxygen availability are critical for microbial growth and biosurfactant production. *Pseudomonas* species developed the most biosurfactant in

the pH range of 6.0 to 6.5, but it fell sharply above pH 7 [18].

• **Dietary factors:** *P. aeruginosa's* complex metabolic capabilities necessitate the use of a wide range of substrates, some of which are difficult to degrade by other organisms [19].

#### • **Source of carbon**

In the production of biosurfactant by microbes, the carbon source used in the culture medium plays an important role. It is divided into three categories: carbohydrates, hydrocarbons, and vegetables. Water immiscible substrates such as alkanes, fuel oil, gasoline, crude oil, olive oil, or oily sludge generated significantly less biosurfactant. *B. licheniformis* has been shown to produce more biosurfactant when grown on mineral salt medium supplemented with glucose and yeast extract. Because of the biosurfactant product, the surface tension of the medium was reduced from 70 to 28 mN/m.

#### • **Phosphorus and other forms of nitrogen**

Biosurfactant processing is influenced by medium constituents other than carbon sources. When the culture reached nitrogen limitation after 30 hours of development, rhamnolipid production began and continued to increase until 60 hours of fermentation [20]. When growth slowed due to nitrogen limitation, a simultaneous

increase in biosurfactant production and glutamine synthetase activity was observed in *P.aeruginosa*. It was noted that the nitrogen source had no significant impact on biosurfactant production by *P. aeruginosa*, and that the amount of nitrogen source present in Bushnell-Hass medium was adequate for optimum biosurfactant production [21]. Odium nitrate was identified as the best nitrogen source for biosurfactant production (0.34 g/l) by *P. aeruginosa* [22]. The final culture pH calculated after 3 days suggested the high biosurfactant yield resulted from the prevention of pH decrease. fed-batch activity under nitrogen limitation conditions was found to be a promising method for biosurfactant production, with a 3.8-fold increase in biosurfactant.

#### **Advantages and Uses of Biosurfactant:**

Biosurfactant (Microbial Surface Active Agents) differs from chemical synthetic surfactant because of its specific properties, such as low toxicity, relative ease of preparation, and widespread applicability. It has recently emerged as an important biotechnology commodity for industrial and medical applications, allowing it to replace chemical synthetic surfactant. They can be used as emulsifiers, de-emulsifiers, wetting agents, spreading agents, foaming agents, functional food ingredients, and detergents in a variety of industries, including Petroleum and Petrochemicals,

Organic Chemicals, Foods and Beverages, Cosmetics and Pharmaceuticals, Mining and Metallurgy, Agrochemicals and Fertilizers, Environmental Control and Management, and so on.

Biosurfactants have a number of benefits over chemically synthesised surfactants. Here are a few examples:

- 1) **Biodegradability:** As compared to chemical surfactants, it is really biodegradable [10].
- 2) **Low toxin levels**
- 3) **Biocompatibility and digestibility,** which enables them to be used in cosmetics, pharmaceuticals, and seasonings for food.
- 4) **Easily accessible raw materials:** The raw materials required for the production of biosurfactant are readily available; biosurfactant-producing microorganisms can be isolated from industrial waste, such as oil-contaminated soil and petrol-pump spills, as well as municipal waste.
- 5) **Use to monitor the atmosphere.** Industrial emulsions, oil spill prevention, biodegradation and toxicity reduction of industrial discharges, and bioremediation of contaminated soil may all benefit from biosurfactants.
- 6) **Biosurfactants are also unique in their action because they have a**

specific organic functional group. This is especially useful for lowering pollutant toxicity, improving emulsification properties, and serving as a raw material in cosmetic, medicinal, and food applications.

## CONCLUSION

Using kerosene oil as the sole carbon source, a biosurfactants-producing bacterial strain was isolated and screened from oil soil. For the screening of biosurfactant-producing bacteria, oil spreading technique, blood hemolysis test, foaming activity, and emulsification activity were used, and positive oil spreading technique was detected in bacterial strains B1 and B2. The isolated bacterial strains B1 and B2 are both Gram-positive and have a rod or circular shape, respectively. The isolated strain may be bacillus type bacteria based on the results of various biochemical and cell morphological characterizations. Bacillus type bacteria and Gram-positive bacteria can make lipopeptides type biosurfactant, according to a review of the literature [7]. Isolated bacteria can be used to extract biosurfactants of the lipopeptides type. The growth of bacterial strains is dependent on pH and incubation temperature, according to a study of growth kinetics. For bacterial strain B1, maximum growth was found at pH 7 and 30°C, while it was found at pH 8 and 30°C for bacterial

strain B2. Based on a review of the literature, it was discovered that bacterial strains like bacillus grow best at pH 7-8 and temperatures of 30-35 °C.

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