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Review Article

Microbial Biosurfactants: An Overview of their Uses, Classification, Types, Properties, and Biosynthesis

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Abstract

Various environmental problems are established because of the accidental oil leakage as well as the hydrocarbon waste disposal on the land, which makes the surrounding area polluted and can cause damage to the ecosystem. Remediation must be carried out in order to repair these types of damages. Several oil-degrading microorganisms exist in the environment, and they also produce biosurfactant as a secondary metabolite. Biosurfactants are surface-active agents with an amphiphilic nature. These compounds are classified on the basis of their microbial origin and molecular weight. Among all the types of biosurfactant, rhamnolipid and surfactin are the most widely known, which are produced by *Pseudomonas aeruginosa* and *Bacillus subtilis*, respectively. Biosurfactants have more beneficial properties than synthetic surfactants, like being less toxic, easily biodegradable, and eco-friendly. They can also survive in extreme conditions and can tolerate high salt levels. Microbes show different metabolic pathways for biosurfactant production according to substrate, given that either it is water soluble or water insoluble hydrocarbons. Biosurfactants also have potential applications in various fields. In this review work, we attempted to describe the classification of biosurfactants, their kinds and qualities, as well as their biosynthetic pathways. Biosurfactants have many applications in bioremediation fields; they also have advanced uses as bioemulsifiers in food biopreservation.

Keywords: Oil Spilling; Hydrocarbon; Pollution; Oil Degrading Microorganism; Rhamnolipid; Metabolic Pathway

Introduction

Biosurfactants, widely known as surface-active agents of biological origin, have carved a niche for themselves in the market due to their unique environment-friendly properties. They have come a long way since first biosurfactant "surfactin" was purified and characterized by Arima., *et al*. (1968). Surfactants with microbiological origins are typically referred to as biosurfactant [24]. Many microorganisms are known to create biosurfactants in significant relative amounts, and extracellular synthesis accounts for the majority of biosurfactant production by microbes. Some have business-related appeal [39]. Biosurfactants are secondary metabolites produced by microorganisms. By adjusting environmental variables and growing conditions, biosurfactant production may be kept at a high level [8].

A significant amount of trash is produced and released into the environment as a result of the increased use of crude oil and other oil-related products [24]. These pollutants cause further harm to the terrestrial and aquatic ecosystems by containing hazardous heavy metals and petroleum hydrocarbons. The greatest barrier to recovering contaminated habitats is thought to be their rising levels and lingering leachates [2].

People all around the world exploit various natural resources through exploration and intervention operations as a result of technological advancement. The world's present economy is increasingly reliant on crude oil-related goods like kerosene, diesel, gasoline, fossil fuel excavation, and different agricultural, pharmaceutical, and chemical items. Unfortunately, due to the careless use of resources like solvents, chemicals, and heavy metals, some interventions have now become harmful to the environment [10]. Also, they produce undesirable compounds or pollutants that have the potential to negatively influence both terrestrial and aquatic ecosystems, as well as people and other animals. Unwanted toxic chemicals that are present in the environment can harm people by absorbing through the skin, inhaling, or building up in bodily parts [46].

Nowadays, oil tankers are used for the majority of oil transportation, and ocean ship trafficking resulted from this issue. These traffic often causes accident in waters and cause oil leakage and damages the marine ecosystem as well as various aquatic species. Marine petroleum hydrocarbon pollution has led to serious environmental and health issues [31]. A leak of nearly 300,000 gallons of marine fuel oil happened on January 18, 2000, at Guanabara Bay, Rio de Janeiro, Brazil, reaching Guapimirim Environmental Protection Area mangroves. Mangrove sediments are abundant in organic matter due to their low hydrodynamics and great biodiversity. Due to their large molecular weight, hydrophobicity, and solid state, petroleum hydrocarbons have a propensity to adsorb organic materials from surfaces, such as mangrove sediments [40]. As a result, these ecosystems are extremely vulnerable to hydrocarbon pollution [19]. Also, this oil leak affects human life by contaminating beaches, forests, and wildlife habitats from the ocean to the soil. As the buildup of pollutants in animal and plant tissue may result in death or mutation, soil pollution with hydrocarbons causes significant harm to the surrounding ecosystem. Low-molecular-weight hydrocarbons are volatilized whereas polar components are dissolved in water when oil spreads in an environment. Due to their limited solubility, the majority of oil hydrocarbons, however, stay on the water's surface or cling to soil particles. The ultimate and total deterioration of oil is mostly carried out by microbiological activity, with evaporation and photo-oxidation playing a significant part [37]. Also, this oil leak affects people indirectly by entering the food chain and building up in fish, fruits, and vegetables, increasing toxicity, and it may even be fatal [1]. In other words, a complete ecosystem becomes poisoned over a longer period of time by oil spills [43,49].

Now days, in most developing nations, heavy metals from sewage and industrial effluents cause major water contamination. The ongoing infiltration and contamination of heavy metals into natural water sources may significantly increase the likelihood that humans will come into contact with these metals through ingestion, inhalation, or skin contact. Over time, this contact may cause liver damage, cancer, and other serious conditions.

The largest environmental disaster in Brazilian history occurred in August 2019 when oil spilled accidentally in Brazil and impacted more than 3000 kilometers of the northern and southern coastlines [21]. In Mexico in 2010, there was a second oil leak that resulted in the deaths of thousands of fish, sea turtles, marine animals, and birds [29].

The area of Nigeria where oil is produced is called the Niger Delta. It is a marsh where the bulk of the population makes a living via fishing and subsistence farming. Many virgin arable areas have become contaminated with hydrocarbons as a result of the exploration and extraction of crude oil in this area, making the land unusable for farming [27]. Due to the direct effects of hydrocarbon contamination on the economic, social, and health welfare of the local inhabitants, this area is now tumultuous. Throughout the area, an estimated 400,000 metric tons of oil have been spilled, and less than 70% of that oil has been collected, according to the United Nations Development Programme (2006) [26].

The Exxon Valdez disaster in 1989, the British Petroleum Deep water Horizon spill in 2010, the catastrophe in Dalian, China, in 2010, and others are examples of hydrocarbon spills that have had serious effects on the whole world [46]. According to Readman., *et al.* [33] and Montagnolli., *et al.* [22], oil output might reach 3 billion metric tonnes per year, and accidents result in the loss of roughly 2 million metric tonnes of hydrocarbons. Obviously, with the serious social and economic effects of oil spills, there is a need to deploy alternate cleanup technologies that would not have a negative impact on the environment [27].

Gasoline and oil, aromatic hydrocarbons, and polycyclic aromatic hydrocarbons are the three categories of hydrocarbon compounds included in the regulation of the Polish Ministry of Environment about the assessment of the earth's surface contamination. Every time a release of hydrocarbons into the environment occurs, there is a major interference, and this group of chemicals primarily ends up in the soil and water habitats [47,49].

Petroleum-derived chemicals in the soil prevent gas exchange, lower the oxygen content in the soil's air and water, alter the homeostasis of the soil environment, and, as a result, impede or even kill edaphon and flora growth in the polluted region. Moreover, chemicals generated from petroleum, particularly Polycyclic Aromatic Hydrocarbons (PAHs), have hazardous, carcinogenic, and mutagenic properties. Skin cancer and systemic malignancies, including bladder, stomach, and lung cancer, are made more likely by PAH exposure [17].

Technology for oil pollution treatment has grown in relevance on a global scale. As most hydrocarbons are insoluble in water, bacteria that can break them down are crucial in the fight against environmental pollution. There is a popular belief that organic contaminants, such as crude oil, provide bacteria with an unfavourable environment in which to grow. However, despite its extreme toxicity and hydrophobicity, accumulating research has shown that crude oil contains biological microorganisms, primarily bacteria. A wide spread of microbial diversity, including *Pseudomonas, Bacillus, Streptomyces,* and *Stenotrophomonas* species, was linked with oil wells [48]. By producing active surfactants, these organisms have the capacity to encapsulate heavy metals and/or hydrocarbons, as well as breakdown a variety of hydrocarbons for use as carbon sources [44].

Mineralization is regulated by soil hydrocarbon desorption. Surfactants can enhance the surface area of hydrophobic chemicals, including insecticides, in water and soil settings, enhancing their water solubility [23]. Surfactants may thereby hasten the degradation of pollutants by bacteria. Utilising biosurfactants to speed up the breakdown of pesticides in soil and water settings has become more crucial in recent years. Therefore, rather than listing the various types of biosurfactants and their characteristics, this study concentrates on the production, characterization, surface tension reduction capability, antimicrobial activity, role in the removal of hydrocarbons from the environment, and effectiveness in the removal of metals [2,40].

Currently, surfactants have been used in the cleanup of the hydrocarbon spill sites. Surfactants are now being employed to clean up the hydrocarbon spill areas. On the other hand, the surfactant has harmful effects on the environment [47]. Certain chemical surfactants have qualities like foaming, lower surface tension, amphiphilic nature, include both hydrophobic and hydrophilic groups, are anti-adhesive, etc. that make them useful for removing oil pollution, but they also have negative impacts on the environment. It also contains hazardous components; as a result, it is necessary to replace synthetic surfactants with eco-friendly, non-toxic alternatives to chemical ones [2].

Biosurfactants, as opposed to synthetic surfactants, offer benefits. High biodegradability, low toxicity and irritancy, compatibility with human skin, and the capacity to maintain activity in adverse conditions are only a few of the benefits that biosurfactants have [6,8,24,28].

The advantages that biosurfactants have over chemically produced alternatives include the following:

- **Biodegradability**: The chemicals are readily destroyed and do not linger in the environment due to their low toxicity and simple chemical structure, which prevents issues by means of buildup [18].
- **Biocompatibility and digestibility**: Because of their biological origin, these substances have an innate compatibility that permits their unrestricted use in medications, cosmetics, and food additives [2,18].
- **Raw material accessibility**: Biosurfactants can be made from readily accessible, reasonably priced raw materials. For microbial production, the carbon sources hydrocarbons, carbohydrates, and lipids - can be employed singly or in combination [18,32].
- **Appropriate production economics**: When allowed by the intended application, biosurfactants may still be generated in large quantities from industrial wastes and byproducts (for use in petroleum recovery) [18].
- **Environmental control**: The use of biosurfactants can be advantageous in processes for stabilising industrial emulsions, preventing oils pills, biodegrading and detoxifying industrial effluents, and bioremediating polluted soil [2,18].

- **Specificity**: Unique functional groups are included into the biosurfactant molecules to enhance their unique functions. This capability may be essential for the de-emulsification of industrial emulsions, the detoxification of specific pollutants, the production of specific cosmetic goods, and the creation of specialised medical and food applications. One of the most crucial characteristics of these microbial compounds is their effectiveness at extremes in temperature, pH, and salinity [32]. The unique structure that differs dramatically from the typical surfactants can promote alternative usage for which the regular surfactants are useless. The characteristic that distinguishes biosurfactants from conventional surfactants is that they reduce surface and interfacial tension using the same mechanisms. Unlike artificial ones, these chemicals may be produced via microbial fermentation processes using less expensive agro-based substrates and waste products [18].

In recent years, a great deal of research has been done on the creation of biosurfactants by diverse microorganisms. Also, a number of elements of biosurfactants have lately been examined, including their biological and therapeutic qualities, natural functions, manufacturing utilising affordable alternative substrates, and economic possibilities.

Biosurfactant

During microbial growth, a set of surface-active molecules with a variety of structural characteristics is produced. Extracellular substances known as bio-surfactants are produced by microorganisms that feed on decomposing hydrocarbons. Biosurfactants are secondary metabolites and amphiphillic in nature. Surfactants are substances with surface activity that lessen the friction at the interface of two liquids or between a liquid and a solid. Surfactants are organic compounds with hydrophobic (the surfactant's tail portion) and hydrophilic (the surfactant's head portion) moieties. Therefore, a surfactant has both a water soluble, or water loving, group and a water insoluble, or water repelling, group [39,42].

Aerobic bacteria in aqueous environments primarily create microbial biosurfactants to aid in the translocation of insoluble substrates across cell membranes, which helps the microorganism proliferate [38]. The microorganisms used in the production of biosurfactants range in molecular weight from low to high and come from a variety of genera, including *Clostridium, Brevibacterium, Pseudomonas, Rhodococcus, Acinetobacter, Thiobacillus, Bacillus, Leuconostoc, Lactobacillus, Enterobacter, Saccharomyces, Aspergillus, Ustilago* and *Penicillium* [7].

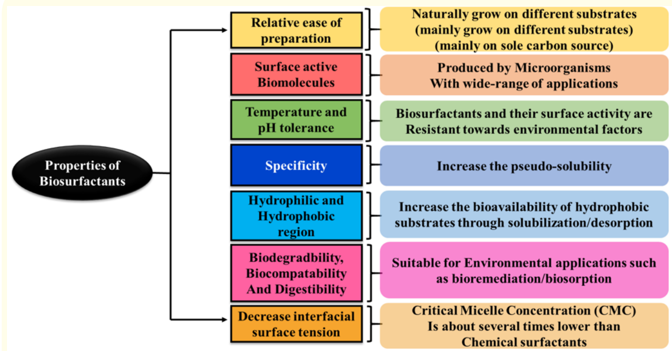

Figure 1: Properties of Biosurfactant.

Classification

Biosurfactants are classified on the basis of the diversity of their structures and their microbial origins. They contain a hydrophilic group, that contain an acid, peptide cations, or anions, mono-, di- or polysaccharides and a hydrophobic group of unsaturated or saturated hydrocarbon chains or fatty acids [34]. Biosurfactants' level of solubility in water is a result of their hydrophilic component. The capillary action is caused by the lipophilic component. The two components are attached to one another by one of the following mechanisms: (a) ester linkage (including lactones with organic and inorganic acids); (b) amide linkage (single and peptide); or (c) glycosidic linkage (sugar-sugar and sugar-hydroxy fatty acids). Surface activity is significantly influenced by the ionisation of functional groups, particularly when simple carboxylic acids are acting at the water-oil interface. Biosurfactants can be classified logically depending on their molecular weight or charge [41].

Classification based on molecular weight

- **Low-molecular-weight biosurfactants:** These substances work at the air/water interface to reduce surface and interfacial tension. Glycolipids or lipopeptides are typically

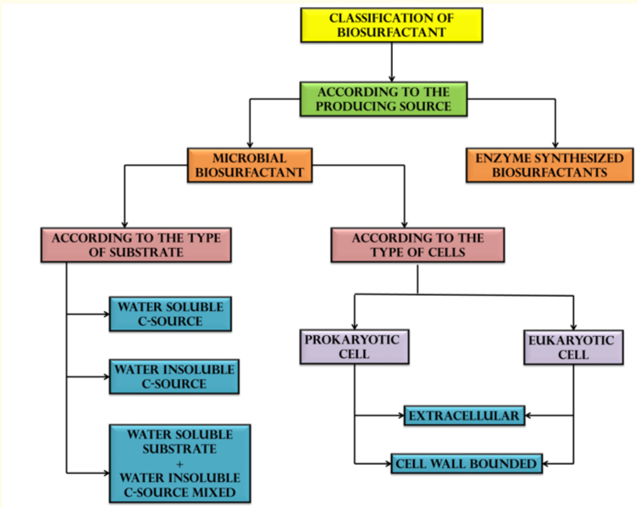

Figure 2: Classification of Biosurfactant.

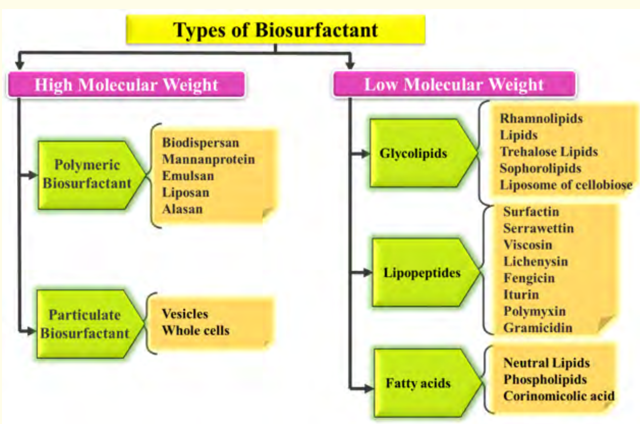

Figure 3: Types of Biosurfactant.

the low molecular weight biosurfactants. Rhamnolipids, trehalolipids, and sophorolipids are disaccharides that have been acylated with long-chain fatty acids or hydroxy fatty acids and are the glycolipids that have undergone the most research [5].

- **High-molecular-weight biosurfactants:** Also known as bioemulsans in most cases. They work better at stabilising emulsions of oil and water. These are extremely effective emulsifiers with great substrate specificity that function at low concentrations. According to Ron and Rosenberg, several bacterial species from various genera create exocellular polymeric surfactants made of proteins, lipopolysaccharides, polysaccharides, or complicated combinations of these biopolymers. Most biosurfactants are either anionic or neutral when categorized based on their polar groupings, with the hydrophobic moiety made up of long chain fatty acids or fatty acids derivatives, and the hydrophilic part made up of carbohydrates, amino acids, phosphate, or cyclic peptides. According to Desai and Banat, a biosurfactant's structure typically consists of a hydrophilic moiety made up of amino acids or peptides, anions or cations, mono-, di-, or polysaccharides, and a hydrophobic moiety made up of derivatives of unsaturated, saturated, or fatty acids [9].

Classification based on chemical composition:

Glycolipid

The majority of biosurfactants are glycolipids. One or more carbohydrates may be combined with one or more fatty acids, hydroxy fatty acids, or fatty alcohols to form glycolipids. They are the most promising for commercial production and usage because of their high production yield and the ability to employ renewable resources for this. The best researched glycolipids are those produced by *Pseudomonas* sp. [13]. The most well-known glycolipids are sophorolipids, trehaloselipids, and rhamnolipids.

Rhamnolipids

Rhamnolipids are glycolipids that include one or two rhamnose molecules connected to one or two hydroxydecanoic acid molecules. They are widely studied and commonly produced by *Pseudomonas aeruginosa* [20].

Sophorolipids

They are glycolipids made by yeasts, and they are composed of a dimeric carbohydrate called sophorose coupled by a glycosidic bond to a long-chain hydroxyl fatty acid. For many applications, the lactone form of sophorolipids typically a combination of at least six to nine distinct hydrophobic sophorolipids is preferred [3].

Trehaloselipids

This is a different category of glycolipids. Most varieties of *Mycobacterium, Corynebacterium*, and *Nocardia* are associated with the disaccharide trehalose, which is linked at C-6 and C-6 to mycolic corrosive. Long-chain, widely dispersed, and hydroxy unsaturated lipids are known as mycolic acids. Trehalolipids from various living things differ in terms of mycolic corrosive size and structure, the number of carbon molecules they contain, and the level of unsaturation. Trehalose lipids obtained from *Arthrobacter* sp. and *Rhodococcus erythropolis* reduced the interfacial strain and surface pressure in the culture stock [25].

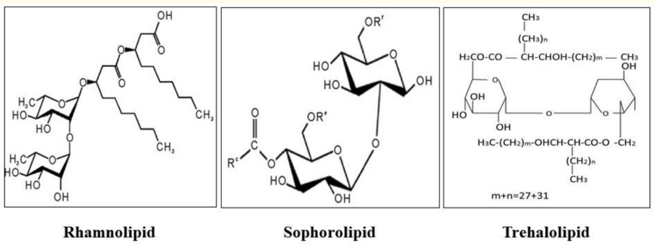

Figure 4: Structures of Glycolipid type of Biosurfactant.

Lipopeptides and lipoproteins

Cyclic substances known as lipopeptide biosurfactants are mostly obtained from bacteria of the Bacillus and Pseudomonas species. Hydrophilic peptides, which are typically between 7 and 10 amino acids long and coupled to a hydrophobic fatty acid structure, make up the majority of lipopeptides. The Surfactin, Iturin, and Fengycin families are the three main subgroups of the Bacillus cyclic lipopeptides. The most often researched protein is surfactin, which has seven cyclic sequences of amino acids coupled to a C13-C16 fatty acid [14].

Surfactin

One of the most promising biosurfactants is surfactin, a cyclic lipopeptide produced by the bacteria *Bacillus subtilis*. It is composed of a fatty-acid chain connected to a seven-amino-acid ring structure by a lactone bond [32].

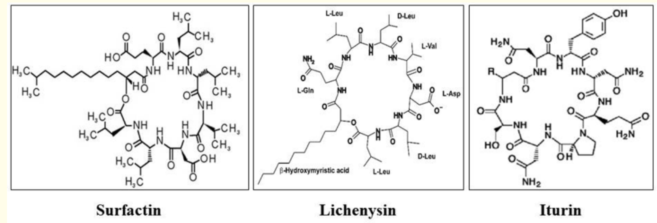

Figure 5: Structures of Lipopeptide type of Biosurfactant.

Iturin

Iturin is a member of the second family of lipopeptides that are made by some strains of the *Bacillus subtilis* genus and other closely related Bacillus species, such *B. amyloliquefaciens*. At the beginning, iturin was isolated from a soil sample taken in Ituri, Zare (now known as the Democratic Republic of Congo) [30].

Lichenysin

The bacterium Bacillus licheniformis produces a number of biosurfactants that work in concert and have excellent temperature, salt, and pH stability. They also resemble surfact in in terms of their structure and physio-chemical characteristics [35].

Neutral lipids, phospholipids, and fatty acids

Many yeasts and bacteria, including *Acinetobacter sp*., create phosphatidylethanolamine-rich vesicles that dissolve alkanes in water to form optically transparent micro-emulsions. Byproducts of microbial oxidation from alkanes include fatty acids and phospholipids, which are regarded as biosurfactants [14]. While fatty acids are extensively employed in the food sector, gene carrier systems have found usage for phospholipids because of their membrane nature. Phospholipid biosurfactants include, for example, lecithin and lysolecithin [29,36].

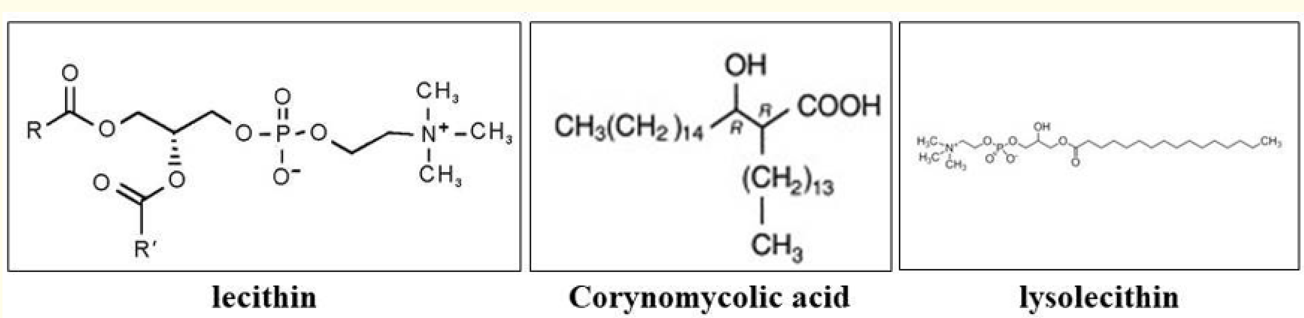

Figure 6: Structures of Phospholipid type of Biosurfactant.

Polymeric biosurfactant

High weight molecular biopolymers known as polymeric biosurfactants can be composed of a variety of biopolymers, including proteins, lipopolysaccharides, polysaccharides, and lipoproteins. Polymer biosurfactant is produced by a broad range of microorganisms. The characteristics of a polymer biosurfactant include high viscosity, tensile strength, and resistance to shear. The following are illustrations of several polymeric biosurfactant types. Emulsan, Alasan, Liposan, and Lipomanan are a few examples, and these substances are frequently generated by *Acinetobacter calcoaceticus, A. radioresistens, C. lipolytica, and C. tropicalis*, respectively [16].

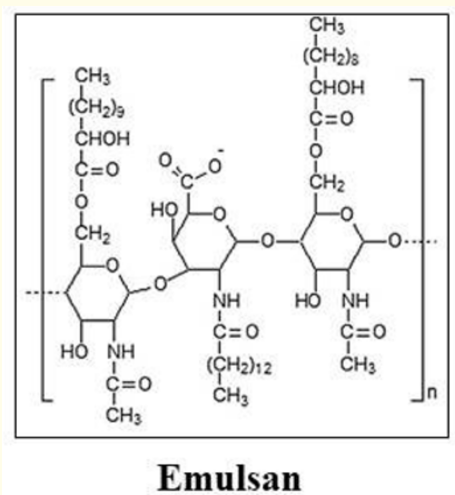

Figure 7: Structures of Polymeric type of Biosurfactant.

Particulate biosurfactants

Particulate biosurfactants are extracellular membrane vesicles that divide hydrocarbons into a microemulsion, which is crucial for microbial cells to absorb alkanes. *Acinetobacter* sp. vesicles have a buoyant density of 1.158 cubic g/cm, a diameter of 20-50 nm, and a composition of protein, phospholipids, and lipopolysaccharide [36].

Biosynthesis mechanism of biosurfactant production:

Many biosurfactants are produced as a result of inadequate understanding of basic biosynthetic pathways and a dearth of molecular structures.

Four main scenarios for the production of such amphiphilic compounds are generally accepted:

- Separate routes are used for the de novo synthesis of hydrophilic and hydrophobic molecules;
- The hydrophilic moiety is created by de novo synthesis, and the hydrophobic moiety is induced by the substrate;
- while the hydrophobic moiety is created by de novo synthesis, and the hydrophilic moiety requires a substrate for synthesis;
- The synthesis of both residues relies on the carbon substrate employed.

Biosurfactant hydrophilic moieties are formed by microorganisms from hydrophobic substrates like oils and fats, whereas hydrophobic moieties are produced from hydrophobic substrates like water-soluble substrates [8,45]. The creation of the precursors for the generation of biosurfactants involves several metabolic processes. For example, carbon flux will be regulated by two major pathways, namely (1) Glycolytic pathway (hydrophilic moiety generation) and (2) Lipogenic pathway (lipid generation). One of the important factors is the carbon source that can be found in the culture medium. Microbial metabolism limits both of these mechanisms [12].

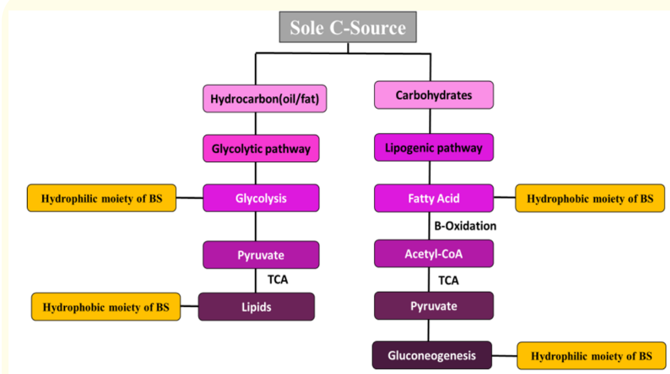

Figure 8: Pathway followed by microbes according to the C-Source in the medium.

The intermediate product glucose-6-phosphate (G6P) is produced during the breakdown of the water-soluble source glucose in the glycolytic pathway. One of the main sources of carbohydrates in the hydrophilic region of the biosurfactant is this glucose-6-

phosphate. The catalysis of Glucose-6-phosphate to create various hydrophilic moieties of trehalose, sophorose, rhamnose, mannose, and polysaccharides is carried out by a number of enzymes. The conversion of glucose to pyruvate produces a hydrophobic moiety. The subsequent conversion of pyruvate to acetyl Co-A produces malonyl Co-A. The subsequent conversion of oxaloacetate and malonyl Co-A into fatty acids serves as a precursor for the synthesis of lipids [12,26].

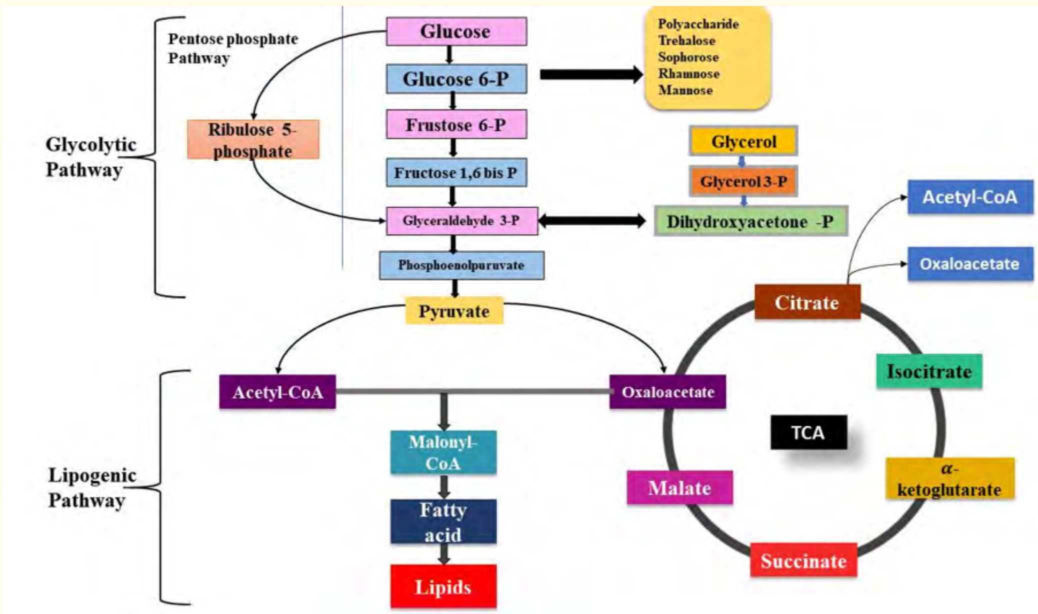

Figure 9: Metabolic pathways involved in synthesising biosurfactant using water-soluble substrates

When made with glycerol as a substrate instead of sugarcane molasses, biosurfactants have a higher lipid content [23]. This might be as a result of the microbes' targeting of the gluconeogenesis and lipogenic pathways. When hydrophobic substrates such as hydrocarbons, oils, and fats are employed as a substrate, the metabolic route may convert into the lipolytic pathway and gluconeogenesis, which controls the synthesis of hydrophobic moieties of the biosurfactant. In this instance, gluconcogenesis will be used to generate the hydrophilic moieties from scratch. The first step in the production of glucose is the oxidation of fatty acids to produce acetyl Co-A. Pyruvate was converted into Glucose-6-phosphate by a number of enzymes that are also engaged in glycolysis. Moreover, certain biosurfactant synthesis processes need for multienzyme complexes to complete the process. The process was first deciphered for rhamnolipids produced by *Pseudomonas aeruginosa* and surfactin produced by *Bacillus subtilis* [26].

Conclusion

One important advantage of biosurfactants over chemical surfactants is that they are biodegradable, which considerably lessens their environmental effect. Yet it is their other, well-acknowledged, effective uses that, in our opinion, will increase their usage in the petroleum industry. Also, several types of biosurfactants are synthesized by microorganisms and may be used in a variety of circumstances to treat hydrocarbon contaminants. Several microorganisms, like *Pseudomonas* and *Bacillus* species, are showing high amounts of biosurfactant production. According to their microbial origin and chemical structure, biosurfactants are classified. Rhamnolipid is produced by *Pseudomonas aeruginosa* and has great commercial uses. We can also produce biosurfactants

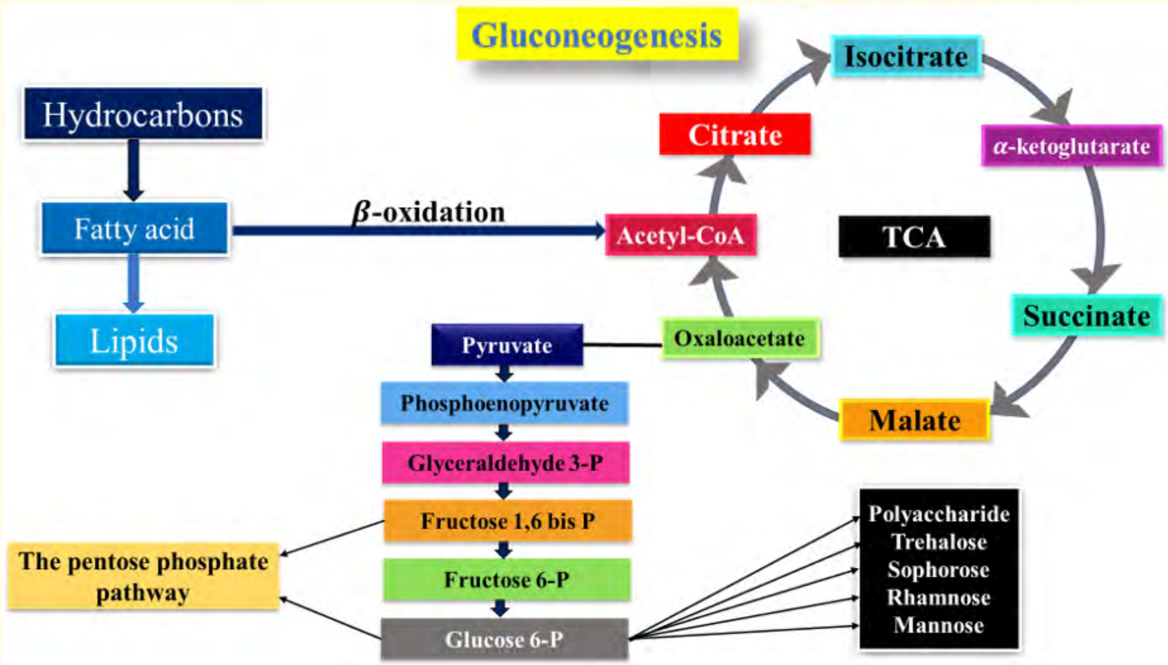

Figure 10: Metabolic pathways involved in the synthesis of biosurfactant using Water-insoluble (hydrocarbon) substrate.

through a variety of metabolic pathways by employing various substrates as carbon sources. They are helpful in many remediation procedures and have a broad application spectrum.

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