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Microbial Biosurfactant: An intermediate with enhanced degradative potential

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Abstract
Studies addressing ecological compatible products have increased over a period of time, especially, in the biosurfactant field. Biosurfactants are extracellular amphiphilic compounds that are mainly produced by microorganisms and are classified into five main groups, including the glycolipids one. Therefore, it is possible to reduce the rhamnolipids production cost, since this has been the main bottleneck for replacing the chemical surfactants. In addition, to meet a bonafide industrial application some limitations such as low productivity as well as recovery and/or purification that represent from 60 to 80% of total production cost should be improved. Therefore, this review covers different ways for producing rhamnolipids covering their application in many fields such as pharmaceutical, agricultural, petrochemical and so on; demonstrating the versatility of these biological compounds. The marine environment is often affected by petroleum hydrocarbon pollution due to industrial activities and petroleum accidents. This pollution has recalcitrant and persistent compounds that pose a high risk to the ecological system and human health. For this reason, the world claims to seek to clean up these pollutants. Bioremediation is an attractive approach for removing petroleum pollution. It is considered a low-cost and highly effective approach with fewer side effects compared to chemical and physical techniques. This depends on the metabolic capability of microorganisms involved in the degradation of hydrocarbons through enzymatic reactions. Bioremediation activities mostly depend on environmental conditions such as temperature, pH, salinity, pressure, and nutrition availability. Understanding the effects of environmental conditions on microbial hydrocarbon degraders and microbial interactions with hydrocarbon compounds could be assessed for the successful degradation of petroleum pollution. The current review provides a critical view of petroleum pollution in seawater, the bioavailability of petroleum compounds, the contribution of microorganisms in petroleum degradation, and the mechanisms of degradation under aerobic and anaerobic conditions. We consider different biodegradation approaches such as biostimulation, bioaugmentation and phytoremediation.

Keywords: *Biosurfactant, Bioremediation, Amphiphilic, Biodegradation, Pollutant*.

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I. INTRODUCTION

Due to their amphiphilicity, surfactants are chemical compounds made from petroleum derivatives that have the ability to lower the surface tension between two immiscible phrases. A surfactant molecule is structurally composed of a hydrophobic and hydrophilic moiety [1][2]. Carbon chain can be formed the a polar portion, whereas carbohydrate, amino acids, carboxylic acids, phosphate Or alcohols can be formed the polar moiety [3]. In contrast, biosurfactants are metabolites produced by bacteria, yeasts and filamentous fungi. These extracellular, amphiphilic molecules, which have several advantages over chemical surfactants, we're discovered in the 1960 by fermenting hydrocarbons. Due to their low toxicity, high selectivity biodegradability, low critical micellar concentrate (CMC) and stability under extreme pH, salinity and temperature conditions, biosurfactants have received a lot of interest in last 10 years [4][5]. When bacteria interact with an interface and create a biofilm, they produce biosurfactants that change the surface water ability and other characteristics. *Pseudomonas aeruginosa,* a marine bacterium isolated from oil polluted sea water, has demonstrated the ability to break down hexadecane, octadecane heptadecane and nonadecane after 28 days of incubation. The creation of a biosurfactant by this bacterium has demonstrated its capacity for degradation. Additionally, it was demonstrated that *Pseudomonas aeruginosa* has successfully destroyed a verity of hydrocarbons, including 2-methylnaphthalene, tetradecane and pristine [6].

Surfactant are compounds that reduce the surface tension between two liquids that differ in their chemical nature. They have ability to reduce surface tension in both aqueous and hydrocarbon mixture [7].Many hypersaline environments, such as natural salt lakes, saline lakes brins, salt marshes, saline industrial currents And oil fields are high levels of petroleum are contaminated with hydrocarbons. Because Conventional microbiological processes do not work well elevated salinity can hypersaline bioremediate the atmosphere.[8].

This characteristic is essential in application requiring lubrication, foaming, solubilization of immiscible compounds dispersion and emulsification [9].Their uses and prospective commercial application have been described a number of industries, including emulsifiers in the food industry and surfactant assisted flooding for enhanced oil recovery in the oil industry [10][11]. Since the biosurfactant of these organisms have specific adaptation to increase stability in adverse environment, the search for biosurfactants in extremophiles appears to be especially promising. These environments are likely to be harsh, where biotechnology applications for these substances are required [12][13]. There are very few reports on biosurfactant producers in hypersaline environments [14]. Biosurfactant are secondary metabolites,diverse group of surface-active agents produced by many living organisms.Halophiles which have a unique lipid composition (phytanlglycerol) may have an important role to play as surface active agents [15].

1.1 Biosurfactants :

Biosurfactant are microbially produced surface active compound have amphiphilic molecules these amphiphilic particles have both hydrophobic and hydrophilic regions making them total at interfaces between fluids with various polarities like water and hydrocarbon [16].Biosurfactants are amphiphilic compound consists of hydrophilic polar moiety as oligo Or monosaccharide and proteins as well as polysaccharides Or peptides and the hydrophilic moiety has unsaturated,saturated fatty alcoholls Or hydroxylated fatty acids [17].One of the key features of biosurfactants is the hydrophilic- lipophilic balance which makes the hydrophobic as well as hydrophilic still up in the air in substance that are surface active Because of the amphiphilic structure, biosurfactant not just the ability to increase the hydrophobic substance surface region yet additionally can change the property of cell surface of the microorganisms [6].

Many of of the biosurfactants producing microorganisms are additionally hydrocarbon degrades. Many studies have showed the impacts of microbially produced surfactants not only on bioremediation as well as on enhanced oil recovery [16].Biosurfactants show selectivity of the substrate to degrade and functionally active at extreme conditions of high temperatures ,high salt concentrations as well as pH that can be attributed by the products and generated waste from industries [18].

Numerous substances are utilized by microbes as a source of carbon and energy for development. While some yeast and bacteria also diffuse biosurfactants that can emulsify hydrocarbons in the media, microorganisms diffuse a variety of compounds as biosurfactants if carbon is an insoluble hydrocarbon [19]. Because biosurfactants-producing bacteria have the unique ability to employ natural waste and hydrocarbon waste as source materials, bioremediation of waste water effluents should be feasible [6].

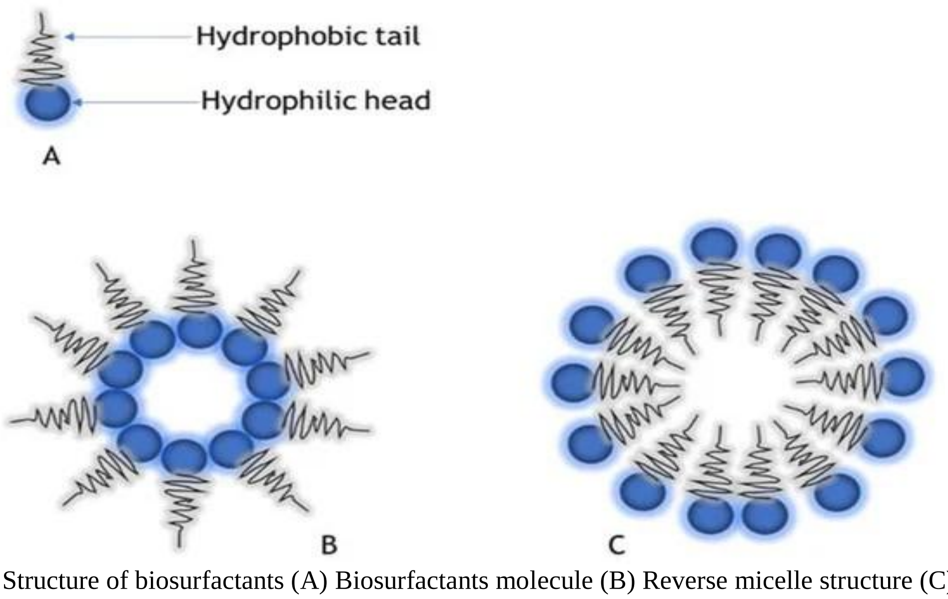

Figure 1: Structure of biosurfactants (A) Biosurfactants molecule (B) Reverse micelle structure (C) Micelle structure [45].

1.2 Source of biosurfactant

Manyof the biosurfactant-producing microorganisms are found to be hydrocarbon degraders [20][21]. However, in the past decades, many studies have shown the effects of microbially produced surfactants for both bioremediation and enhanced oil recovery [21][22]. Table 1 lists the biosurfactants produced from microbial sources.

1.2.1Bacterial Biosurfactants: As a source of carbon and energy for growth, microorganisms make use of a wide variety of organic substances. Microorganisms produce a variety of chemicals, known as biosurfactants, to enable their diffusion into the cell when the carbon source is in an insoluble form like a hydrocarbon.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 biosurfactants are rhamnolipids that are produced by different Pseudomonas spp. [23][24] or sophorolipids that are produced by several Torulopsis spp.[25][26].

Other bacteria have the capacity to alter the composition of their cell walls through the production of non-ionic or lipopolysaccharide surfactants. These are some examples of this group: Various *Mycobacterium species,* including *Rhodococcus erythropolis*, and *Arthrobacter species* create nonionic trehalose corynomycolates. [27][28][29].There are lipopolysaccharides, such as emulsan, produced by *Acinetobacter spp.* [29]and lipoproteins such as surfactin and subtilisin, that are produced by *Bacillus subtilis* [26].

1.2.2Fungal biosurfactants: The synthesis of biosurfactants by bacterial species has received much study, although comparatively few fungi are known to do so**.** Amon*g fungi, Candida bombicola* [30], *Candida lipolytica* [31], *Candida ishiwadae* [32], *Candida batistae* [33], *Aspergillus ustus* [34] and *Trichosporon ashii* [35] are the explored ones. Many of these are known to produce biosurfactants 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 [36].

Table 1: Biosurfactants derived from bacteria and fungi

Organisms	Biosurfactants	Reference
Bacteria : *Serratia marcescens*	Serrawettin	[37]
Rhodococcus erythropolis, Arthrobacter spp.	Trehalose lipids	[38]
Pseudomonas spp. *Agrobacterium spp.*	Ornithine lipids	[39]
Pseudomonas fluorescence	Viscosin	[40]
Pseudomonas aeruginosa	Rhamnolipids	[41]
Fungi : *Torulopsis bombicola*	Sophorose lipid	[42]
Candida bombicola	Sophorolipids	[30]
Candida liplytica	Protein lipidpolysaccharide	[31]
Candida ishiwadae	Glycolipids	[32]
Candida batistae	Sophorolipid	[33]
Aspergillus ustus	Glycolipoprotein	[34]

II. Classification of biosurfactant

Surfactants can be classified according to the nature of the charge on individual polar moieties. Anionic surfactants are negatively charged usually due to a sulphonate or sulphur group. Non – ionic surfactants contain no ionic constituent and the majority of all non- Ionics are polymerization products of 1,2- epoxyethane. Cationic surfactants are characterized by a quaternary ammonium group which is positively charged. Lastly, amphoteric surfactants have both positively and negatively charged moieties in the same molecule [43]. Biosurfactants can also be classified into two catogerized, namely, low-molecular-massmolecules with efficient lower surface and interfacial tensions and high- molecular- polymers, which bind tightly to surfaces [44]. There are classified into 5 major groups: 1) Glycolipids, 2) Phospholipids, 3) Lipopeptides, 4) Polymeric and 5) Particulate [16].

Glycolipids, Lipopeptides and Phospholipids have the lowest mass biosurfactant while large amounts of biosurfactants contain Particulate surfactants and Polymeric microbial surfactants [6].

2.1 Glycolipids :

The most known biosurfactants are glycolipids. They Consist of mono-, di-, tri- and tetrasaccharides which Include glucose, mannose, galactose, glucuronic acid, rhamnose and galactose sulphate. The fatty acid component usually has a composition similar to that of phospholipids of the same micro-organism [48]. They are made up of carbohydrates in combination With long-chain aliphatic acids or hydroxyaliphatic acids [50]. Among the glycolipids, the best known are the rhamnolipids, trehalolipids and Sophorolipids whilst the best-studied glycolipid bioemulsifiers, rhamnolipds, trehalolipids and sophorolipids [50,51]are disaccharides that are acylated with long-chain fatty acids or hydroxyl fatty acids [52].

2.1.1 Rhamnolipids:

Bacteria of the genus *Pseudomonas* are known to produce glycolipid surfactant containing rhamnose and 3-hydroxy fatty acids [53]. It is the widely studied biosurfactant which are the principal glycolipids produced by *Pseudomonas aeruginosa* [55].Disaccharide rhamnolipids are Formed by condensing two moles of rhamnose sugar an acetal group links the hydrophobic group. However, the lipid part of the molecule contains ester and carboxyl groups. Rhamnolipid produced by *Pseudomonas aeruginosa* strains are among the most effective surfactants when applied for the removal of hydrophobic compounds from Contaminated soils [56].

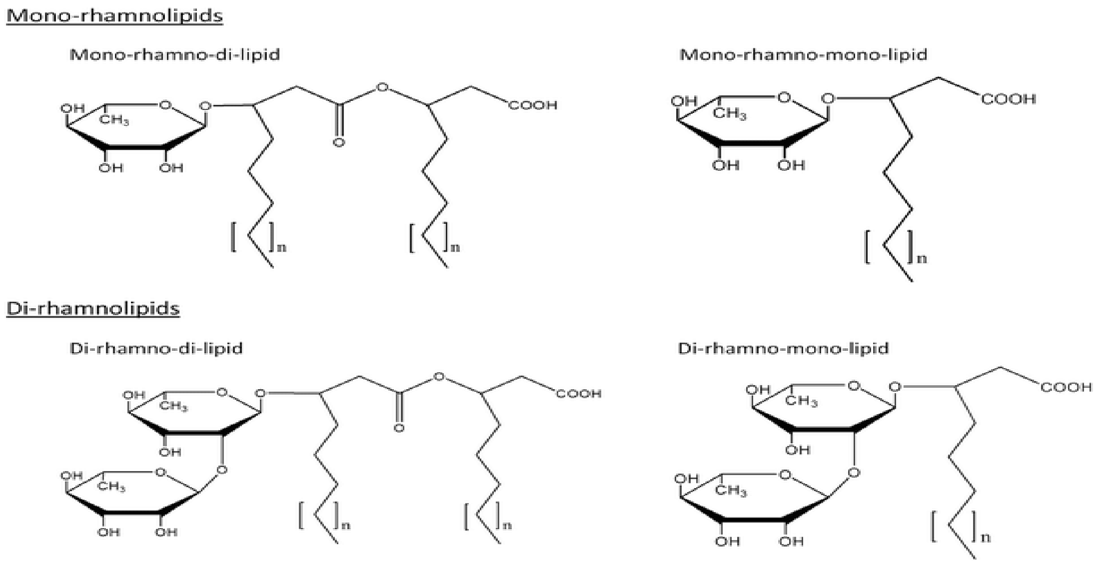

Figure 2 : Rhamnolipid chemical compositions. Based on the quantity of l-rhamnose residues, rhamnolipids are divided into mono- and di-rhamnolipids. There are rhamnolipid species with only one fatty acid chain (mono-rhamno-mono-lipid and di-rhamno-mono-lipid) in addition to the normal rhamnolipid species with two 3-hydroxyfatty acids (mono- and di-rhamno-di-lipid). While organisms from the genus Burkholderia produce rhamnolipids with longer alkyl chains and typical lengths between C10 and C16 (n = 3-9), rhamnolipids from *Pseudomonas aeruginosa* typically contain fatty acids with chain lengths between C8 and C14 (n = 1-7)[46].

2.12. Sophorolipids:

Sophorolipids are group of biosurfactants produced by Torulopsis sp. Sophorolipids consist of a dimeric sugar (sophorose) and a hydroxyl fatty acid, linked by a glycosidic bond [57].According to Hu and Ju, (2001) there are two types of sophorolipids namely, the acidic (non-lactonic) sophorolipids and the lactonic sophorolipids.Sophorolipids, generally a mixture of at least six to nine different hydrophobic sophorolipids and lactone from of the sophorolipid is preferablefor many application [58]

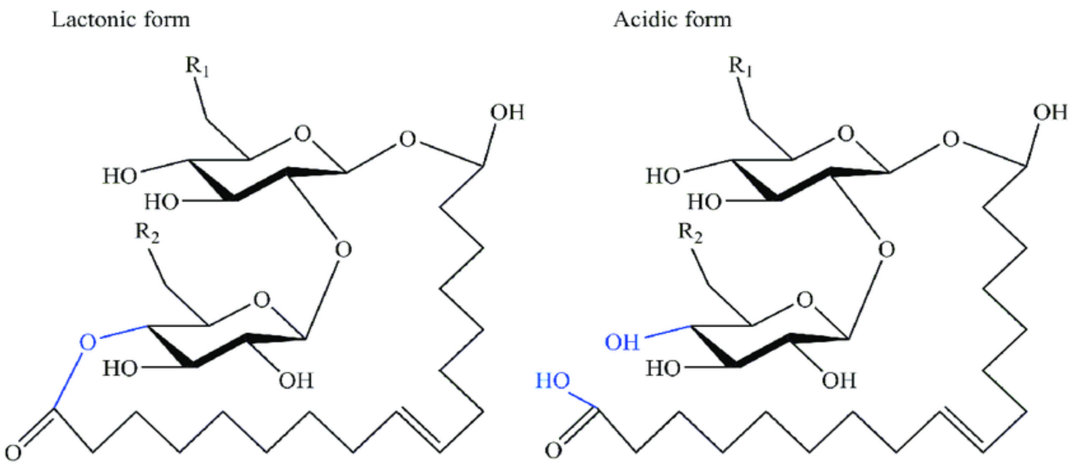

Figure 3 : Common structures of sophorolipids: lactonic form and acidic form. R1 = OH or OCOCH3, R2 = OH or OCOCH3.[41].

2.2 Phospholipids :

A phospholipid is made up of 2 fatty acid tails and a phosphate group head. Fats are long chains that are mostly made up of hydrogen and carbon, while phosphate groups include a phosphorus molecule with 4 oxygen particles connected. These two parts of the phospholipid are connected by means of a third molecule, glycerol (Figure 5).[] Phospholipids are known to form major components of microbial membranes. When Certain hydrocarbon-degrading bacteria or yeast are grown on alkane substrates, the level of phospholipid increases greatly. Phospholipids have been Quantitatively produced from *Thiobacillus*, *Thiooxidans* that are responsible for wetting elemental sulphur necessary for growth [60]. Phosphatidylethanolamine produced by *Rhodococcus erythropolis* grown on n-alkane resulted in the lowering of interfacial tension between Water and hexadecane to less than 1 mNm^{-1} and CMC Of 30 mgL_{-1} [61].

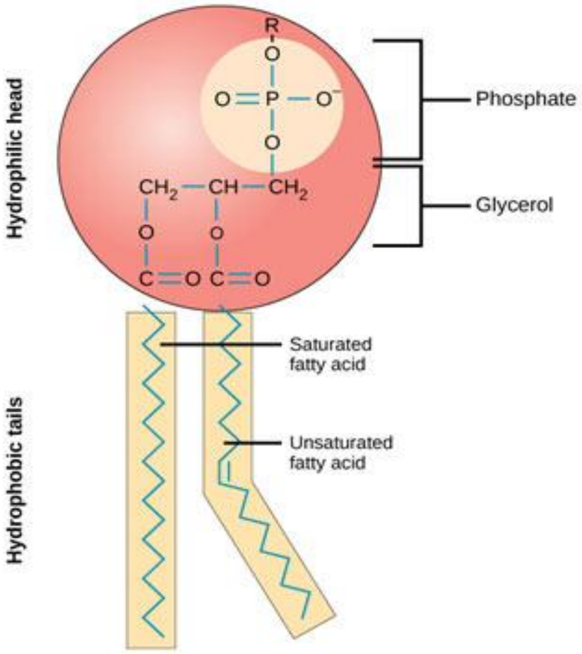

Figure 4: Structure of phospholipid

2.3 Lipoproteins and Lipopeptides :

Lipopeptides also called surfactins, have been produced by *Bacillus sp*. contains seven amino acids bonded to carboxyl and hydroxyl groups of a 14-Carbon acid [62]. Thelipopeptide biosurfactant enhanced the degradation of polycyclic aromatic compounds and showed well emulsification activity with various hydrocarbons [63].Another important characteristic of surfactin is its ability to lyse mammalian erythrocytes and to form spheroplasts [64].

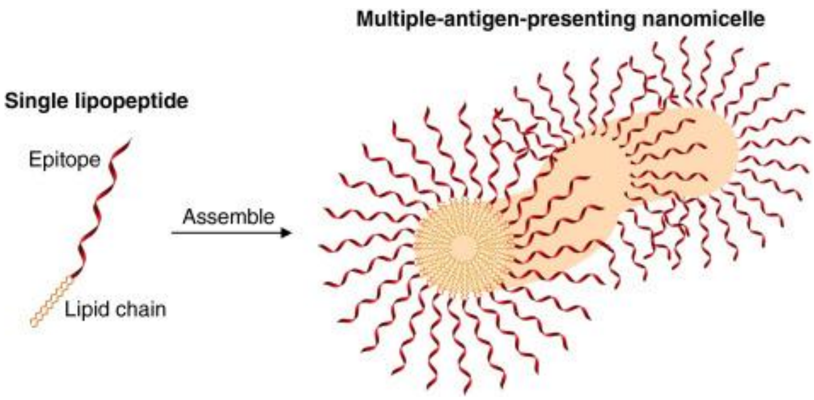

Figure 5 : Structure of lipopepetides [65].

2.4 Polymeric Compounds :

Emulsan, liposan, mannoprotein and Polysaccharide-protein complexes are known to be the best-studied polymeric biosurfactants [66].[67] synthesized liposan, an Extracellular water-soluble emulsifier using *Candida Lipolytica*. It composed of 83% carbohydrate and 17 protein with the carbohydrate portion being a heteropolysaccharide consisting of glucose, galactose,galactosamine and galactoronic acid [68] reported production of large amounts of mannoprotein from *Saccharomyces cerevisiae* with Excellent emulsifying activity toward several oils, alkanes and organic solvents. When purified, the emulsifier contains 44% mannose and Extracellular vesicles from *Acinetobacter sp.* when grown on hexadecane, accumulated extracellular vesicles of 20 to 50 mm diameter with a buoyant density of 1.158 g/cm3 . These vesicles appear to play a role in the uptake of alkanes by *Acinetobacter s*sp [66].Partition of alkane by an extracellular vesicle derived from hexadecane-grown Acinetobacter. Most hydrocarbon-degrading microorganisms, many nonhydrocarbon degraders, some species of *Cyanobacteria*, and some pathogens have a strong affinity for hydrocarbon-water and air-water interfaces. In such cases, the microbial cell itself is a surfactant [69].

III. Significance of biosurfactants

The chemically synthesized surfactants are expensive and which cause environmental pollution. The biosurfactants of microbial origin comprises unique properties that chemically synthesized surfactants do not have [69].The major advantages of biosurfactants over chemically synthesized surfactants are:

• Biodegradability: Biosurfactant are produced by biological processes, which are excreted as extracellularly by microorganism and can be easily degradable.

• Biocompatibility: Biosurfactants produced from biological or natural origin are establishing more environment friendly in nature.

• Availability of raw materials: Biosurfactants can be produced from economically cheap raw materials, which are easily available in large quantities. Also, they can be produced from industrial wastes and their by-products.

• Low toxicity: Biosurfactants of microbial origin having less toxicity.

• Environmental management: Biosurfactants can be used in handling industrial emulsions, biodegradation and detoxification of industrial effluents.

• Stability: Biosurfactants are capable of stable at high range of pH, temperature and salt concentration.

• Bioremediation: Biosurfactants considered as environment friendly because, they can undergo in-situ bioremediation, restores the soil structure and requires less energy input compared to chemical surfactants.[71].

IV. Application of biosurfactants

In the recent years, there has been an increasing demand for biosurfactant as emulsifiers, de-emulsifiers, wetting agents, foaming agents, spreading agents, detergents and functional food ingredients [72].There are numerous chief advantages of biosurfactant over chemical surfactant such as, lower toxicity, biodegradability, high environmental compatibility and production from renewable raw substrates. Therefore, the biosurfactant has been become the imperative area of extensive research and application from food industries to oil Industries [73].

The critical micelle concentration is commonly used to measure the effectiveness of biosurfactant. The potent biosurfactant should have a low critical micelle concentration, which means that a reduced amount of biosurfactant is required to reduce the surface tension. Microemulsion are produced when one liquid phase is discrete as droplets in other liquid phase, for example water dispersed in oil or oil dispersed in water [66].The effectiveness of the biosurfactant is determined by measuring its capability to alter surface and interfacial tension, stabilization of emulsion and by learning it's hydrophilic – lipophilic balance. This hydrophilic-lipophilic baknce value is a measure to designate whether a biosurfactant is associated to oil in water or water in oil emulsion. This factor can be used to find out the suitable applicability of biosurfactant [74].

4.1 Agriculture

There are many interesting properties of biosurfactant, generated particular interests in finding potential application in agricultural field. These bio- molecules play an important role in motility, signifying that biosurfactant holds plant microbe interaction. In agriculture field, it makes the availability of nutrients for plant and also used for elimination of plant pathogens. Agriculture important products like pesticides produced with the assistance of biosurfactant can be extensively used on agricultural field [67].

4.2 Bioremediation

Biosurfactants are potential useful tools for controlling oil spill pollution by enhancing the degradation of hydrocarbons in the environment [75].The Bioremediation of hydrocarbon contaminated sites are limited due to the low water solubility, which reduces their availability to microorganisms. It has been assumed that, biosurfactants can be used to improve the bioavailability of hydrophobic compounds [74].The most significant application of biosurfactants in bioremediation process is removing heavy metals from soil [76].

4.3 Biomedical and pharmaceutical applications

Biosurfactants possesses numerous bioactivities such as antibacterial, antifungal, antiviral,antibiotics, anti-adhesive, anti-biofilm, haemolysin, tumor growth inhibition, immune stimulant, anti-asthma, food digestion, migration of human neutrophils and many bio regulatory effect [77]. The wide range use and commercial applications of biosurfactants in the medical and pharmaceutical fields has increased during the past decades.

They have a potential to be used in a variety of industries like agricultural, cosmetics, pharmaceuticals, food preservatives and detergents [67].

4.4 Additives in healthcare and cosmetic products

The biosurfactants apply numerous properties such as emulsification, de-emulsification,water binding, spreading, wetting, foaming and product stability can be efficiently exploited by cosmetic industries. Recently, the use of surfactants as emulsifiers, wetting agents,foaming agents, cleansers, solubilizers, antimicrobial agents and mediators of enzymes action in different dosages forms like lotions, creams, pastes, liquids, gels, powders, sticks,sprays and films may be replaced by biosurfactants [78][79].Additionally, biosurfactants could be used in insect repellents, antacids, antidandruff products, bath products, acne pads, hair colours, contact lens solution and health care products such as deodorants, body massage accessories, nail care, lip markers, eye shades, polishes, soaps, shampoos, tooth pastes, mouth cleansers, foot care, antiperspirants, conditioners, Antiseptics, baby products, moisturizers and many health and beauty products [80][81].

Biosurfactants are known to have various advantages over chemically synthesized surfactants such as better moisturizing properties, low or anti-irritancy effects and compatibility with skin. The most commonly used biosurfactant in cosmetic products are glycolipids, which include sophorolipids, rhamnolipids and mannosylerythritol lipids. Sophorolipids are skin compatible and have excellent moisturizing properties. Rhamnolipids are good emulsifiers that can replace the usage of petrochemical based surfactants in many cosmetic products. Mannosylerythritol lipids are commonly used as the active ingredient in skin care formulations to prevent skin roughness [82].

V. CONCLUSION :

Marine environments polluted by hydrocarbons and their products are priority pollutants as they extend from marine organisms to human health. Bioremediation is recognized as an effective performance technique (low cost, and low in products),used to remove crude oil contamination from marine environments. Many factors affect the bioremediation of crude oil, such as the nature and composition of petroleum, distribution, and fate In marine environments, mechanisms of degradation (aerobic or anaerobic), oxygen availability, temperature, pH, pressure, biosurfactant production, and petroleum availability. All of these factors influence the success of the bioremediation process. Biodegradation is initiated when favorable environmental conditions are present. Owing to the low number of microbial hydrocarbon degraders in marine environments and the variable conditions, many strategies have been proposed to enhance bioremediation, such as biostimulation, bioaugmentation, and phytoremediation. All these techniques can be used successfully In the bioremediation of marine oil pollution. However, the environmental conditions that affect microorganism growth and Activity are critical in determining the most suitable technique.

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