#### Original Article

# An Overview of Biosurfactants: Structures, Functions, and Applications in Hydrocarbon Bioremediation

Abdulrahman Mohammed Alhudhaibi1\*

Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU),

Riyadh 11623, Saudi Arabia

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#### **Abstract:**

This review evaluates the potential of biosurfactants for hydrocarbon bioremediation, aiming to clarify how their structural diversity and functional properties support environmentally sustainable applications. It outlines the major biosurfactant classes and summarizes the key mechanisms—particularly their ability to enhance the solubility and bioavailability of hydrophobic contaminants—that facilitate more efficient microbial degradation of hydrocarbons. The scope further includes an assessment of their broader industrial relevance, encompassing roles in enhanced oil recovery, wastewater treatment, and formulation of products in the food, cosmetic, and pharmaceutical sectors.

The review emphasizes the advantages of biosurfactants over synthetic surfactants, including their biodegradability, low toxicity, and performance under variable environmental conditions, which collectively position them as strong candidates for green technological solutions. It concludes that biosurfactants hold significant promise for pollution mitigation and sustainable bioprocessing, while highlighting the need for continued advances in cost-effective production, strain optimization, and scalable manufacturing to enable wider commercial adoption.

#### **Keywords:**

Microbial surfactants, bioremediation, PAHs, CMC, hydrocarbon degradation.

#### Address for correspondence:

E-mail: amalhudhaibi@imamu.edu.sa

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#### **Definition and classification of biosurfactants**

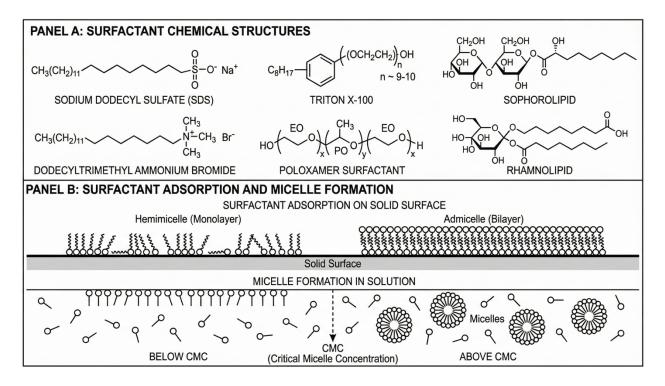
Biosurfactants are compounds synthesized by aerobic-growing microorganisms in aqueous environments using carbon source feedstocks, including hydrocarbons, carbohydrates, oils, and fats, which facilitate the movement and transfer of insoluble substrates across cellular membranes [1]. Biologically synthesized surfactants are classified into two categories: nonionic and anionic. No literature exists on cationic ones; however, in certain cases, the addition of nitrogen-harboring groups confers a degree of cationic properties to specific parts of the molecule, thereby influencing phenomena such as particle flocculation and adsorption on dispersed particles. Biosurfactants, like all agents that are surface-active, comprise one or more hydrophilic and lipophilic

components. The lipophilic ones may consist of a peptide or protein characterized by a significant presence of side chains with hydrophobic properties, typically relating to a fatty acid containing from 10 to 18 carbon atoms acting as the hydrocarbon chain, though fatty acids with elevated molecular weights have also been documented. On the other hand, the hydrophilic component may consist of a hydroxy group, ester, carboxylate group, phosphate group, or carbohydrate [2].

Surfactants are known as compounds with amphiphilic properties that consist of two components; one of them is hydrophobic, and the other is hydrophilic [3, 4]. As a result, these molecules typically aggregate at interfaces, leading to a reduction in interfacial tension (Figure 1 A and B). Surfactants exhibit a significant characteristic whereby, upon reaching a certain concentration known as the critical-micelle-

concentration (CMC), they form stable molecules that aggregate together, forming micelles of 10 to 200 molecules. These micelles can improve the solubility of hydrophobic organic compounds (HOCs) [5, 6] as can be explained in Figure 2. The

CMC represents the precise concentration of surfactants in a liquid where micelle formation leads to a significant change in the relationship between the concentration and certain physicochemical properties, such as the surface tension [5, 7].



**Figure 1**. Polymolecular and molecular structures of surfactants. (A) represents the molecular structures of biosurfactants and specific synthetic surfactants, while (B) represents unassociated surfactants and its micelle in a solid-aqueous environment (upper photo) and aqueous-only environment (lower photo); CMC is the abbreviation of critical micelle concentration, and ATCC is the abbreviation of American Type Culture Collection adopted from [7].

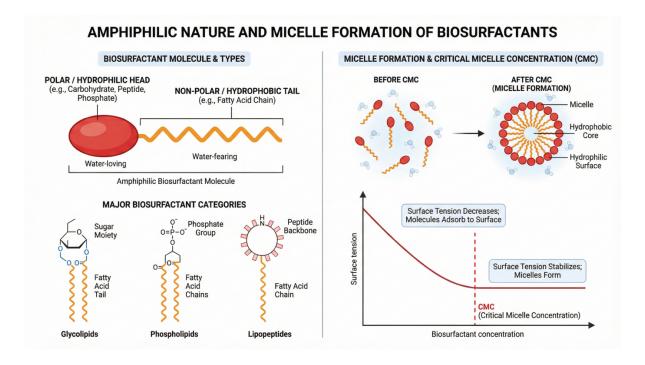


Figure 2. Microbial surfactants and their micelle, adopted from [8].

#### **Types of biosurfactants**

Bacteria synthesize low molecular weight compounds that significantly decrease surface and interfacial tensions, alongside high molecular weight polymers that exhibit strong adhesion to surfaces [9-12].

Biosurfactants with low molecular weight typically consist of glycolipids, which are formed by the combination of carbohydrates and lipopeptides or long-chain aliphatic acids. For instance, trehalose lipids, sophorolipids, and rhamnolipids consist of disaccharides acylated with hydroxy fatty acids or long-chain fatty acids and belong to the glycolipids. Rhamnolipid is a glycolipid extensively studied and produced by various species of Pseudomonas, consisting of two moles of  $\beta$ -hydroxydecanoic acid and two moles of rhamnose [13, 14].

A large number of bacterial species from different genera are known for the production of high molecular weight surfactants. They are composed of lipopolysaccharides, proteins, polysaccharides, lipoproteins, or complex combinations of these biopolymers. Surfactants with high molecular weight exhibit reduced efficacy in lowering interfacial tension; however, they are effective in inhibiting their coalescence through their surroundings to the oil droplets [12, 15].

High molecular weight bio-emulsifiers represent notable substrate selectivity. Certain agents can effectively emulsify mixtures of aromatic (or cyclic alkane) and aliphatic hydrocarbons while failing to emulsify aromatic, pure aliphatic, or cyclic hydrocarbons. Conversely, some agents can emulsify pure hydrocarbons, especially those with high molecular weight. The bioemulsans produced by various Acinetobacter species are the most extensively studied biosurfactants [12, 16] in addition to the recent exopolysaccharides produced by Rhizobium tropici [17].

- [18] classify biosurfactants into five specific categories: (1) Glycolinids include trehalose, sophorose, rhamnolinids.
- (1) Glycolipids include trehalose, sophorose, rhamnolipids, and mannosylerythritol lipids.
- (2) Liposaccharides, including emulsifiers that are water-soluble, extracellular, and have elevated molecular weight. They are produced by hydrocarbon-consuming bacteria such as Acinetobacter calcoaceticus and known as emulsans.
- (3) Lipopeptides include subtilysin and ornithine lipids, which are synthesized by Bacillus subtilis.
- (4) Phospholipids are prevalent in all microorganisms; however, cases of extracellular synthesis are infrequent, with Corynebacterium lepus being the most notable example due to its production of biosurfactants.
- (5) Neutral lipids and fatty acids include corynomycolic acids, ustilagic acid, lipoteichoic acids (or glycolipids), and proteins with a hydrophobic nature.

The types of microorganisms that are able to produce biosurfactants, in addition to the types of the produced biosurfactants, are represented in Table 1.

**Table 1.** Major types of biosurfactants and their producing microorganisms

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Biosurfacta nt type	Chemical nature	Major producing microorganisms	Representati ve references
Rhamnolipi ds	Glycolipids	Pseudomonas aeruginosa, Pseudomonas putida	[12, 19]
Sophorolipi ds	Glycolipids	Candida bombicola, Candida apicola	[20, 21]
Surfactin	Lipopeptide	Bacillus subtilis	[13, 22]
Lichenysin	Lipopeptide	Bacillus licheniformis	[23]
Trehalose lipids	Glycolipids	Rhodococcus erythropolis, Mycobacterium spp.	[24]
Emulsan	Lipopolysac charide	Acinetobacter calcoaceticus RAG- 1	[16]
Alasan	Complex bioemulsifi er	Acinetobacter radioresistens	[25]

Each biosurfactant has its own potential to form a different CMC value. For example, rhamnolipids typically exhibit CMC values ranging from 10 to 200 mg/L, while surfactin shows much lower CMC levels (20–25 mg/L), indicating stronger surface activity [26, 27]. Sophorolipids generally present higher CMC values but excel in emulsification stability [28]. These differences are now explicitly compared in terms of their implications for hydrocarbon solubilization and bioremediation efficiency [8].

Moreover, the efficiency of each selected biosurfactant and its physicochemical properties are presented in Table 2.

**Table 2.** Physicochemical properties and efficiencies of selected biosurfactants

Biosurfactant	Typical CMC (mg/L)	Surface tension reduction (mN/m)	Key functional advantage	Reference
Rhamnolipid	10–200	~25–30	Strong solubilization of PAHs	[29, 30]
Surfactin	20–25	~27	Very low CMC, strong surface activity	[13]
Sophorolipid	40–150	~30–35	High emulsification stability	[17]
Trehalose lipid	30–100	~28–32	Effective desorption from soils	[24]
Emulsan	Not defined (polymeric)	Limited tension reduction	Excellent emulsion stability	[16]

lipophilic component generally comprises an alkyl tail that may be saturated, hydroxylated, branched, unsaturated, or terminating with a group with an acidic nature. The nonhydrophilic fatty acid is linked to the hydrophilic group through an amide bond or a glycosidic ester. Biosurfactants predominantly exhibit a negatively charged or neutral nature, with their anionic characteristic resulting from the presence of carboxyl groups that bear a pKa lower than the solution pH. Microorganisms produce a variety of potentially advantageous agents that are surface-active, including both nonionic and ionic molecules, which range in size from large polymers to short-chain fatty acids. The three main categories of biosurfactant chemical structures are phospholipids, glycolipids, and fatty acids, in addition to lipoproteins and lipopeptides. These structures can produce biosurfactants in the form of singular macromolecules, polymeric formations, or particulate entities [31]. The main classes of biosurfactants can be shown in Figure 3.

#### **Composition of biosurfactants**

Biosurfactants are produced by various microorganisms, particularly when growing on water-immiscible substrates, and display a diverse array of molecular structures. The

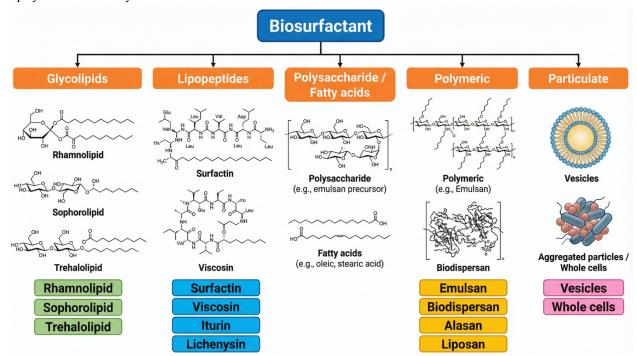


Figure 3. Main classes and structures of biosurfactants adopted from [32].

### Mechanism of biosurfactant activity in Enhancing Hydrocarbon Bioavailability

The influence of surfactants on the bioavailability of organic contaminants can be explained through several interrelated mechanisms that collectively enhance the transfer of hydrophobic compounds to microorganisms (Figure 4). These mechanisms include micellar solubilization, dispersion of nonaqueous-phase liquids (NAPLs), modification of microbial cell-surface hydrophobicity, and enhanced desorption of pollutants from solid matrices.

#### 1. Micellar Solubilization

Micellar solubilization occurs when biosurfactant concentrations exceed the critical micelle concentration (CMC), leading to the formation of micelles that encapsulate hydrophobic organic compounds within their hydrophobic cores. This process increases the apparent aqueous solubility of poorly water-soluble contaminants such as polycyclic aromatic hydrocarbons (PAHs), thereby improving their accessibility to microorganisms [33]. Microbial uptake can occur either directly from the micellar phase or following the release of solubilized contaminants into the aqueous phase, facilitating subsequent biodegradation [34] (Figure 4A).

#### 2. Dispersion of Nonaqueous-Phase Liquids (NAPLs)

Biosurfactants can significantly reduce interfacial tension between aqueous and nonaqueous phases, resulting in the dispersion of NAPL droplets into smaller units with increased surface area. This mechanism is operative primarily in systems containing a separate organic phase and enhances mass transfer of hydrocarbons from the NAPL phase into the aqueous environment [33]. The increased interfacial area accelerates the rate at which hydrocarbons partition into the water phase, promoting microbial degradation. However, the relative contribution of NAPL dispersion versus micellar solubilization may be difficult to distinguish, as both processes enhance contaminant flux to the aqueous phase (Figure 4B).

## 3. Modification of Microbial Cell-Surface Hydrophobicity Certain biosurfactants interact directly with microbial cell membranes, altering cell-surface hydrophobicity and

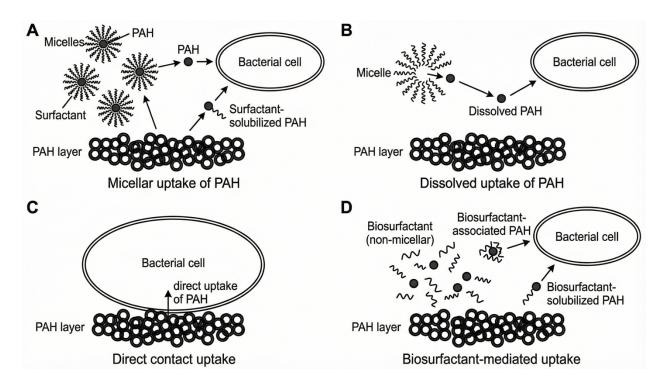
enhancing adhesion to hydrophobic substrates. This mechanism facilitates direct contact between microorganisms and NAPL droplets, reducing diffusion limitations and improving substrate uptake [34]. The modification of cell hydrophobicity has been demonstrated to increase microbial attachment to hydrocarbons, thereby supporting efficient biodegradation, particularly in oil-contaminated environments [35-37] (Figure 4C).

#### 4. Enhanced Desorption from Solid Matrices

Biosurfactants can promote the desorption of hydrophobic pollutants that are strongly sorbed to soil or sediment particles. By reducing surface tension at solid-liquid interfaces and interacting with both the contaminant and the solid matrix, biosurfactants decrease the binding strength of organic compounds, facilitating their transfer into the aqueous phase [35-37]. Additionally, surfactants may enhance microbial adsorption to pollutant-coated soil particles, effectively reducing the diffusion distance between the sorbed contaminant and microbial uptake sites [35-37] (Figure 4D).

In summary, these mechanisms often operate simultaneously, and their relative importance depends on factors such as surfactant concentration, contaminant type, soil characteristics, and microbial community composition. Understanding the dominant mechanisms under specific environmental conditions is essential for optimizing biosurfactant-assisted bioremediation strategies.

In addition, each surfactant can act with a specific mechanism; for instance, rhamnolipids primarily promote micellar solubilization. On the other hand, other surfactants enhance desorption from soil matrices (e.g., trehalose lipids), and others can modify cell-surface hydrophobicity to facilitate direct hydrocarbon uptake (e.g., surfactin) [38, 39]. In addition to the non-micellar pathways that have recently gained attention [40].



**Figure 4.** Mechanisms proposed for enhancing the bioavailability of PAH through surfactant application. The figure depicts (A) the direct uptake of PAH from micelles, (B) uptake of PAH linked to the aqueous phase subsequent to micelle release, (C) enhanced direct uptake of PAH through PAH-surfactant-cell interaction, and (D) proposed augmentation of nonmicellar biosurfactant to solubilization of PAH adopted from [34].

#### Benefits of biosurfactants

Biologically produced surfactants are more effective than surfactants that are artificially synthesized in improving the bioavailability of substances with hydrophobic nature. Additionally, they are environmentally friendly, selective, and typically demonstrate reduced stability relative to the majority of synthetic surfactants. Recent studies have shown that the high molecular weight bioemulsifiers, such as Alasan significantly improves the biodegradation rate of various PAHs [25, 41].

The use of emulsifiers is valuable in situations where the growth of bacterial cells is limited, such as at reduced temperatures or high pollutant concentrations, or when dealing with persistent pollutants like PAHs. Bio-emulsifiers can serve as an assistant to improve the bioremediation process [12, 42]. Biosurfactants possess several unique properties and advantages, including the following:

1- Superior interface and surface activity compared to sulphonated anionics: at lower surfactant concentrations they are able to reduce the surface tension [43, 44] [45].

2- Temperature resistance: certain biologically produced surfactants remain unchanged at temperatures above 90°C

3- Ionic strength tolerance: biosurfactants remain soluble in saline solutions of up to 10%, whereas 2 to 3% salt is adequate to inactivate the chemically synthesized surfactants [8]. 4- Biodegradability: biosurfactants are easily decomposed in

aquatic and terrestrial environments, thereby presenting no further pollution risk [46].

- 5- Emulsion disruption: emulsions formulated with biosurfactants can be readily separated with the introduction of enzymes. For instance, minimal quantities of the depolymerase enzyme can decompose the hydrocarbons existing in oil emulsions [47].
- 6- Non-toxic: these biosurfactants are entirely non-toxic to multiple microorganisms and thus improbable to impede the bio-removal of PAHs [31].
- 7- More economical: they are more cost-effective than chemically synthesized surfactants and can be readily produced in situ at contaminated locations using affordable basic materials. [29] reported that molasses, which is a cheap and highly available sugar industry byproduct, can hugely increase the production of biosurfactants and hence will enhance the oil recovery-mediated microbes in soil columns [48, 49].

The suggested method for improving the PAHs-bioavailability involves the use of biosurfactants, which primarily enhance microbial activity in environments with hydrophilic-hydrophobic interfaces [50]. Biosurfactants possess the ability to solubilize hydrocarbons, providing advantages over synthetic surfactants, which positions them as promising candidates for bioremediation strategies [21].

#### **Drawbacks of synthetic surfactants**

Surfactants derived from petroleum can exhibit toxicity and resistance to biodegradation, often leading to their expensive

synthesis and making them difficult to execute on-site, in addition to producing hazardous waste by-products [7,51]. The often limited efficacy and comparative toxicity of chemically synthesized surfactants at reduced concentrations limit their applicability in PAH-contaminated environments [7,52].

In contrast, some studies indicated that synthetic surfactants had an inhibitory effect. Various explanations have been proposed to clarify this inhibitory effect. A theory posits that limited direct access of microorganisms to PAHs within micelles may restrict the degree of mass transfer between the aqueous phase and these micelles, thereby constraining The second biodegradation. theory suggests biodegradation inhibition may occur if the surfactant is detrimental or if microorganisms preferentially utilize it as a sole carbon source [53, 54]. Table 3 is a comparative table that contrasts synthetic surfactants and biosurfactants across key parameters, including biodegradability, toxicity, critical micelle concentration (CMC), performance under extreme environmental conditions, production cost, scalability, environmental persistence, and suitability for in situ bioremediation.

**Table 3**. Comparison between synthetic surfactants and biosurfactants in hydrocarbon bioremediation

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Parameter	Synthetic surfactant s	Biosurfactant s	Reference s
Source	Petroleum -derived	Microbial origin	[7]
Biodegradabilit y	Often low or slow	Readily biodegradabl e	[47]
Toxicity	Moderate to high	Low or negligible	[52]
СМС	Generally higher	Often lower	[29]
Environmental persistence	High	Low	[50]
Effect on microbes	May inhibit activity	Generally stimulatory	[54]
Cost (current)	Lower	Higher	[55]
Field applicability	Limited by toxicity	More suitable for in situ use	[21]

#### **Applications of Biosurfactants**

Biosurfactants have attracted considerable attention due to their amphiphilic properties, biodegradability, and effectiveness under extreme environmental conditions. Their capacity to enhance hydrocarbon solubilization, dispersion, and microbial accessibility has enabled broad applications in environmental remediation and multiple industrial sectors [12, 21].

#### 1. Oil and Petroleum Industry Applications

In the petroleum industry, biosurfactants are primarily applied in enhanced oil recovery (EOR) and petroleum tank cleaning. By reducing interfacial tension between oil and water, biosurfactants mobilize residual oil trapped in reservoir pores, leading to improved oil recovery under high salinity and temperature conditions [56]. In petroleum storage tanks, biosurfactants facilitate the removal of heavy oil fractions and sludge through emulsification, enabling oil recovery while minimizing hazardous waste generation [57, 58]. Field-scale and semi-industrial applications have demonstrated the technical feasibility of biosurfactant-assisted sludge remediation in crude oil storage facilities [59].

#### 2. Soil and Sediment Bioremediation

Biosurfactants are widely employed to enhance the bioremediation of hydrocarbon- and PAH-contaminated soils and sediments. They increase pollutant bioavailability through micellar solubilization, desorption from soil matrices, and dispersion of nonaqueous-phase liquids, thereby accelerating microbial degradation [7, 40]. Studies have reported significant improvements in the degradation of PAHs such as phenanthrene and naphthalene in biosurfactant-amended soils and slurry bioreactors, particularly when rhamnolipids, sophorolipids, surfactin, and trehalose lipids are employed [19, 22, 23, 30]. Their low toxicity and biodegradability make biosurfactants especially suitable for in situ soil remediation strategies [50]. Various biosurfactants have been employed in environmental remediation, particularly in improving the bioremediation of hydrocarbons with hydrophobic nature in soil slurries within bioreactors and oil-contaminated coastal soils [60]. A lot of studies on enhanced bioremediation depending on the use of biosurfactants have employed wellinvestigated biosurfactants, such as sophorose lipids from Candida apicola [20], rhamnolipids from Pseudomonas aeruginosa [19], lichenysins from Bacillus sp. [23], trehalose dimycolipids from Rhodococcus erythropolis [24], and surfactin from Bacillus subtilis [22] (Figure 5).

#### 3. Wastewater Treatment

In wastewater treatment, biosurfactants enhance the removal of hydrophobic organic pollutants from industrial effluents by improving emulsification, solubilization, and microbial uptake [61]. They have been successfully applied in the treatment of effluents from oil refineries and petrochemical industries, where they promote oil–water separation, improve sludge dewatering, and reduce membrane fouling [60, 62]. Importantly, biosurfactants are compatible with biological treatment systems and generally do not inhibit microbial activity, making them suitable for integration into activated sludge and biofilm-based processes [12].

#### 4. Non-Environmental Industrial Applications

Beyond environmental remediation, biosurfactants are increasingly used in cosmetics, food processing, pharmaceuticals, and drug delivery systems due to their biocompatibility, mildness, and multifunctional properties [55, 63]. Sophorolipids and surfactin, in particular, have been

incorporated into personal care formulations, antimicrobial products, and pharmaceutical carriers for dermal and transdermal drug delivery [13, 64]. Despite their promising performance, high production and purification costs continue to limit their large-scale adoption in these non-environmental sectors [10, 21].

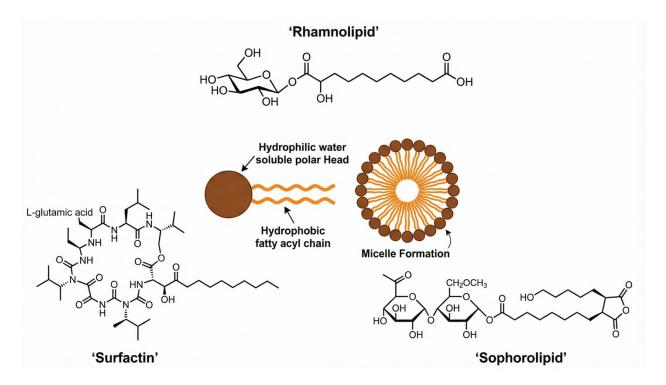
Overall, biosurfactants exhibit broad applicability across environmental and industrial domains, with particularly strong potential in the petroleum industry and hydrocarbon bioremediation. Continued progress in strain development, process optimization, and cost-effective production is expected to further expand their practical and commercial use.

However, some of these studies are reporting their degradation outcomes. For instance, rhamnolipid-enhanced systems achieved up to 60–90% removal of PAHs such as phenanthrene [19, 30], while surfactin-producing Bacillus strains increased degradation of crude oil components by 40–70% [22, 23]. These comparisons clarify which biosurfactants are more effective under specific environmental conditions.

In general, the applications of biosurfactants in the bioremediation process are represented in Table 4.

**Table 4**. Applications of biosurfactants in bioremediation and industry

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Application area	Biosurfactant used	Observed effect	Reference
PAH- contaminate d soils	Rhamnolipids	60–90% enhancement of phenanthrene degradation	[19, 30]
Crude oil bioremediati on	Surfactin	Increased oil dispersion and degradation rates	[22]
Petroleum tank cleaning	Mixed biosurfactants	Sludge mobilization and oil recovery	[57, 58]
Enhanced oil recovery	Rhamnolipids, surfactin	Improved oil mobilization	[56]
Wastewater treatment	Sophorolipids	Improved hydrocarbon removal	[61]



**Figure 5**. Structure of some common biosurfactants adopted from [65].

Biosurfactants have gained considerable interest in the field of environmental biotechnology and industry as well in recent years. Their multiple applications include enhancing the recovery of oil, lubricants for crude oil extraction, healthcare, and food processing [64] Additional applications of biosurfactants include formulations for cosmetics and soaps, food products, and systems for dermal or transdermal drug delivery [63].

### The function of biosurfactants in the solubilization of hydrocarbons

Bioremediation is restricted by the recalcitrance of target pollutants, attributed to their chemical stability, as well as the toxicity of these substances, especially the limitation of the bioavailability of hydrophobic contaminants (poorly-water-soluble contaminants) like PAHs. It is well known that the biologically active reactions can occur in or at the interface of the aqueous phase, while surfactants can disperse and desorb limitedly soluble substances within small micelles with high surface area in the water phase. The vulnerability of these substrates to microbial degradation can be increased by surfactants [66-68].

Oil pollution represents a growing environmental concern. Microorganisms that degrade hydrocarbons and are adapted to thrive in oil-rich environments are essential for the biological remediation of this pollution. The bioavailability of different oil fractions serves as a limiting factor in this process. Microorganisms capable of hydrocarbon degradation produce biosurfactants characterized by diverse chemical compositions and molecular sizes. These surfactants elevate the surface area

of such hydrophobic substrates with water-insoluble properties, thereby improving their bioavailability and increasing the growth of the bacteria that are able to enhance the rate of the bioremediation process [12, 38, 69].

Bioemulsifiers are synthesized by bacteria that are able to metabolize hydrocarbons, and they have been shown to promote bacterial proliferation and accelerate bioremediation processes [11, 12]. All these chemicals have limited solubility in water [12]. The requirement for bacteria to directly interact with hydrocarbon substrates is highlighted by the necessity of a membrane-bound oxygenase in the initial phase of hydrocarbon biodegradation. The hydrocarbons can be emulsified through a biological technique that enhances the interaction between the hydrocarbon (with water-insoluble properties) and the microorganism. Thus, it is expected that the microorganism thriving on crude oil can produce a potent emulsifier. Surfactants promote oil dispersion, improve microbial growth through increasing the surface area, and aid in the detachment of bacteria that already bind to the oil droplets, especially after the depletion of available hydrocarbons [12, 70-72].

Oil-degrading bacteria metabolize a limited range of hydrocarbons; consequently, those bacteria that adhere to and proliferate on the oil droplets become nutrient-deficient once this targeted group of hydrocarbons is consumed. The cell-bound biosurfactant can increase the hydrophobic nature of the surface on the microbial cell, as determined by its orientation. In the case of Pseudomonas aeruginosa, it was observed that the hydrophobicity of the cell surface was hugely increased by the presence of rhamnolipid, which was bound to the cell [38, 39, 73], while in the case of Acinetobacter strains, it was found

that the existence of cell-bound emulsifier resulted in their cell surface hydrophobicity [74]. The data demonstrate that microbes can employ their biosurfactants to alter their cellsurface properties for attachment or detachment from surfaces as necessary. Evidence for the ability of A. calcoaceticus RAG-1 to degrade and use crude oil as its sole carbon source has been documented [72]. During the exponential growth of the bacteria, it was noticed that the emulsan is attached to the cell surface in the form of minicapsules. This microorganism was able to grow through the utilization of a sole carbon source, including long-chain alkanes. Upon the application of these compounds, RAG-1 is rendered deficient, yet it remains linked to the oil droplets, which have a lot of cyclic and aromatic paraffins. The absence of RAG-1 leads to the detachment of the emulsan minicapsule. The released emulsan was able to form polymeric coatings on the oil droplets rich with n-alkanes, resulting in the desorption of the starving cell [74]. The emulsifier facilitates cellular access to novel substrates. The oil droplet is classified as utilized, having developed a hydrophilic surface that inhibits bacterial adhesion. The isolation of microorganisms from the depleted oil droplet facilitates their migration to other droplets, resulting in the metabolization of specific types of usable hydrocarbons [12, 75].

The long-time existence of hydrophobic substances with high molecular weight primarily results from their decreased water solubility, which results in enhancing their sorption of these substances to other surfaces and reducing their availability to the bioremediating microbes. The irreversible binding of organic molecules to surfaces hinders biodegradation

[40, 76]. Biosurfactants boost the growth on bound substrates by increasing their actual water solubility or as a result of desorption from surfaces [77]. Surfactants that effectively lower interfacial tension are adept at mobilizing bound hydrophobic compounds, thereby enhancing their biodegradation [12, 70]. The bacterial growth rate on hydrocarbons may be reduced by the interfacial surface area between oil droplets and water [38, 78]. When surface area serves as a limiting factor, biomass exhibits an arithmetic increase rather than an exponential one [12, 79].

Recent studies indicate that biosurfactants can boost the degradation rates of PAHs by aiding microbial access to insoluble substrates [80, 81]. The efficacy of biosurfactants in soil remediation is contingent upon their capacity to enhance dissolution and desorption, alongside facilitating the biodegradation of pollutants. The parameters might be interrelated, as boosted dissolution, desorption, or transport could improve the bioavailability of the pollutant, thereby accelerating biodegradation rates [7, 61].

[30] examined the effect of biosurfactants produced by P. aeruginosa UG2 on the degradation of phenanthrene in soil slurries, employing Pseudomonas sp. UG14. An increase in phenanthrene biodegradation was noted, along with a reduction in the lag phase prior to the beginning of biomineralization.

[82] reported the potency of PAH-degrading bacteria to express their biosurfactants from phenanthrene and naphthalene.

These biosurfactants that were obtained from these two substrates were identified as responsible for the increased actual aqueous content of naphthalene. [83] employed Tween 80 as a synthetic surfactant in conjunction with glycolipids from Rhodococcus sp. H13A to enhance the solubility of naphthalene and methyl-naphthalene. Both surfactants lowered the surface tension of the aqueous solution to 30 dynes/cm; however, the used biosurfactants exhibited greater effectiveness in increasing the obvious solubility of PAHs. The effect was reducibly pronounced in naphthalene compared to methyl-naphthalene. The increased solubilization capacity of biosurfactants can be ascribed to their significant micellar volume, allowing for the incorporation of a greater quantity of replaced PAHs.

## **Identification of knowledge gaps and research challenges:** In the field of biosurfactant synthesis and applications, there are still unresolved issues such as

Limited understanding of biosurfactant performance in heterogeneous soils and fluctuating moisture regimes [40, 80]. Insufficient linkage between biosurfactant structure and biodegradation kinetics [15].

Lack of long-term ecological safety assessments [50].

Scarce techno-economic evaluations for industrial-scale production [9].

## Limitations regarding scale-up and real-world applications:

The scaling-up and real-world applications of biosurfactants are facing major barriers, including:

Low yields and high downstream purification costs [10, 59].

Performance inconsistencies in field soils due to sorption or biodegradation of the biosurfactant itself [40, 81].

Challenges with in situ delivery and stability at high salinities or varying pH [8].

Commercial adoption remains limited, reflecting high production costs compared to synthetic surfactants [7, 55].

#### **Future Perspectives and Research Directions**

Despite the demonstrated potential of biosurfactants in hydrocarbon bioremediation, several scientific and technological challenges remain that warrant focused future research. Addressing these gaps will be essential for advancing biosurfactants from laboratory-scale studies to reliable, large-scale environmental applications.

#### 1. Structure-Function Relationships

Future studies should aim to establish clearer correlations between biosurfactant molecular structure, physicochemical properties (e.g., CMC, emulsification index, surface tension reduction), and biodegradation performance. Comparative investigations using standardized experimental conditions are particularly needed to determine how specific biosurfactant classes and congeners influence hydrocarbon solubilization and microbial uptake.

#### 2. Performance in Complex Environmental Matrices

Most existing studies have been conducted under controlled laboratory conditions. Future research should prioritize field-

scale and mesocosm studies to evaluate biosurfactant behavior in heterogeneous soils, sediments, and groundwater systems. Factors such as soil organic matter, salinity, pH, temperature, and indigenous microbial communities must be systematically examined to better predict real-world performance.

#### 3. Ecotoxicological and Long-Term Environmental Impacts

Although biosurfactants are generally considered environmentally benign, comprehensive assessments of their long-term ecological effects remain limited. Future work should focus on chronic toxicity, biodegradation pathways, metabolite formation, and potential impacts on non-target organisms to ensure environmental safety during prolonged or repeated applications.

#### 4. Cost-Effective Production and Scale-Up

High production costs and downstream processing remain major barriers to commercial adoption. Research efforts should focus on strain improvement through metabolic engineering, optimization of fermentation conditions, and the use of low-cost or waste-derived substrates. Integrated bioprocessing strategies that reduce purification requirements may further enhance economic feasibility.

#### 5. Integration with Advanced Bioremediation Strategies

Future research should explore synergistic approaches that combine biosurfactants with other remediation technologies, such as bioaugmentation, phytoremediation, nanotechnology, and electrokinetic remediation. Understanding how biosurfactants interact with these systems may lead to more efficient and resilient remediation strategies.

#### 6. Standardization and Comparative Assessment

The lack of standardized testing protocols limits direct comparison among studies. Establishing uniform methodologies for evaluating biosurfactant efficiency, biodegradation enhancement, and environmental impact will improve reproducibility and facilitate regulatory acceptance.

#### Conclusion

Biosurfactants represent a promising class of biologically derived surface-active compounds with significant potential for hydrocarbon bioremediation and a wide range of industrial applications. Their amphiphilic nature, structural diversity, and capacity to enhance the bioavailability of hydrophobic pollutants enable effective mechanisms such as micellar solubilization, NAPL dispersion, desorption from solid matrices, and modification of microbial cell-surface hydrophobicity. Collectively, these properties distinguish biosurfactants from conventional synthetic surfactants and underscore their suitability for environmentally sustainable remediation strategies.

Despite these advantages, several challenges continue to limit the large-scale and commercial deployment of biosurfactants. High production costs and relatively low yields remain major barriers, largely due to suboptimal microbial strains, expensive substrates, and energy-intensive downstream purification processes. In addition, variability in biosurfactant composition, reduced effectiveness in heterogeneous field environments, and uncertainties surrounding long-term environmental

behavior complicate scale-up and real-world application. Regulatory challenges, including the absence of standardized evaluation frameworks and approval pathways for environmental use, further constrain commercialization and widespread adoption.

Future research should therefore prioritize the development of cost-effective production strategies, including strain improvement through metabolic engineering, utilization of low-cost or waste-derived substrates, and simplified recovery processes. Greater emphasis on pilot-scale and field-based studies is needed to validate laboratory findings and assess performance under realistic environmental conditions. Establishing standardized testing protocols and regulatory guidelines will also be critical to facilitate market entry and practical implementation.

In conclusion, while biosurfactants offer substantial environmental and technological benefits, overcoming economic, technical, and regulatory challenges will be essential to fully realize their potential as sustainable alternatives in hydrocarbon bioremediation and bio-based industrial applications.

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