Biosurfactants aided Bioremediation mechanisms: a mini-review

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Abstract

Biosurfactants can be used for bioremediation as amphiphilic compounds that have shown good tolerance to changes in temperature, salt concentration, pH, and other environmental factors. They have received increased attention in bioremediation because they are biodegradable and do not generate any secondary contaminants. Biosurfactants are used to remove organic molecules for example hydrocarbon contaminants through various mechanisms such as surface tension reduction, emulsification, and micelle formation. They can also play a role in removing heavy metals by increasing contact with the surface of heavy metal deposits and forming complexes and micelle formation. In this review, we focus on the role of biosurfactants in improving the efficacy of bioremediation.

Keywords: Bioremediation; Biosurfactants; Oil pollution; Emulsification, Biodegradation

1. Introduction

The release of potentially hazardous organic and inorganic pollutants into the ecosystem has been a topic of interest for years (1). Organic compounds and heavy metals are, usually present in the soil and high concentrations the inorganic contaminants are known to have the greatest risk to humans (2). The accumulation of non-degradable pollutants as heavy metals in biological systems would ultimately lead to the contamination of the entire food chain (3). In 2015 it was reported that pollution-induced diseases resulted in an estimated 9 million premature deaths representing 16% of all deaths in the world (4). The physical approaches of bioremediation are mainly method of eliminating or degrading mainly organic pollutants present in the soil by different methods including physical techniques such as soil replacement processes, landfill barrier methods, and thermal desorption approaches (5). Chemical cleanup meanwhile consist mainly of soil solidification-stabilization, leaching, and oxidation-reduction processing. The solidification-stabilization technique could establish long-term stability of pollutants in contaminated media (6). Physical and chemical techniques of remediation/cleanup are usually costly and do not result in complete pollutants removal and often require management of a significant amount of harmful waste generated (7).

Alternative bioremediation strategies to eliminate pollution are considered as relatively new sustainable recent approaches. There are three types/approaches for bioremediation: phytoremediation, animal remediation and microbial remediation (8). Phytoremediation, a practical, cheap and environmentally friendly rehabilitation strategy, is a bioremediation technology that involves plants to remove either organic and inorganic contaminants, particularly from the soil environment (9). Animal remediation is also used widely to remedy soil and water contamination at specific locations (10). Microbial remediation process in comparison is mainly carried out by microorganisms or parts thereof to remove or clean up contaminants through their effective degradation and/or enhancing their bioavailability and breakdown. This process is the main approach for removing many environmental pollutants, such as products from the petroleum industry (11). In some experiments, the polluted hydrocarbon soils were augmented with biosurfactants producing bacterial species which resulted in enhance hydrocarbons degradation (2, 12, 13). Plants, animals, and microbes are known to synthesize biosurfactants (14). Biosurfactants are known as amphiphilic compounds consisting of hydrophilic and hydrophobic moieties (15)) . This composition gives them surface-active properties, including reduction of surface and interfacial tension in aqueous solutions and mixtures of hydrocarbons (2, 16). This indicates that biosurfactants can be used to enhance bioremediation processes. Hence, in this review, we focus on the above issues through a discussion bioremediation with biosurfactants and their mechanism.

2. Pollution

Water and soil are the main recipients for different pollutants that influence their quality, nature, and performance. Hydrocarbon pollutants may include alkanes, aromatic compounds, chlorinated hydrocarbons, heterocyclic nitrogen, and nitroaromatics (17). Metal pollution in the ecosystem has also increased as a result of increased industrial activity. Heavy metals are present in soil and are known to be the inorganic pollutants with the greatest significant hazard to humans (18, 19). Metal toxicity is not only related to the exposure level but also to the metallic chemical species involved, which has an effect on stability and bioavailability within the ecosystem (20). Due to the propensity of such substances to bioaccumulation, they impose a considerable risk for food safety and all living organism (21, 22).

Hydrocarbons are the most common organic contaminant in soil and water which are of growing concerns (11). Numerous oil spills repeatedly displayed the hazard effects hydrocarbons have on the environment. Oil pollution therefore needs solutions that are quick and economical (23). The persistence of organic polluting compounds within the natural environment depends on many factors, including chemical composition, distribution, and concentration (24). In the event of an oil spill, physicochemical remedies are usually applied; however, these procedures are very costly and more strategies may be required depending on the chemical agents selected as surfactants or catalysts (25). Chemical remediation requires the addition of chemical compounds to degrade pollutants or turn them into substances that are less hazardous to the environment. Oxidation, reduction, polymerization, and precipitation are the most widely used methods in this process (26).

Conventional physical remedies that separate/isolate soil and pollutants without chemical destruction or modifications of the oils are also common. Many of the petroleum products are trapped in the soil matrix, thereby reducing each remediation method performance. Biological processes on the other hand offers efficient remediation methods, as they combine efficiency and cost-effectiveness. Among many novel strategies, bioremediation consistently emerges as the least aggressive and often the most suitable method for maintaining the ecological balance (27).

3. Bioremediation methods

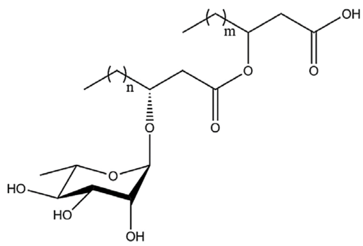
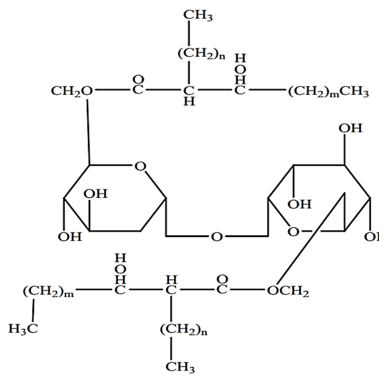
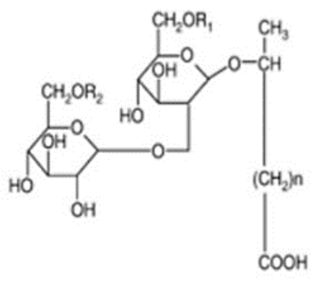
The use of biological processes to remove or transform pollutants in the environment to either safe levels or to turn pollutants into acceptable forms is known as bioremediation (8, 20). The definition involves biodegradation, which relates to the transformation or detoxification of contaminants partially or completely by biological systems (28). Bioremediation is therefore a method that improves the effectiveness of the natural biodegradation process (29). This technique involves low-technology and is generally more economical and can often be carried out on-site or *in situ* (30, 31). The purpose of bioremediation is to reduce contamination levels to less toxic or safe levels, compared to the limits set by regulatory agencies or, preferably, complete mineralization to water and carbon dioxide (28). Bioremediation is also beneficial because of its environmentally sustainable nature, as it does not involve the introduction of foreign or hazardous chemicals to the polluted site. Environmental sustainability is as a result of using natural reproducible additives that do not entail any damage to the natural ecosystem that often results from chemical and physical remediation methods. Bioremediation enables biological organisms to degrade toxic hydrocarbons into simple compounds that do not pose risk to human health and minimizes the need to eliminate and transfer harmful substances to another location (32). Biosurfactants producing bacterial species were shown to enhance hydrocarbon degradation through increased hydrocarbon removal compared to common chemical surfactants uses (12, 33). Therefore, biosurfactants applications are attractive in bioremediation processes(34).

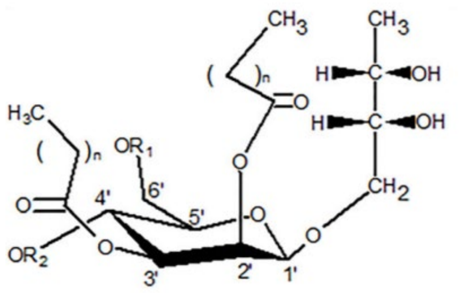
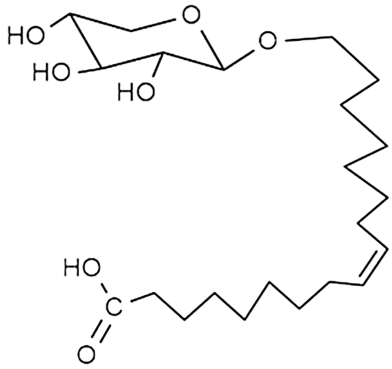
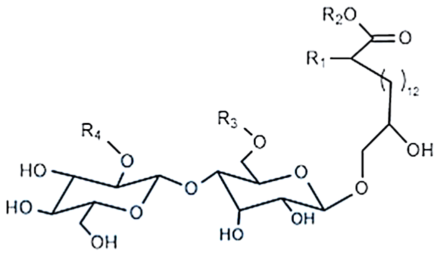
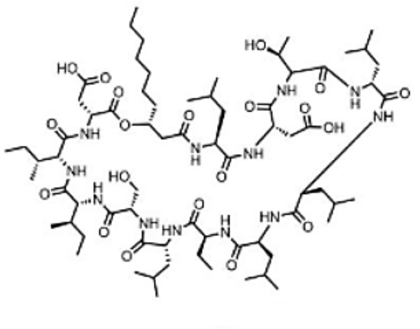
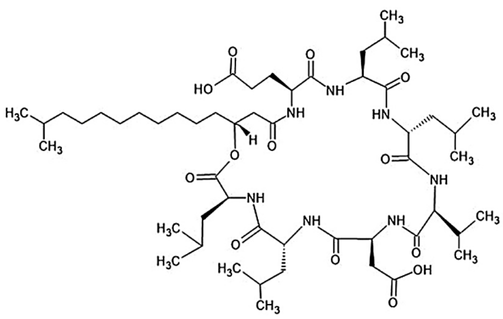
4. **Biosurfactants properties and types**

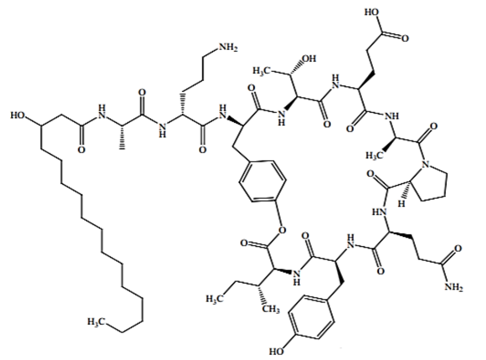
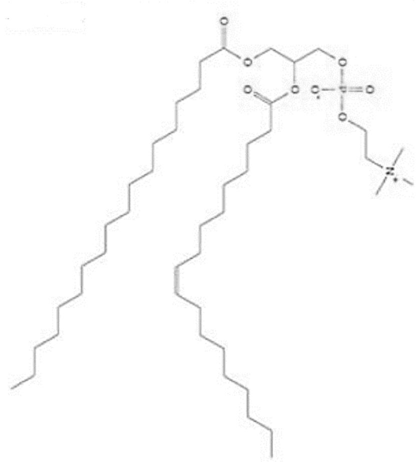
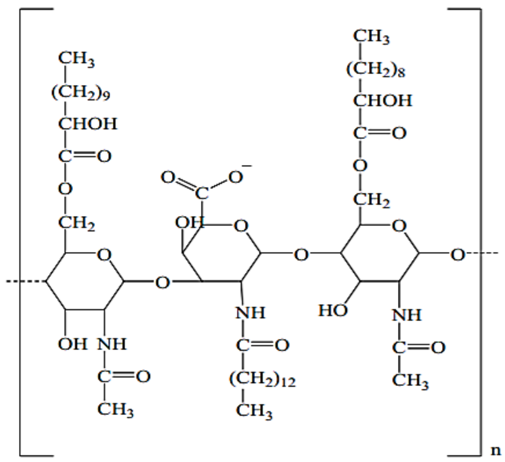
Biosurfactants are amphiphilic compounds containing both lipophilic and hydrophilic groups. This structure gives them surface-active features such as surface and interfacial tension reductions in mixtures of waters and hydrocarbon (16). Plants, animals and microbes are reported to synthesize biosurfactants (14). These can have lower critical micelle concentration (CMC) values than synthetic surfactants which improves their performance in different applications. Microbial biosurfactants are divided into two main groups high molecular weight (HMW) polymeric compounds, e.g. polysaccharides, proteins or combined lipoprotein and lipopolysaccharide types and low molecular weight (LMW), e.g. lipopeptides and glycolipids (35). HMW biosurfactants can adhere very firmly to different surfaces and act as bio emulsifiers. LMW biosurfactants such as rhamnolipids and sophorolipids that are disaccharides with long chain acetylated fatty acids or hydroxyl fatty acids. They significantly reduce surface and interfacial tension (36).

Unlike chemically synthesized surfactants, which are classified according to the nature of their polar group, biosurfactants are categorized mainly by their chemical composition and their microbial origin. In general, their structure includes a hydrophilic moiety consisting of amino acids or peptides, anions or cations; mono-, di-, or polysaccharides; and a hydrophobic moiety consisting of unsaturated and saturated fatty acids (37, 38). Glycolipids are the most known biosurfactants. They are conjugates of carbohydrates and fatty acids. The linkage is by means of either an ether or an ester group (39, 40). Lipopeptides can also be classified as biosurfactants (41). A summary of the classification of biosurfactants and their structure is shown in Table 1.

Table 1. Classification of biosurfactants and their structure

Biosurfactant types	Biosurfactants subtypes	Chemical structure example	Reference
	Rhamnolipids		(42)
Glycolipids			(43, 44)
	Sophorolipids		(45)

	Mannosylerythritol Lipids		
	Xylolipids		(47)
	Cellobiose lipids		(47)
Lipopeptides			(48)
	Surfactin		(49)

	Fengycin		(44)
Fatty Acids and Phospholipids	Phospholipids		(50)
Polymeric Biosurfactant	Emulsan		(44)

4-1. Glycolipid biosurfactants

4-1-1. Rhamnolipids

Rhamnolipids are glycolipid biosurfactants, mainly produced by *Pseudomonas aeruginosa* which are well known for their potential industrial, bioremediation and environmental uses (51, 52). They are composed of one or two rhamnose sugar groups linked to one or two fatty acid chain of mainly 10-14 carbon atoms, forming mono or di-rhamnolipid molecules (53).

4-1-2. Trehalolipids

Trehalolipids are disaccharide trehalose linked to mycolic acids. Trehalolipids from different organisms differ in the size and structure of mycolic acid, the number of carbon atoms, and the degree of unsaturation (54). Trehalolipids are produced by different species of *Mycobacterium, Nocardia*, and *Corynebacterium*. For example, trehalose dimycolate produced by *Rhodococcus erythropolis* (43, 55-57).

4-1-3. Sophorolipids

Sophorolipids are extra cellular glycolipids consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxy fatty acid by a glycosidic bond. These biosurfactants are a mixture of at least six to nine different congeners, and showed application related to the oil bioremediation (58, 59). The purified sophorolipids were more surface active, less water soluble and showed stronger cytotoxic effects. Although, sophorolipids can lower surface and interfacial tension, they are not effective emulsifying agents (60). Sophorolipids are produced mainly by yeasts such as *Torulopsis bombicola*, *T. petrophilum* and *T. apicola* (61-63).

4-1-4. Mannosylerythritol Lipids

biosurfactants containing mannose, erythritol, and two fatty acid chains are known as mannosylerythritol lipids. These glycolipids are centered around the disaccharide mannosylerythritol. The 2′ and 3′ positions of two fatty acids are linked to mannose by ester bonds and a bond at the 1′ position links erythritol to mannose (64, 65).

4-1-5. Xylolipids

A xylolipid is a glycolipid biosurfactant with a xylose head and fatty acid tails. The bacteria that produce xylolipids are usually lactic acid bacteria (66). Although a few yeast species such as *Pichia caribbica* are reported to be able to synthesize xylolipids. In Joshi-Navare *et al,* research xylolipids as biosurfactants can reduce the surface tension to 35.9 mNm^{-1} with a CMC of 1 mgL^{-1} (67).

4-1-6. Cellobiose lipids

Cellobiose lipids are a group of biosurfactants produced by microbes as secondary metabolites. It is usually produced as a mixture of different acylated low molecular weight D-glucolipids, linked to a hydroxyl palmitic acid via their ω-hydroxyl groups (68, 69). Cellobiose lipids were produced by *Cryptococcus humicola* JCM 1461 and the structure of main product was 16-O-(2”,3”,4”,6’-tetra -O-acetyl-β-cellobiosyl)-2-hydroxyhexadecanoic acid. The CMC was 3.3×10^{5} M and 4.1×10^{4} M in pH 4.0 and 7.0 respectively (69).

4-2. Lipopeptides biosurfactants

The group of Lipopeptide/lipoproteins presents a heterogeneous class of biologically active peptides and most of them are known to possess antimicrobial activity. Arthrofactin (AF) and surfactin (SF) are the most effective cyclic lipopeptide biosurfactants ever reported (49, 70).

4-2-1. Arthrofactin

Arthrobacter and Actinomyces and Streptomyces produced arthrofactin, a lipopeptide biosurfactant type (71). The surface and interfacial behavior of arthrofactin is noteworthy as this cyclic lipopeptide (at a concentration of 100 µM) can reduce the surface tension of water from 72 to 24 mNm-1 (72). Effects on biofilm formation, in addition to a wide range of industrial applications relevant for medical applications were reported for arthrofactin (72, 73).

4-2-2. Surfactin

Surfactant is a cyclic lipopeptide consisting of a hydrophobic tail that is thirteen to fifteen carbons long chain with seven amino acids produced by *Bacillus subtilis* is the most effective biosurfactant with low toxicity (74, 75). The amphiphilic nature helps surfactin to exist and function in both hydrophobic and hydrophilic environments. Surfactin is a commonly used biosurfactant with detergents, antimicrobial, antibacterial, and antiviral properties in a variety of industries and formulations of cosmetic products, and oil bioremediation (76).

4-2-3. Fengycin

In fengycin as cyclic lipopeptide, decapeptides are joined to a linear chain of β-fatty acid by cyclization between the phenol side chain at position 3 and the C-terminus of an amino acid at position 10 (77). Bacillus species are the primary producers of fengycin (78). It has been demonstrated that fengycin readily interacts with the lipid bilayer and have antimicrobial effects (79). A lipopeptide closely related to fengycin has been identified and referred to as plipastatin. In fengycin and plipastatin, the Tyr position differs (80, 81).

4-3. Fatty Acids, and Phospholipids biosurfactants

Several bacteria and yeasts produce large quantities of fatty acid and phospholipid surfactants during growth on n-alkanes. The HLB is directly related to the length of the hydrocarbon chain in their structures. These are usually organisms which produce surface-active lipids when growing on hydrocarbon substrates. Several different types of biosurfactants have been isolated and characterized. These include glycolipids, lipopeptides, phospholipids, and neutral lipids. The complex lipids all contain fatty acids and these fatty acids often have a hydroxyl function on the carbon β to the carboxyl group or farther along the chain (82, 83). One of the most popular phospholipid biosurfactants is produced by *Corynebacterium Lepus* (83).

4-4. Polymeric biosurfactants

Extracellular polymeric substances (EPSs) such as emulsan are involved in both detrimental and beneficial consequences of microbial aggregates such as biofilms, flocs and biological sludge. In biofouling, they are responsible for the increase of friction resistance, change of surface properties such as hydrophobicity, roughness, color, etc. In bio corrosion of metals, they are involved by their ability to bind metal ions. In bio weathering, they contribute by their complexing properties to the dissolution of minerals. The EPSs represent a sorption site for pollutants such as heavy metal ions and organic molecules (84, 85).

5. Biosurfactants in bioremediation

Biosurfactants can withstand high temperatures, high salt concentrations and harsh conditions and remain stable. Remediation techniques using biosurfactants and microorganisms generating biosurfactants help to detoxify petrolatum and heavy metals from the contaminated environment (86-89). Biosurfactants produced by *Serratia marcescens* ZCF25 are lipopeptide. This microorganism was isolated from oil sludge. Biosurfactants were highly stable in harsh environments, reduce surface tension and have a bioremediation application (90) *Stenotrophomonas* sp. S1VKR-26 produces biosurfactants that can be used for bioremediation of petrolatum contamination in wastewater (91). *Bacillus cereus* UCP 1615 biosurfactant are lipopeptide type with potential for oil spills remediation (92). The extracted biosurfactant from *Rhodococcus erythropolis* HX-2 increase the solubility of the hydrophilic compound and enhances petroleum biodegradation (93).

Some studies and research with regard to biosurfactants which improved the biological degradation of contaminants are shown in Table 2.

Table 2. Numerous studies shown biosurfactants improved the biological degradation of contaminants.

Microorganisms	Pollutants	Biosurfactant Type	Mechanisms of Effects	Reference
Bacillus sp.	Zinc, lead, chromium and copper	Lipopeptide	Complex (biosurfactant–metal) formation, then the complex form micelles and mobilize (leaves the soil)	(86)
Acinetobacter sp. *Pseudomonas putida*	Lead, zinc and copper	Rhamnolipid	Wetting, interaction to the sediment surface and metal separation from the sediment	(94)
Isolates of KDM3, KDM 4, KDM 6	Zinc, lead and chromium	Biosurfactant (not specified)	No purpose mechanism	(95)
Pseudomonas sp. CQ2	Cd, Cu and Pb	Biosurfactant (not specified)	Metals complex with carboxyl functional groups in biosurfactants	(33)
Achromobacter xylosoxidans *Stenotrophomonas maltophilia*	Polychlorinated biphenyls	Saponin Rhamnolipid	Direct bacterial cell absorption of pollutants from the micellar core, increased mass transfer of contaminants to the aqueous phase,	(96)

			and modifying cell surface and cell lipophilicity	
Saccharomyces cerevisiae	Biodiesel	Mannoprotein	Emulsifying contaminant with soil particles, thereby promoting biodegradation	(97)
Mixed culture microflora	Biodiesel and diesel oil	Rhamnolipid	Increase bioavailability of organic compounds solubilized in micelles to microbial cells	(98)
Achromobacter sp. A-8	Petroleum	Biosurfactant (not specified)	Decreases surface tension and high performance in oil displacement	(87)
Serrati sp.	Hydrocarbon	Lipopeptide	Reduced surface and interfacial tension increasing hydrocarbons surface area, which makes them accessible to the microbe	(99)
Bacillus cereus	Oil	Lipopeptide	Increase of lipophilic substrates' bioavailability	(100)
Shewanella sp.	Oil	Rhamnolipid	Improving the rate of mass transfer and microbial adhesion	(88)

Wickerhamomyces anomalous	Crude oil	Lipopeptide	Reduction in surface tension	(101)
Bacillus algicola (003-Phe1), *Rhodococcus soli* (102-Na5), *Isoptericola chiayiensis* (103-Na4), and *Pseudoalteromonas agarivorans* (SDRB-Py1)	Crude oil	Rhamnolipid	Increasing the emulsification of crude oil	(102)
Bacillus sp. and *Acinetobacter* sp.	Oil	Lipopeptide Emulsan	Micelles formation	(89)
Serratia marcescens UCP 1549	Burned motor oil	Lipopeptide	emulsify oil, improve water solubility and decrease the surface tension	(103)
Bacillus methylotrophicus UCP1616	Motor oil	Lipopeptide	Increases the surface area of the hydrocarbons and enhances the interaction of the hydrophobic contamination and the microbial cell membrane	(104)

Paenibacillus sp. D9	Diesel and motor oil	Biosurfactant (not specified)	Increase the solubility of the hydrophobic contaminated aqueous environment, thereby improving biodegradation	(105)
Acinetobacter sp. Y2	Hydrocarbon	Lipopeptide	Improve the solubility and bioavailability of lipophilic compounds	(106)
Bacillus stratospheric strain FLU5	Motor oil	Lipopeptide	Micelles formation and increases the solubility of pollutions	(107)
Staphylococcus epidermidis EVR4	Diesel oil	Biosurfactant (not specified)	Oil pollutants become more soluble by biosurfactant	(108)

6. Mechanism of biosurfactants in bioremediation

One variable affecting the process of microbial degradation is the bioavailability of the polluting compounds to the degrading bacteria (109). Biosurfactants increase the hydrophobic pollutants bioavailability to microbes for biodegradation through enhancing their solubility (97). One application of biosurfactants is improving the biodegradation process of insoluble organic contaminations. Biosurfactant influences the rate of biodegradation of hydrocarbons and increases the decomposition process through two mechanisms; by increasing the solubility of petroleum hydrocarbons and by controlling the interaction between bacterial cells and petroleum substances reducing the surface tension among two phases (17). Biosurfactants as amphiphilic structure accumulate and form micelles in the hydrophilic environment at bulk concentrations above the CMC. Micelles are thermodynamically stable structures, and micelle formation is an equilibrium process. In micelle structures, hydrophobic groups of surfactants contact/orients towards the hydrophobic environment and hydrophilic groups contact/orients towards the aqueous phase; thus, hydrophobic contamination become dispersed and soluble in the aqueous solvent. On the other hand, micelle can increase the rate of absorption of substance to microbial cells (17, 107, 110).

Biosurfactants can enhance biodegradation of poorly soluble substances by two main processes which enhance/increased bioavailability:

- Improving the solubility by emulsifying hydrophobic compounds, making it more accessible to microbial attack.
- Facilitating transfer of hydrophobic contamination by micelle formation, providing greater access to bacterial cells (17).

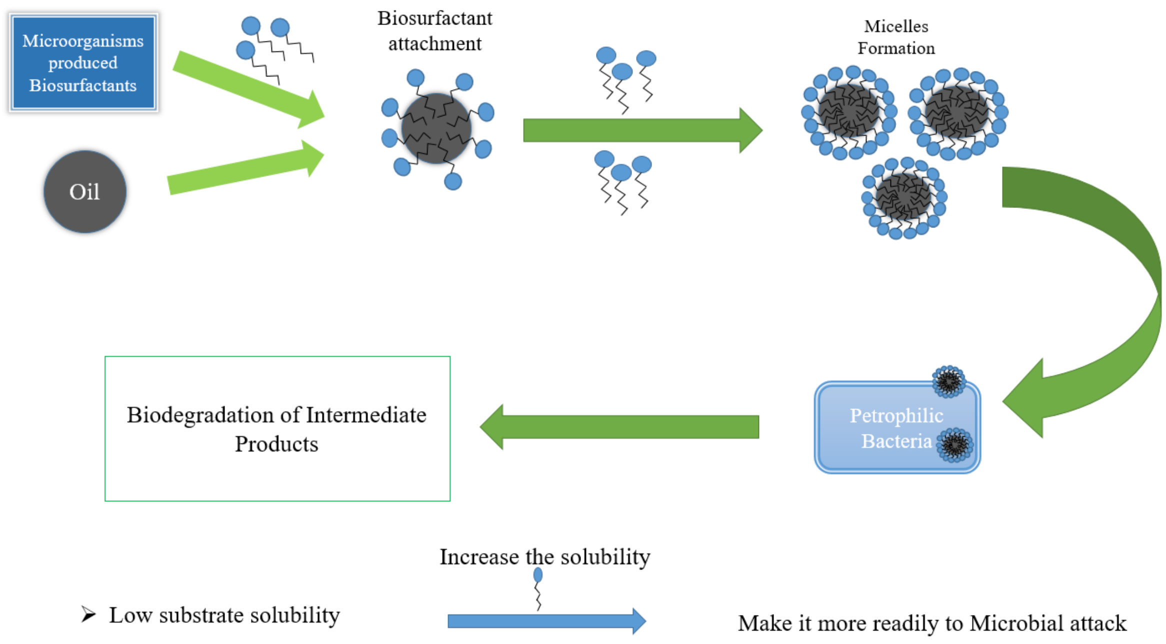


Fig.1 shown how biosurfactants can be increased bioremediation.

Fig.1 Mechanisms of microbial degradation of the hydrophobic compound with the aid of biosurfactants

Biosurfactants with complexation or surface sorption can aid heavy metal remediation. The anionic biosurfactant has a strong affinity to cationic heavy metals such as Zinc, Lead, Chromium and Copper and complexed with them, then biosurfactant–metal complex leaves the soil surfaces and form micelles. Based on this, biosurfactants can use for heavy-metal pollution remediation (33, 86, 111). Smaller micelles are more beneficial to biosurfactant diffusion in the soil, which increases the contact area with heavy metals in the soil and thus improves bioremediation performance (33).

7. Conclusion

The biosurfactants can be used as a low-cost method without the need for special equipment and in situ techniques to degrade organic contaminants such as petroleum and mobilise/collect inorganic contaminants such as heavy metals. Biosurfactants increase solubility by emulsifying hydrophobic pollution and providing greater access of microorganisms to contamination, complexed with heavy metals and micelle formation, lead to the removal of contamination without creating a new toxic product.

8. **Future prospects**

The use of biosurfactants is an attractive option because of its versatility, biodegradability, ecological safety and environmental acceptance. Due to its higher production cost, purification and low yield, biosurfactant used has limited. For produce a high yield of surfactants and lower cost biosurfactants; renewable substrates, alternative purification technologies, genetic and metabolic engineering tools, and statistical methods can be applied. More efforts are required to evaluate biosurfactants in situ and their effect on indigenous microorganisms.

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