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**Review Article**

# **The Definition, Preparation and Application of Rhamnolipids as Biosurfactants**

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**To cite this article:**

Kamal-Alahmad. The Definition, Preparation and Application of Rhamnolipids as Biosurfactants. *International Journal of Nutrition and Food Sciences*. Vol. 4, No. 6, 2015, pp. 613-623. doi: 10.11648/j.ijnfs.20150406.13

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**Abstract:** Rhamnolipids mainly are produced by *Pseudomonas aeruginosa* and another microorganism that have been found to have good surface activity. Rhamnolipids are used in various application areas, including environmental, health, food, cosmetic, oil industries, pharmaceuticals and environmental bio remediation particularly in enhanced oil recovery (EOR) and cleaning of oil spills. Many kinds of bio surfactant such as, rhamnolipids are already being used in industry but it is important to develop indigenous technology for the production of rhamnolipids produced by the microorganisms of local origin which would be more suitable for the application to that specific areas. Rhamnolipids have several beneficial uses: they are easily degradable, nontoxic, nonmutagenic, and have the highest surface-tension-reduction index of any surface-tension reducing agent currently in use. In this review, I summarize the definition, preparation, properties, and the application in different areas especially in food and agriculture, and industrial potential of rhamnolipids, as the next generation of biosurfactants.

**Keywords:** Rhamnolipids, Biosurfactants, *Pseudomonas Aeruginosa*, Application, Characteristics, Production of Rhamnolipids

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## **1. Introduction**

In the recent years, an increase in environmental awareness has led to much more interest in the use of renewable-based, biodegradable and more environmentally friendly surfactants. The market for these “green” alternatives to synthetic surfactants was 344 kilo tons in 2013, and it is expected to reach 462 kilo tons and 2308 million USD by 2020 (Grand View Research, 2014). Among them, biosurfactants, surface active molecules produced by diverse groups of microorganisms (bacteria, yeasts and filamentous fungi), have attracted considerable attention as alternatives to their synthetic counterparts, owing to their unique properties, that include low toxicity, high biodegradability, high selectivity, low critical micelle concentrations (cmc) and effectiveness at extreme temperatures, PHS and salinities. Due to these properties, biosurfactants comprise a variety of potential applications in agriculture, bioremediation, petrochemical, pharmaceutical, cosmetics, and detergent or food industries.

Biosurfactants or microbial surfactants are surface-active bimolecular that are produced by a variety of microorganisms [1-2]. Biosurfactants offer advantages over synthetic surfactants in terms of their derivation from renewable resources, low or non-toxicity, biodegradability, excellent surface activity, possible reuse through regeneration, high specificity, and effectiveness under extreme temperature and pH conditions [3-4]. The major functions of biosurfactants include solubilization, emulsification, dispersion, wetting, foaming, and detergent capacity, as well as antimicrobial activity in some cases. 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 biosurfactants “surfactin” was purified and characterized by Arima. (Biosurfactants have been researched thoroughly and satisfactorily since then by many research groups across the world yet there are aspects that elude our understanding.

There are five major categories of biosurfactants viz. glycolipid, phospholipids and fatty acids, lipopeptides and lipoproteins, polymeric biosurfactants and particulate biosurfactants that have found applications in agricultural, pharmaceutical, food, cosmetics, and detergent industries. Data reveals there are more than 250 patents obtained on these wonder biodegradable molecules so far. It has also been observed that microbial biosurfactants are advantageous over plant-based surfactants because of the scale-up capacity, rapid production, and multi-functional properties. Several plant-based biosurfactants for example saponins, lecithins, and soy proteins have excellent emulsification properties but are expensive to produce at industrial scale and have other debatable issues such as solubility and hydrophobicity. Among the various categories of biosurfactants the glycolipid biosurfactants “rhamnolipids” stand apart. Rhamnolipids, primarily a crystalline acid, is composed of  $\beta$ -hydroxy fatty acid connected by the carboxyl end to a rhamnose sugar molecule. Rhamnolipids are predominantly produced by *Pseudomonas aeruginosa* and classified as: mono and di-rhamnolipids. Other *Pseudomonas* species that have been reported to produce rhamnolipids are *P. chlororaphis*, *P. plantarii*, *P. putida*, and *P. fluorescens*. Some bacteria are known to produce only mono-rhamnolipids while some produce both. The ratio of mono and di-rhamnolipids can also be controlled in the production method.

## 2. Type of Biosurfactants

The major classes of biosurfactants include glycolipid, lipopeptides and lipoproteins, fatty acids, phospholipids and neutral lipids, and polymeric surfactants [12-13].

### 2.1. Glycolipid

Most known biosurfactants are glycolipid. They are carbohydrates in combination with long chain aliphatic acid or hydroxyaliphatic acid. Among glycolipid, the best known are rhamnolipids, trehalolipids and sophorolipids.

### 2.2. Lipopeptides and Lipoproteins

A large number of cyclic lipopeptides including decapeptide antibiotic (gramicidins) and lipopeptide antibiotics (polymyxins) produced by *Bacillus brevis* and *B. polymyxa*, respectively. The cyclic lipopeptide surfactin produced by *B. subtilis* ATCC 21332, is one of the most powerful biosurfactants.

### 2.3. Fatty Acids, Phospholipids and Neutral Lipids

Several bacteria and yeast produce large quantities of fatty acid and phospholipids surfactants during growth on n-alkanes. In *Acinetobacter* sp. strain H01-N phosphatidyl ethanol amine rich vesicles are produced. The potent surfactant properties of these vesicles are evident from the observation that they are able to generate optically clear microemulsions of alkanes in water.

### 2.4. Polymeric Surfactants

The best-studied polymeric biosurfactants are emulsan, liposan, mannoprotein and other polysaccharide protein complexes. Novonvenezia *et al.* described the isolation of alasan, an anionic alanine containing heteropolysaccharide protein biosurfactant with a molecular approximately 1 MDa produced by *Acinetobacter radioresistens* KA-53. Alasan produced by a strain of *Acinetobacter radioresistens*, is a complex of an anionic polysaccharide and protein. The polysaccharide component of alasan is unusual in that it contains covalently bound alanine. The protein component of alasan appears to play an important role in both the structure and activity of the complex.

*Rhamnolipids* is a class of glycolipid produced by *Pseudomonas aeruginosa*, amongst other organisms, frequently cited as the best characterised of the bacterial surfactants [5-6-7]. They have a glycosyl head group, in this case a rhamnose moiety, and a 3-(hydroxyalkanoyloxy) alkanic acid (HAA) fatty acid tail [8-9]. Specifically there are two main classes of rhamnolipids, mono-rhamnolipids and di-rhamnolipids; consisting of one or two rhamnose groups respectively [10]. Rhamnolipids are also heterogeneous in the length and degree of branching of the HAA moiety which varies with the growth media used and the environmental conditions [11].

Rhamnolipids are extracellular glycolipid composed of L-rhamnose and 3-hydroxyalkanoic acid that are produced by that has been conducted on rhamnolipids to date has *Pseudomonas* spp. Much of the research focused on determining potential applications. Jarvis and Johnson first isolated and described rhamnolipids from *Pseudomonas aeruginosa* in 1949[14].

Rhamnolipids are synthesized when one or two rhamnose sugar molecules fuse with one or two  $\beta$ -hydroxy 3-hydroxy fatty acids [15]. There are four types of rhamnolipids: mono-rhamnolipids (Rh1), which contain one rhamnose sugar attached to two molecules of  $\beta$ -hydroxydecanoic acid; dirhamnolipids (Rh2), which contain two rhamnose sugars attached to two molecules of  $\beta$ -hydroxydecanoic acid; tri-rhamnolipids (Rh3), which contain one rhamnose sugar attached to one molecule of  $\beta$ -hydroxydecanoic acid; and tetrarhamnolipids (Rh4), which contain two rhamnose sugars attached to one molecule of  $\beta$ -hydroxydecanoic acid [16]. The length of the carbon chains found on the  $\beta$ -hydroxyacyl portion of the rhamnolipids can vary significantly. However, rhamnolipids produced by *Pseudomonas aeruginosa* predominantly contain a 10-C molecular chain [17]. Glycolipid in which one or two rhamnose molecules are linked to one or two molecules of  $\beta$ -hydroxydecanoic acid have been the most studied. The OH - group of one of the acids forms a glycosidic bond with the reducing end of the rhamnose disaccharide, while the OH - group of the second acid is involved in ester formation [18]. The Rh1 L-rhamnosyl - L rhamnosylb - hydroxydecanoyl -  $\beta$  - hydroxydecanoate, and L - rhamnosyl -  $\beta$ hydrodecanoyl -  $\beta$  hydroxydecanoate, an Rh2, (Fig. 1) are the principal glycolipid produced by *P. aeruginosa* [19].

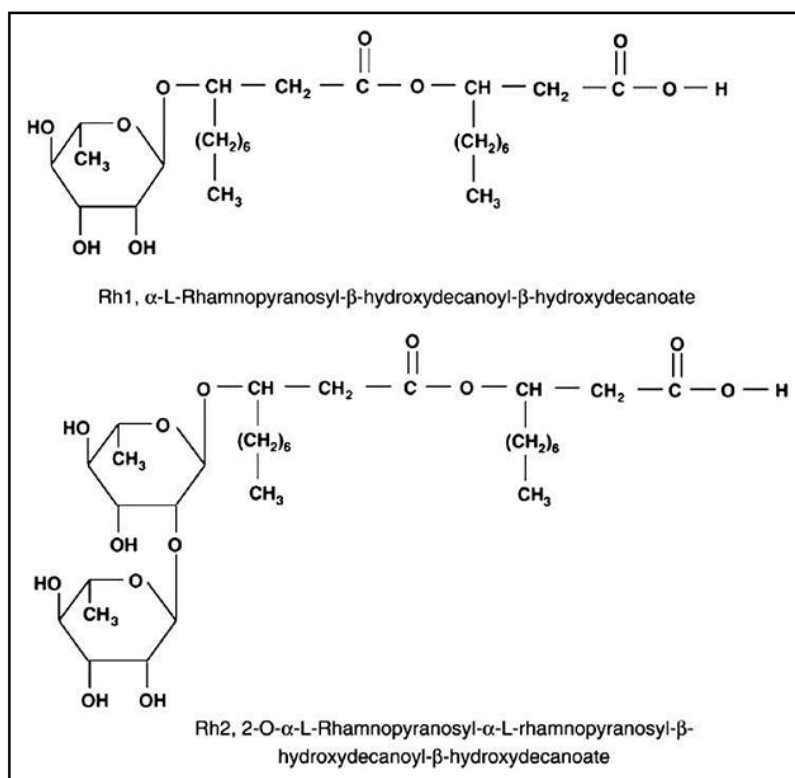


Fig. 1. Chemical structures of rhamnolipid 1 (Rh1) and rhamnolipid 2 (Rh2).

### 3. Properties of Rhamnolipids

Rhamnolipids are of increasing interest for commercial use because of their biological compatibility. Rhamnolipids used as biosurfactants and derived from natural sources have many advantages compared to their chemically synthesized counterparts. They can be obtained from different microbes, including those originating from industrial and environmental wastes[20-12]. Rhamnolipids have several beneficial properties. These include the ability to reduce surface tension. Rhamnolipids from *P. aeruginosa* can decrease the surface tension of water from roughly 72 millinewtons (mN)/m to 25–30 mN/m, and the interfacial tension of water/hexadecane to < 1mN/m [22-23]. Rhamnolipids are easily degradable and particularly suited for environmental applications such as bioremediation and dispersion of oil spills.[24-25]. Rhamnolipids biosurfactants have been shown to be nontoxic and non-mutagenic, compared to the toxicity and mutagenicity associated with chemically derived surfactants [26]. Rhamnolipids also exhibit excellent emulsification properties and have the highest emulsification index against toluene (86.4%).[27] Rhamnolipids induces a remarkably larger reduction in the surface tension of water from 72 to values below 30 mN/m and it reduces the interfacial tension of water/oil systems from 43 to values below 1 mN/m. Rhamnolipids also has an excellent emulsifying power with a variety of hydrocarbons and vegetable oils [48]. The increasing ecological concern with using synthetic chemical

surfactants has led us to propose rhamnolipids as environmentally benign substitute, although it will be necessary to reduce production costs. The use of renewable low-cost substrates, such as plant oil and grain starch, and even lignocelluloses biomass, could dramatically increase the economics of rhamnolipids production [49]. Although rhamnolipids is an effective biosurfactants and is well suited for applications in bioremediation of oil pollutants, the major hurdle for commercial application of the biosurfactants has been the low yield and high production cost. Therefore, there is an urgent demand to develop an efficient biosurfactants producer and a cost-effective bioprocess for the production of rhamnolipids. Biosurfactants as a rhamnolipids are mainly used in the petrochemical industry to enhance oil recovery and for hydrocarbon. These biological compounds also have potential applications in agriculture, cosmetics, pharmaceuticals, detergents, food processing, laundry supplies and paint industries [50].

### 4. Production of Rhamnolipids Surfactant

Rhamnolipids are produced by fermentation process similar to those used to produce beer or other fermented products. Bacteria is added to a fermentation tank and provided a nutrient source, generally a food grade olive, or soy oil, and under properly controlled conditions the result will be Rhamnolipids. In production of rhamnolipids we can use

agriculture waste to eliminate the need for expensive nutrient sources. Rhamnolipids are extracted within a short cycle, processed to remove any residual bacteria, purified, and the resultant mixture diluted into a final product. The amount and mixture of Rhamnolipids material in the final solution can be precisely controlled. The Rhamnolipids mixture can be further refined to produce certain foaming characteristics, mixed according to customer specifications, including production as a solution or as a powder.

*Pseudomonas* sp. are well known for their ability to produce rhamnolipids biosurfactants with potential surface active properties when grown on different carbon substrates and, therefore, are promising candidates for large scale production of biosurfactants [28-29]. In addition to tensioactive properties, rhamnolipids are compounds which play a vital role in regulating the cell population density-dependent control of genes expression, termed quorum sensing (QS) or cell-to-cell communication [30]. Except these, biosurfactants in the mentioned physiological process are involved as transcription factors, signal molecules and as a range of other secondary metabolites, among others extracellular lipase, the expression of which on a genetic level is regulated together with the rhamnolipids themselves [31].

Rhamnolipids biosurfactants are surface-active compounds produced by *Pseudomonas aeruginosa*. They can reduce surface tension, stabilize emulsions, promote foaming and are generally non-toxic, non-hazardous and biodegradable [32]. Due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, effectiveness under extreme conditions in small quantities, production on renewable sources and potential applications in environmental protection, biosurfactants are gaining prominence over chemical surfactants. In addition, the valuable carbohydrate moiety, rhamnose of the rhamnolipids biosurfactants is used for the transport of insoluble drugs in humans and acts as precursor for high – quality flavor components.

Rhamnolipids enhance emulsification of hydrocarbons, and therefore have the potential to solubilize hydrocarbon contaminants and increase their availability for microbial degradation. Hence, biosurfactants producing microorganisms may play an important role in the accelerated bioremediation of hydrocarbon contaminated sites [33-34]. Due to the high cost of remediation processes and other potential applications, the need for increasing the yields of biosurfactants is inevitable. Although the potential for biosurfactants production is determined by the genetics of the microorganisms, other factors like environmental conditions and nature of the substrate also influence the level of expression. Hence, optimization of these conditions may lead to high and safe biosurfactants production.

Most of the studies to date describe biosurfactants production by bacteria grown on hydrocarbons but a few have reported biosurfactants produced by bacteria growing on carbohydrates [35]. Most of the world's total oil and fat production is derived from plants. Hence these hydrophobic substrates may be used for bulk production of rhamnolipids biosurfactants. There are reports regarding the production of

biosurfactants from different substrates like glycerol [36], soybean oil [37], olive oil [38], corn oil [39], canola oil [40], ethanol [41], sucrose and whey [42] by different strains of *Pseudomonas aeruginosa* like *Pseudomonas aeruginosa* GS9-119 and *Pseudomonas aeruginosa* DS10-129. Use of water miscible wastes like molasses, whey and distillery wastes, wastewater from olive oil processing has also been reported [42-43-44-45].

In another study they found that is a Rhamnolipids, a glycolipid-type biosurfactants primarily produced by *Pseudomonas aeruginosa*, is among the most effective biosurfactants and has been applied in various industries and bioremediation. Rhamnolipids surfactants were found to be able to release three times as much oil as water alone from the oil-contaminated beaches in Alaska [46]. Previous work showed that *P. aeruginosa* is able to produce six types of rhamnolipids, which possess similar chemical structure and surface activity and have an average molecular weight of 577. Rhamnolipids can reduce surface tension of water from 72 to 30 mN/m with a critical micelle concentration of 27–54 mg/L. Although rhamnolipids is not the strongest biosurfactants available, it is well suited for applications in bioremediation of oil pollutants due to having high emulsification activity and minor antibiotic effects [47].

Some examples for production rhamnolipids from different microorganisms:

Production of rhamnolipids from *Pseudomonas aeruginosa* san-ai:

The aim of this method was to optimize the medium with regard to sources of carbon and nitrogen for improved production of rhamnolipids by the strain *P. aeruginosa* san-ai and to characterize the obtained rhamnolipids by FTIR and MS analysis. This is the first investigation of the production of rhamnolipids by a strain isolated from an unusual environment, *i.e.*, an extremely alkaline environment with a high amount of hydrocarbons. The dynamics of the production of RL by *P. aeruginosa* san-ai during submerged growth, as well comparison of the productivity of a referent strain and strains isolated from similar environments, was also investigated. Strain *P. aeruginosa* san-ai, isolated from an unusual extremely alkaline environment with high content of hydrocarbons (mineral cutting oil), was investigated to determine its capability to produce rhamnolipids (RLs) on different sources of carbon and nitrogen. Potential of *P. aeruginosa* san ai to produce RLs was compared to that of a referent strain ATCC 27853 and the strain *P. aeruginosa* 67, isolated from a biopile with a high level of petroleum hydrocarbons. *Pseudomonas* sp. produced rhamnolipids as secondary metabolites and the production, among other things, depends on the general medium composition, particularly on the sources of carbon and nitrogen, as well as the total C/N ratio [51-52]. This study showed that good substrates for the production of RL were vegetable oils, including oil wastes, as a carbon source and peptone I as an organic nitrogen source, which differs significantly from producing strains reported in the literature. Namely, glucose, glycerol and olive oil in combination with inorganic nitrogen were found to be

preferential sources for RL production giving a high yield of 1.2–7.6 g /L of RL, respectively [53]. In addition, industrial waste and byproducts, such as whey waste, with yield of 0.92 g/L of RL, and molasses and corn steep liquor, both with yield of 0.25 g L<sup>-1</sup> of RL, showed themselves to be relatively good substrates.

Production of rhamnolipids from *Serratia rubidaea* SNAU02:

Biosurfactants production by *S. rubidaea* SNAU02 under SSF using different substrates showed, mahua oil cake as best substrate, which resulted maximum production of biosurfactants (EI24 30%) when compared other substrates (data not shown). Selection of a proper substrate is another key aspect of SSF. In SSF, solid material is non-soluble that acts both as physical support and source of nutrients. Solid material could be a naturally occurring solid substrate such as agricultural crops, agro-industrial residues or inert support [54]. Mahua oil cake performed best at biosurfactants production compared to other substrates (groundnut oil cake, coconut oil cake, gingelly oil cake, castor oil cake, palm oil cake, sunflower oil cake). Mahua oil cake is non-edible oil cake and is not fed to animals but can be used as organic manure, due to its high NPK content. Hence, mahua oil cake (*M. indica*) was chosen as the substrate for the production of biosurfactants [55]. Reported mahua oil cake suitable for biosurfactants production due to its rich organic nature content (total sugars 73%, reducing sugars 62.7% and protein 8.6%)

Production of Rhamnolipids by indigenous *Pseudomonas aeruginosa* J4 originating from petrochemical wastewater:

In this study, an indigenous bacterial strain capable of producing biosurfactants was isolated from waste water of a local petrochemical factory. The strain, identified as *P. aeruginosa* J4, was found to be able to produce rhamnolipids and was thus evaluated for its potential for commercial-scale production of the biosurfactants. The isolated strain was cultivated with different media and conditions to determine the optimal culture strategy for rhamnolipids production. Different carbon sources, especially oils (e.g., diesel, kerosene, olive oil, sunflower oil, grape seed oil, etc.), were adopted to explore their effects on the yield of rhamnolipids. The rhamnolipids present in the supernatant of *P. Aeruginosa* J4 culture was purified and identified by NMR and mass spectrometry. Surface activity of the rhamnolipids produced from *P. aeruginosa* J4 was analyzed in terms of surface tension reduction and emulsification activity for oil substrates. The objective of this work was to assess the potential and commercialized feasibility of using the indigenous bacterium for the production of rhamnolipids. Two complex media (LB and CMS) and a defined medium (GMS) were used to grow *P. aeruginosa* J4. The effect of those media on rhamnolipids production was examined. LB medium was used since it is one of the most commonly adopted culture media for *P. aeruginosa* strains. The CMS medium, made from wastes of molasses fermentation, is considered an economically feasible culture medium for industrial-scale fermentation due to its low cost and high nutrient contents. The results show that *P. aeruginosa* J4

strain was able to grow and produce rhamnolipids efficiently with GMS medium, whereas rhamnolipids production was significantly lower when the two complex media were used. *P. aeruginosa* J4 was able to produce rhamnolipids at a concentration of 1733, 773 and 206 mg/L when it was grown in GMS, LB, and CMS medium, respectively. The culture with GMS medium also attained a lower surface tension (30.6 dyn/cm) and a higher emulsion index (80%) than those with LB and CMS media. This further supports that GMS medium gave higher biosurfactants production. The poor performance of the rich media (LB and CMS) on rhamnolipids production may be attributed to their abundance in nitrogen sources, which are known to limit rhamnolipids production [56].

A bacterial strain (*P. aeruginosa* J4) capable of producing rhamnolipids effectively from various carbon sources was successfully isolated. The J4 strain can degrade vegetable oils as well as mineral oils (e.g., diesel and kerosene) to produce biosurfactants. Hence, the strain itself or its biosurfactants product has the potential to be applied in bioremediation of those oil pollutants. Among the seven carbon substrates examined, olive oil was the most efficient one for rhamnolipids production. At a concentration of 10%, olive oil gave a maximum production level of 3600 mg/L and an optimal production rate of 26 mg/h/L. Rhamnolipids production was optimal in batch cultures when the temperature and agitation rate were controlled at 30 °C and 200 rpm, respectively. Rhamnolipids produced by *P. aeruginosa* J4 had a critical micelle concentration of ca. 50 mg/L and exhibited an excellent emulsification activity for diesel and kerosene (E24 = 70 and 78%, respectively). Rhamnolipids in the culture broth was purified primarily via solvent extraction. The NMR and mass spectrometry analysis shows that the purified product contained two types of rhamnolipids compounds (namely, RL1 and RL2).

## 5. Applications of Rhamnolipids

Biosurfactants as rhamnolipids are promising surfactants and are useful in the fields of cosmetics, food processing, pharmaceuticals and environmental bio-remediation particularly in enhanced oil recovery (EOR) and cleaning of oil spills and in agriculture.

The chemical surfactant industry is one of the most rapidly growing industries in the world. The total quantity of surfactants produced during 1989–1990 in the United States was  $7.6 \cdot 10^9$  lb. However, chemical surfactants are synthetic chemicals and non-biodegradable, and thus impose hazardous impacts on the environment. In recent years, attention has been diverted toward biosurfactants because of their broad range of functional properties and renewable production routes based on microbes [56]. Rhamnolipids are powerful natural emulsifiers, can reduce the surface tension of water, can be used to assist in the breakdown and removal of oil spills, and have well-defined roles in medical and agricultural fields due to their antibacterial, antimicrobial, and antifungal properties [57]. Rhamnolipids biosurfactants can be applied to

several industrial sectors, including the handling of industrial emulsions, controlling oil spills, biodegradation, detoxification of industrial effluents, bioremediation of contaminated soil, and in the production of biocompatible cosmetic products [58-59].

### 5.1. *Rhamnolipids in Food Industry*

The biosurfactants as biocompatible, biodegradable, and/or nontoxic compounds have the combination of particular characteristics that exhibit a variety of useful properties for the food industry especially as emulsifiers, foaming, wetting, solubilizers, ant adhesive and antimicrobial agents [63]. Biosurfactants show several properties such as Emulsion-based formulations that have great potential applications in many fields of food industry. An emulsion is a heterogeneous system, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets, having dispersed and continuous phase. The addition of emulsifiers is of special value for low-fat products [64] as it improves the texture and creaminess of dairy products. Biosurfactants act as controlling consistency in bakery and ice cream formulations. They are also utilized as fat stabilizer and anti spattering agent during cooking of oil and fats [65]. The antiadhesive activity of biosurfactants like rhamnolipids used as a new tool to inhibit and disrupt the biofilms formed in food contact surfaces by different groups of bacteria. The bacterial biofilms (group of bacteria that have colonized a surface) present in food industry surfaces are potential sources of contamination, which may lead to food spoilage and disease transmission, thus controlling the adherence of microorganisms to food contact surfaces is an essential step in providing safe and quality products to consumers [66].

Rhamnolipids can be used in the food industry as food additives. They can agglutinate fat globules, stabilize aerated systems, improve texture and shelf-life of starch-containing products, modify rheological properties of wheat dough, and improve the consistency and texture of fat-based products<sup>60</sup>. In ice cream and bakery formulations, rhamnolipids can be used to control consistency, retard staling, solubilize flavor oils, stabilize fats, and reduce spattering<sup>61</sup>. Rhamnolipids can also be used to improve properties of butter cream, croissants, and frozen confectionery products. L-rhamnose has considerable potential as a precursor for flavorings; it is being used as a precursor for high-quality flavor components like Furaneol, which is produced by hydrolyzing rhamnolipids<sup>62</sup>. Recently, a bioemulsifier isolated from a marine strain of (*Enterobacter cloacae*) was described as a potential viscosity enhancement agent of interest in food industry especially due to the good viscosity observed at acidic pH allowing its use in products containing citric or ascorbic acid [67].

### 5.2. *Rhamnolipids in Oil Industry*

Rhamnolipids have potential applications in the oil industry, using whole-cell broth with minimum purity specifications [68]. These rhamnolipids are very selective, required in small quantities, and are effective under a broad range of oil and

reservoir conditions [69]. Specific properties of rhamnolipids—anaerobic, halotolerant, and thermotolerant—make them potential agents for in situ and ex situ microbially enhanced oil recovery [70]. Rhamnolipids can also be used in the microbial remediation of hydrocarbon and crude-oil-contaminated soils [71]. Biodegradation of hydrocarbons by native microbial populations is the primary mechanism by which hydrocarbon contamination can be removed from the environment [72]. Rhamnolipids have been used in contaminated Alaskan gravel to remove substantial quantities of oil from the Exxon Valdez oil spill [73]. Validated the effectiveness of in situ bioremediation of the Exxon Valdez oil spill using rhamnolipids in a large-scale test in 1994 [74]. In another experiment, Shabtai and Gutnick demonstrated a 25–70% and 40–80% increase in the recovery of hydrocarbons from contaminated sandy-loam and silt-loam soil, respectively [75]. In another report, 56% and 73% of the aliphatic and aromatic hydrocarbons, respectively, were recovered from contaminated sandy-loam soil when treated with Rhamnolipids [76]. The ability of rhamnolipids biosurfactants to emulsify hydrocarbon-water mixtures, degrade hydrocarbons in oil spill management, and remediate metal contaminated soil has been well documented [77-78]. There are several strategies involving the use of biosurfactants like rhamnolipids in (MEOR), such as injection of biosurfactants-producing microorganisms into a reservoir through the well, with subsequent propagation in situ through the reservoir rock; or injection of selected nutrients into a reservoir, thus stimulating the growth of indigenous biosurfactants-producing microorganisms; while the other mechanism involves the production of rhamnolipids in bioreactors ex situ and subsequent injection into the reservoir. Many types of surfactants are already being used in oil industry but it is important to develop even more new biosurfactants to broaden the spectrum of specific properties and application [80].

### 5.3. *Rhamnolipids in Cosmetics Industry*

In the cosmetic industry, due to its emulsification, foaming, water binding capacity, spreading and wetting properties effect on viscosity and on product consistency, biosurfactants have been proposed to replace chemically synthesized surfactants. These surfactants are used as emulsifiers, foaming agents, solubilizers, wetting agents, cleansers, antimicrobial agents, mediators of enzyme action, in insect repellents, antacids, bath products, pads, anti dandruff products, contact lens solutions, baby products, mascara, lipsticks, toothpaste, dentine cleansers to mention but a few [81]. So large number of compounds for cosmetics applications are prepared by enzymatic conversions of hydrophobic molecules using various lipases and whole cells. Monoglyceride, one of the most widely used surfactants in the cosmetic industry, has been produced from glycerol-tallow (1.5:2) with a 90% yield using *Pseudomonas fluorescens* lipase treatments. Surfactants in cosmetics applications are required to have a shelf life of more than 3 years, and saturated Acyl groups are preferred over unsaturated compounds. Kanebo Cosmetics Global

Corporation (Tokyo) recently unveiled rhamnolipids biosurfactants-based products, and new research has pointed to rhamnolipids as the only biocompatible and ideal biosurfactants for use in cosmetics [79].

#### 5.4. Rhamnolipids in Agriculture

Surfactants have several functional properties, well known, and exploited in many commercial sectors. There are numerous areas of agriculture which also requires surfactants. A review by Deleu and Paquot (2004) enlists the major area where surfactants are employed. It is reported in year 2004 that approximately  $\pm 0.2$  million tons of surfactants are used in crop protection and agrochemical formulations. Several reports have highlighted the advantages of green surfactants (biosurfactants derived from microbes) over the synthetic surfactant. Since there are fewer reports stating the application of biosurfactants in agriculture, the review emphasizes on the significance of biosurfactants and biosurfactants producing microbes from soil especially rhizosphere in agriculture sector. Taking into account the dual hydrophobic/hydrophilic nature of biosurfactants from microbial sources [82] these green surfactants have more advantages over the chemically synthesized surfactants. These biosurfactants can be exploited in areas related to agriculture for enhancement of biodegradation of pollutants to improve the quality of

agriculture soil, for indirect plant growth promotion as these biosurfactants have antimicrobial activity and to increase the plant microbe interaction beneficial for plant. These biosurfactants can replace the harsh surfactant presently used in pesticide industries as these natural surfactants are found to be utilized as carbon source by soil inhabiting microbes [83-84-85] and this accounts for the biological removal of biosurfactants from the agricultural soil. The following part of the review highlights on the reports on role of biosurfactants and biosurfactants producing microbes in the most important commercial sector in agriculture.

The productivity of agriculture land is affected by presence of organic and inorganic pollutants that impart a biotic stress on the cultivated crop plant. To increase the quality of such soil contaminated by hydrocarbon and heavy metals, process of bioremediation is required. Microorganisms producing biosurfactants such as rhamnolipids and and/or biosurfactants can be effectively used for removal of hydrocarbons heavy metals [86]. As biosurfactants (rhamnolipids) are known to enhance bioavailability and carry out biodegradation of hydrophobic compounds, different technologies such as soil washing technology and clean up combined technology employ biosurfactants for effective removal of hydrocarbon and metal, respectively [87-88-89].

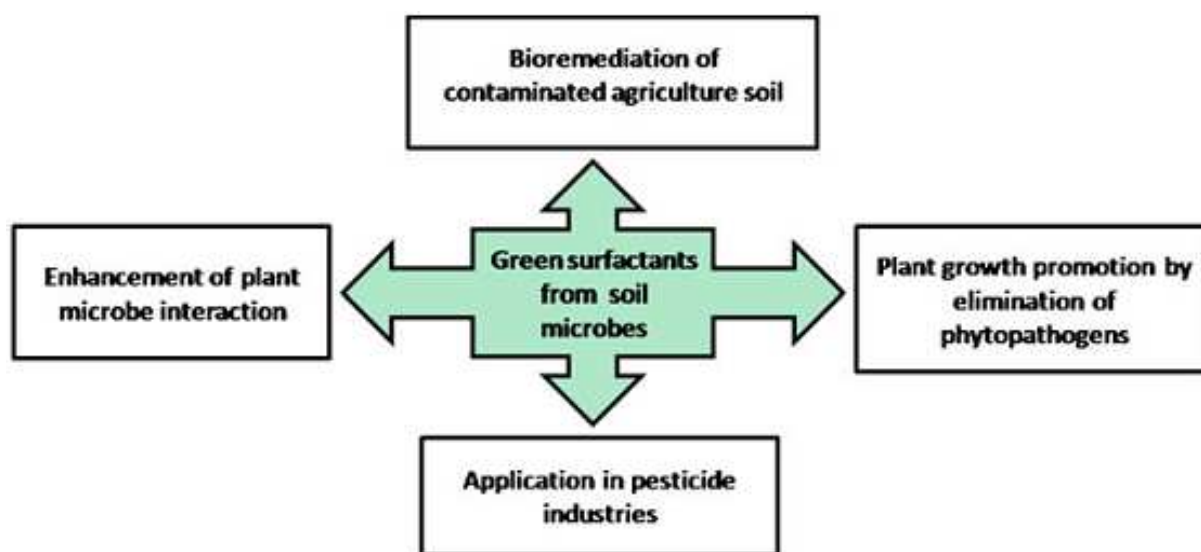


Fig. 2. \*. (Multifunctional prospective of biosurfactants in agriculture).

Many researchers have observed that the efficiency of biosurfactants in removal of organic insoluble pollutants from soil is more as compared to synthetic surfactants [90]. Rhamnolipids are found to be useful in removal of poly aromatic hydrocarbons [91] and pentachlorophenol [92] from soil. Thus biosurfactants can be applied in agriculture soil to enhance soil quality. However, high cost for production of biosurfactants yet restricts the application of these green surfactants for bioremediation of soil contaminated by crude oil and/or petroleum [93]. A recent review summarizes the role of biosurfactants and biosurfactants producing microorganisms in bioremediation of heavy metals and

hydrocarbon pollutants. There are several reports on potential properties of biosurfactants produced by *Pseudomonas* sp, *Bacillus* sp., and *Acinetobacter* sp. for removal of heavy metals from contaminated soil and even acceleration of biodegradation of pesticides [87-94]. Rhamnolipids biosurfactants produced by species of pseudomonads are reported to remove toxic metals from soil [95]. Further, biosurfactants such as rhamnolipids and surfactin are known to remove heavy metals such as Ni, Cd, Mg, Mn, Ca, Ba, Li, Cu, and Zn (ions) from soil with a new method of foaming-surfactant technology [96-97].



## 6. Conclusions

This review on rhamnolipids showed that these biosurfactants have the potential to apply in a range of theatrical chemical surfactants due to their broad application area and significance. In This research exists the well-characterized properties of rhamnolipids and their applications and uses in various areas. They can also be applied in the cosmetics industry, food industry, and in environmental protection also can be used in oil-spill management and in agriculture. Surfactants have several applications in agriculture and agrochemical industries. However, there is rare use of biosurfactants which are more environmental friendly. There is need to work on the production cost of green surfactants to achieve net economic gain from application of biosurfactants in agriculture as well as other sectors. The use of agriculture waste for over production of biosurfactants also requires more serious studies. Biosurfactants (rhamnolipids) show several properties which could be useful in many fields of food industry. Recently, their antiadhesive activity has attracted attention as a new tool to inhibit and disrupt the biofilms formed in food contact surfaces. The Particular characteristics such as emulsifying, antiadhesive and antimicrobial activities presented by biosurfactants suggest potential application as multipurpose ingredients or additives. By using biosymphonic compounds like rhamnolipids we can solve several problems in food industry, oil industry, cosmetics industry, environmental protection and in the agriculture.

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