

A Review: Potentials of Biosurfactants in Remediating Contaminated Sites

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Abstract

Studies based on evaluating the influence of biosurfactants on bioremediation efficiency is constantly growing. Biosurfactants are surface-active substances produced by diverse group of microorganisms. They are non-hazardous, biodegradable, non-toxic and environmentally friendly compounds which may be under *ex-situ* condition cost of effectively produced; at the site of contamination *in-situ* production may be stimulated and can be recovered and recycled. Due to their naturally occurring property their application in bioremediation processes may be more acceptable from a social point of view. Impressive advantages of biosurfactants include their vast structural diversity and broad range of unique properties, their biodegradability and possibility of cost effective production. These properties make biosurfactants a promising choice for applications in bioremediation. This paper reviews the potentials of biosurfactants in remediating contaminated sites.

Keywords: Biosurfactant; Environment; Bioremediation; Biodegradation

Introduction

Inorganic pollutants such as heavy metals and organic pollutants like hydrophobic organic compounds cause the soil and water contamination that became a serious challenges. Cadmium (Cd), Copper (Cu), Lead (Pb), Chromium (Cr), Zinc (Zn) and Nickel (Ni) heavy metals are considered as environmental pollutants and these toxic metals accumulate in soil and water constitute potential health hazard for man and the ecosystem. HOCs (Hydrophobic Organic Compounds) such as Pentachloro Phenol (PCP), Hexachloro Benzene (HCB), Polychlorinated Bi phenyls (PCBs) and Dichloro Phenol (DCP) are known environmental pollutants and their removal from the contaminated site is a major environmental concern [1-3]. Besides the energy resources mainly petroleum and petroleum hydrocarbons are also major pollutants of the environment. The oil and products contamination may cause severe harm and oil products [4].

Bioremediation is termed as any process that uses

microorganisms or their enzymes to return the environment altered by contaminants to its original condition. It can also be defined as the use of biological processes to degrade or break down contaminants from soil and water. It is a natural process which depends on bacteria, fungi and plants to remove contaminants as these organisms carry out their normal life functions [5]. Chemical contaminants in the soil are used by microbes as energy source to mobilize the target contaminants into usable energy during bioremediation. The metabolites discharged into the environments are less toxic than the original contaminants. Petroleum hydrocarbons can be degraded by two ways: Aerobic respiration (sufficient oxygen) and anaerobic respiration (insufficient or lack of oxygen) [6]. The end product of this reaction is usually carbon dioxide and water. The term bioremediation describes the process of using biological agents to remove toxic waste from environment. To manage polluted environments and restore contaminated soil to its original state bioremediation is the most effective management tool. Bioremediation process is an attractive and successful cleaning technique for polluted environments [7-10].

Literature Review

Biosurfactant have attracted much attention and their popularity seems to steadily increase during recent years. Being a frequent object of study, as work dedicated to the isolation and characterization of novel biosurfactant producers is constantly growing. Characterization of biosurfactants through a vast structural diversity and showed a broad range of properties elaborate why this group of molecules continues to entice scientific curiosity [11]. Compared to their synthetic counterparts numerous advantages of biosurfactants like biodegradability, less toxicity and eco-friendliness are yet another reason why these compounds seem so promising. Environmental friendliness combined with the ability to solubilize hydrophobic compounds may well explain why biosurfactants have also been considered as excellent compounds for enhance bioremediation of contaminated sites [12,13]. In bioremediation application of biosurfactants has been believed to be highly beneficial. Regarding the actual efficiency of biosurfactants in bioremediation is the driving force behind this manuscript, which is focused on providing a critical overview on the role of biosurfactant in bioremediation [14].

Definition of biosurfactants

Biosurfactants make for a peculiar group of compound which exhibit notable distinction in terms of chemical structure and the composition and due to this fact they have found numerous types of interesting applications. Various different classes, such as glycolipids, lipopeptides, lipoproteins, phospholipids, fatty acids, as well as complex biopolymers are commonly associated with "biosurfactants". For scientific experiments an extensively studied and reviewed group of compounds i.e. rhamnolipid is often used as a model biosurfactant [15]. Generally, biosurfactants exist either in an anionic or non-ionic form, however in most cases both the hydrophilic and the lipophilic part may be distinguished with relative ease. In bioremediation processes this particular characteristic is essential. Different microorganisms are capable of producing surface active compound-biosurfactants.

Class/type of biosurfactant	Microorganisms
Glycolipids	
Rhamnolipids	<i>Pseudomonas aeruginosa</i>
Sophorolipids	<i>Torulopsis bombicola</i> , <i>T. apicola</i>
Trehalolipids	<i>Rhodococcus erythropolis</i> , <i>Mycobacterium sp</i>
Lipopeptides and lipoproteins	
Peptide-lipid	<i>Bacillus licheniformis</i>
Viscosin	<i>Pseudomonas fluorescens</i>
Serrawettin	<i>Serratia marcescens</i>
Surfactin	<i>Bacillus subtilis</i>
Subtilisin	<i>Bacillus subtilis</i>
Gramicidin	<i>Bacillus brevis</i>
Polymyxin	<i>Bacillus polymyxa</i>
Fatty acids, neutral lipids and phospholipids	
Fatty acid	<i>Corynebacterium lepus</i>
Neutral lipids	<i>Nocardia erythropolis</i>
Phospholipids	<i>Thiobacillus thiooxidans</i>
Fatty acid	<i>Corynebacterium lepus</i>
Polymeric surfactants	
Emulsan	<i>Acinetobacter calcoaceticus</i>
Biodispersan	<i>Acinetobacter calcoaceticus</i>
Liposan	<i>Candida lipolytica</i>
Carbohydrate-lipid-protein	<i>Pseudomonas fluorescens</i>
Mannan-lipid-protein	<i>Candida tropicalis</i>
Particulate surfactant	
Vesicles	<i>Acinetobacter calcoaceticus</i>

Table 1: Main classes of biosurfactants and respective producer microorganisms.

Pseudomonas, *Bacillus*, *Rhodococcus* and *Candida* genera are the most commonly used in the production of diverse biosurfactants. **Table 1** displays a list of microorganisms that produce biosurfactants [16].

Biosurfactants contribution to bioremediation

Bioavailability of the pollutant is the main issue which directly influences the efficiency of biological treatment. Possible sorption of molecules into the soil matrix, interactions with organic matter, formation of non-aqueous phases, biotransformation and contaminant aging are the reasons of limited bioavailability, thus decreasing the efficiency of bioremediation. Enhancing the distribution of contaminants into the aqueous phase and increasing their bioavailability are the most common intended role of biosurfactants [17]. An overview of recent studies on biosurfactant assisted bioremediation was presented in **Table 2**.

At the oil/water interface biosurfactant has a tendency to deposit. Through specific interaction biosurfactants may facilitate the transport of hydrophobic contaminants (i.e., hydrocarbon-based substances) into the aqueous phase which results in solubilization and micellization. Subsequent removal of such pollutants either by soil flushing or potentially makes them more susceptible to biodegradation due to increased mobilization [18]. Additionally, in the structure of biosurfactants heteroatoms are commonly present, in the process of forming complexes with heavy metal ions there are several active chemical groups (such as hydroxyl, carbonyl, or amine) which participate. This process enables removal of heavy metal ions and using biological methods there may be an enhancement of their extraction efficiency [19-21]. At the cellular membrane level biosurfactants showed strong biological activity. Enhanced hydrophobicity results from modifications, in terms of biodegradation efficiency which is considered to be relevant, or change the permeability of cellular membranes, which would potentially be beneficial during bio-extraction [22].

In the actual application of biosurfactants in bioremediation processes the molecules may either be added externally (i.e., influent, spraying, injection) or produced *on-site*, which seems especially promising in case of *in-situ* treatment. In the latter case, the production of biosurfactants may be obtained by bio-augmentation with appropriate microorganisms, since autochthonic microorganisms rarely exhibit satisfying efficiency [23].

Type of biosurfactant	Pollutant	Relevant bioremediator	Established effect	Removal efficiency
Rhamnolipids	Phenanthrene	<i>Sphingomonas</i> sp. monoculture	Positive-solubilization	99% after 10 days compared to 84% without biosurfactant (IC-10 g/l)
Rhamnolipids	Anthracene	<i>Sphingomonas</i> sp. and <i>Pseudomonas</i> sp. monocultures	Positive-solubilization	52% after 18 days compared to 32% without

				biosurfactant for <i>Pseudomonas</i> (IC-25 mg/l)
Rhamnolipid, emulsan and indigenous biosurfactants	Pyrene	<i>Pseudomonas fluorescens</i> monoculture	Positive/negative	98% after 10 days compared to 91% without emulsan (IC-50 mg/l)
Rhamnolipids	Polycyclic aromatic hydrocarbons	Alfalfa+ arbuscular mycorrhizal fungi+ microbial consortium of PAH degraders	Positive-solubilization	61% after 90 days compared to 17% with only phytoremediation (IC-12.85 g/kg of soil)
Sophorolipid	Hydrocarbon mixture	Autochthonous soil microflora	Positive-solubilization and mobilization	Respectively: 95% after 2 days, 97% after 6 days and 85% after 6 days (IC-6 mg/g of soil)
Rhamnolipids	Diesel oil and biodiesel blends	Microbial consortium	Positive/no effect	77% after 7 days compared to 58% without biosurfactants for blends (IC-approx. 15 g/l)

Table 2: An overview of recent studies on biosurfactant-assisted bioremediation.

Environmental contamination by oil spills and biosurfactant-enhanced remediation

Global pollution due to the release of contaminants, such as petroleum and petroleum byproducts, into the environment has become a focus of great concern both in industrialized and developing countries due to the broad environmental distribution in soil, groundwater, and air [24]. Various sources of contamination are: Accidents during fuel transportation by ships and trucks; leakage from underground storage tanks; processing during oil extraction; waste released from industrial waste that use byproducts in the process of plastic, cosmetics and pharmaceuticals. Major hydrocarbon source in the oceans comes from routines operation of ship washing natural oil leakage on the sea bed, and accidents during oil exploration and transportation [25]. Covering 163 km² spills occurred i.e. 5943 L was the most impacting leakage in November 2011 on the Sedco 706 oil rig, operated by Chevron Brazil in Campos Bay. In the Gulf of Mexico in 2010, another largest oil spills in the world occurred due to the explosion of an oil rig off the coast of the states of Louisiana and Mississippi (USA) [26]. In the history of the United States, estimated total of three to four million barrels of oil spilled, making this the largest environmental disaster. To the ocean and coast in Dalian, China in July 2010 an oil spill of 1500 tons of crude oil caused serious environmental problems. Resident organisms are affected to the large amounts of crude

oil entering the marine environment, groundwater and soil [27]. Being a hydrophobic hydrocarbon, petroleum harm the structural and functional properties of cell membranes in living organisms, and contaminate both marine and terrestrial ecosystems. The potential threat to human health posed by hydrocarbons is associated with the physical and chemical properties of these compounds, which are absorbed by the skin and quickly spread through the organism if ingested or inhaled [28-32].

Dispersal of contaminants enhanced by biosurfactants in the aqueous phase and also increase the bioavailability of the hydrophobic substrate to microorganisms, with subsequent biodegradation removal of such pollutants [33]. Diverse examples indicate the potential application of biosurfactants in environmental decontamination. Biosurfactant derived from *Candida sphaerica* removed motor oil from soil and seawater. From clay and silty soil removal rates were 75% and 92% respectively [34]. It is tested about biosurfactants from the *Candida tropicalis* which showed removal motor oil from sand with removal rates of 78% to 97% [36]. Lunasan biosurfactant are derived from *Candida sphaerica* UCP 0995 showed removal of 95% of motor oil adsorbed to sand. Good dispersion effectiveness on crude oil shown by lipopeptides secreted by *Bacillus subtilis* HSO121 [35]. Lipopeptides acted effectively at dispersing oil and performed excellently at stimulating microbial oil biodegradation, which indicated its application in oil spill cleaning. The addition of both a glycolipid biosurfactant and immobilized *Gordonia* sp. JC11 was able to remove 60%-70% of 1 g L⁻¹ clay fuel oil. **Table 3** showed Biosurfactants, producing microorganisms and uses in the bioremediation of oil-contaminated environments [36-40].

Microorganisms	Type of biosurfactant	Applications
<i>Rhodococcus erythropolis</i> 3C-9	Glucolipid and trehalose lipid	Oil spill cleanup operations
<i>Pseudomonas aeruginosa</i> S2	Rhamnolipid	Bioremediation of oil-contaminated sites
<i>Rhodococcus</i> sp. TW53	Lipopeptide	Bioremediation of marine oil pollution
<i>R. wratislaviensis</i> BN38	Glycolipid	Bioremediation applications
<i>Bacillus subtilis</i> BS5	Lipopeptide	Bioremediation of hydrocarbon-contaminated sites
<i>Pseudomonas aeruginosa</i> BS20	Rhamnolipid	Bioremediation of hydrocarbon-contaminated sites
<i>Micrococcus luteus</i> BN56	Trehalose tetraester	Bioremediation of oil-contaminated environments
<i>Nocardioopsis alba</i> MSA10	Lipopeptide	Bioremediation
<i>Pseudomonas alcaligenes</i>	Rhamnolipid	Environmental applications
<i>C. lipolytica</i> UCP0988	Sophorolipids	Oil recovery
<i>C. guilliermondii</i> UCP0992	Glycolipid complex	Removal of petroleum derivative motor oil from sand

<i>C. sphaerica</i> UCP0995	Protein-carbohydrate-lipid complex	Oil removal
<i>C. lipolytica</i> UCP0988	Sophorolipids	Removal of petroleum and motor oil adsorbed to sand
<i>C. glabrata</i> UCP1002	Protein-carbohydrate-lipid complex	Oil removal
<i>Pseudozyma hubeiensis</i>	Glycolipid	Bioremediation of marine oil pollution
<i>Pseudomonas cepacia</i> CCT6659	Rhamnolipid	Bioremediation of marine and soil environments

Table 3: Biosurfactants, producing microorganisms and uses in the bioremediation of oil-contaminated environments.

Discussion

Application in Microbial Enhanced Oil Recovery (MEOR)

Oil spills cause devastating effect on aquatic life on marine environment. Chemically synthesized surfactants had been reported for their toxicity on aquatic organisms, so were, treated them unsuitable for remediation [41,42]. One of the inherent alternatives for this purpose was to find the biomolecules which had surface activity as well as the emulsifying activity along with the low Critical Micelle Concentration (CMC) characteristics. Due to the high cost of chemical tension active agents hinders the widespread use of surfactants in oil recovery processes [43]. Thus, to reduce the interfacial tension between oil/water and oil/rock, biosurfactants have been employed which leads to a reduction in the capillary forces that impede oil from moving through rock pores (**Figure 1**). The biosurfactants emulsify the hydrocarbons in water to form various mixtures and make them water soluble. Lichenysins, rhamnolipids and surfactin are the few surfactants which are found to be successful in the remediation of the oil contamination [44-46]. It has been isolated a bacterium from a crude oil sample which produced by crude oil biosurfactant that had good emulsifying properties on crude oil paraffin [29]. Literature suggested that biosurfactants produced from marine bacterium were capable enough to destroy the oil slicks which float on the surface of water in order to promote the dispersion of oil in water by forming a stable emulsion thereby enhancing the rate of biodegradation. Due to these factors, biosurfactants had shown potential in its applications of cleaning up the oil spills on shorelines and in the sea [47-50].

The ubiquitous presence of the marine bacteria which degrade hydrocarbons have been recognized as hydrocarbonoclastic bacteria [51]. These bacteria degrade the hydrocarbons present in the polluted sites of marine environment degradation [2]. Different study objects revealed by that the mixture of the biosurfactants stimulated the degradation of hydrocarbons in the marine environment [52,53]. Hydrocarbonoclasticity bacterial consortium has a wide range of degradation capabilities on both aliphatic as well as aromatic fractions of crude oil. In general, biosurfactants produced by oil degrading bacteria can enhance the assimilation of the

hydrocarbons as well as the nutrients available in the environment. Emulsifying agents synthesized by some groups of microorganisms, hence emulsifiers have been used for cleaning up the oil as they help in hydrocarbon degradation. In the industrial scale biosurfactants can be largely produced by fermentation process; Lichenysins were produced from *B. licheniformis* JF-2, Lichenysin even at lower concentrations (10-60 mg/l) was able to reduce the surface tension between the interfacial surfaces into ultra lesser values of (10^{-2} mN/m) [54,55]. The range of temperature ($\leq 140^{\circ}\text{C}$), pH (6-10), and salinity (up to 10% w/v NaCl) variation had no effect on its activity. By altering the wettability capacity of the porous media biosurfactant adsorbs the oil. *Acinetobacter venetianus* ATCC 31012 produce an emulsion which at 0.1 mg/ml conc. removes 89% of crude oil which had been reabsorbed to the samples of limestone and at 0.5 mg/ml concentration 98% of removal was achieved [56].

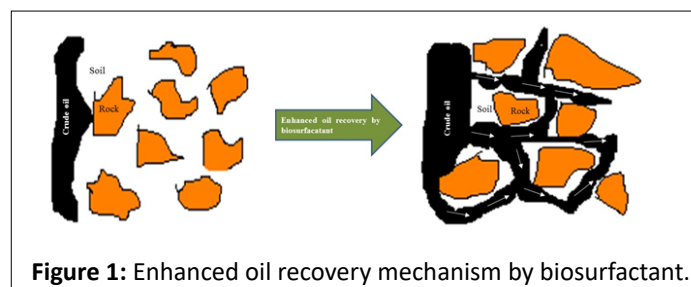


Figure 1: Enhanced oil recovery mechanism by biosurfactant.

Studies have emphasized on the possibility of introducing the bacteria which produce biosurfactants into the infected sites, so that they can utilize the nutrients present in the oil well for their growth, but it was more suitable for the strategy of microbially enhanced oil recovery where the bacteria would metabolically active even at extreme conditions in the petroleum reservoirs [57]. Many bacterial species that produce biosurfactants had been described for the microbially enhanced oil recovery *in-situ* applications that belong to *Bacillus* sp. because of their thermal and halotolerance ability. A typical *Bacillus* strain was grown and produced lichenysin by both anaerobic and aerobic processes at relatively high temperatures ranging from 40°C - 60°C . Different processes can be approached to exploit the biosurfactant producing strains in oil recovery applications [58].

Application of biosurfactants in removing heavy metal ions

Biosurfactant-assisted removal of heavy metal ions by complex formation and subsequent mobilization has received much attention. Heavy metals are the inorganic pollutants that pose the greatest potential risk to human health occurs naturally in rock, soil, plants and animals. Ions dissolved in water, vapour or minerals in rock, sand and soil are the different forms of metals. Metals can also be bonded to organic or inorganic molecules or trapped by air particles. Metals release into the air and water by both natural and anthropogenic processes. Due to number of industrial activities, such as mining, metal forging, the manufacturing of automobile batteries, emissions from vehicles and industrial waste dumps and the dispersion of ash from incineration processes heavy metal contamination occurs [59]. Heavy metals in soil causes serious problems, as they are

non-biodegradable, which causes the contamination of biological systems and the subsoil through the process of lixiviation. For instance, lead is found in 15% of contaminated lands in the USA, followed by chrome, cadmium and copper, which are found in 7% to 11% of soils. In the treatments of soils contaminated by heavy metals numerous methods have been developed and implemented. To treat contaminated soil two major technologies are employed. In the first technology there is immobilization of heavy metals in a solid matrix strongly bonded to the soil to minimize migration [60]. The second technology promotes the mobility of the metal and through desorption and solubilisation its migration to a liquid phase. Most widely applied methods includes the flushing of soil with acids and chelating agents, such as Ethylene Diamine Tetraacetic Acid (EDTA). Flushing with acids reduces the fertility of the soil and leads to changes in the physicochemical solubilisation composite [47]. Moreover, the use of EDTA is not good from the main safety point of view due to its low degradation rate. The difficulty in recovering the heavy metal from the metal-EDTA complex also prohibits the use of this method. For the remediation of soils contaminated by heavy metals and oils the use of surfactants is a potential and permanent solution. In solutions surfactants allow the reuse of the soil by facilitating the solubilisation, dispersion and desorption of the contaminants. In decontamination tests a number of synthetic surfactants have been evaluated. However, the need to replace synthetic compounds with surface active compounds of microbial origin has led to research into the usage of potential biosurfactants. In this respect, studies showed the potential of rhamnolipids, sophorolipids (both of a bacterial origin) and surfactin [61]. The biodegradability, ionic nature, low degree of toxicity and excellent surface properties make biosurfactants promising compounds for the removal of heavy metals from soil and sediment.

The removal of metals by ionic biosurfactants is through three steps occur in the order (Figure 2). According to Mulligan, with different concentrations of biosurfactants removal is possible [10]. Das et al. report that the removal of cadmium and zinc by using an aqueous solution also occurred at concentrations below the CMC, while a concentration fivefold greater than the CMC resulted in the nearly complete removal of 100 ppm of metallic ion [15]. Wen et al. studied the degradation of anionic rhamnolipid in soils contaminated by cadmium and zinc and found that this compound could remain in the soil long enough to enhance the phytoextraction of the metal [57]. Through electrostatic interactions heavy metals are trapped within the micelles and through precipitation or membrane separation methods it can be easily recovered. With metal through ionic bonds anionic biosurfactants create non-ionic complexes. Such bonds are stronger as compare to those which established between the soil and metal [62]. Due to the reduction in interfacial tension the metal-biosurfactant complex is desorbed from the soil matrix. Cationic biosurfactants can replace similarly charged metal ions through competition for some, but not all, the negatively charged surfaces (ion exchange). By surfactant micelles metal ions can also be removed from the soil surface [63]. Biosurfactants offer indisputable advantages, since microorganisms capable of producing surfactant compounds do

not need to have the ability to survive in the soil contaminated by a heavy metal, although the continuous addition of biosurfactant is required in the process. Biosurfactants have also been applied in mining. As compare to conventional chemical reagents, *Pseudomonas* sp. and *Alcaligenes* sp. produced tensioactive compounds which have been used for the floatation and separation of scheelite and calcite, with recovery rates of 95% for CaWO_4 and 30% for CaCO_3 . Biosurfactants are produced by species of candida have been successfully employed in the floatation of heavy metals, demonstrating the ability to remove more than 90% of cations from columns and in dissolved air floatation processes. An anionic polysaccharide produced by *Acinetobacter calcoaceticus* A2, denominated biodispersan, has been used for the prevention of flocculation and dispersion in mixture of calcareous rock and water. Rhamnolipid derived from *Pseudomonas aeruginosa* capable of the removal of chromium containing precipitates [64]. A yeast biosurfactant derived from *Acinetobacter venetianus* was first evaluated in removing heavy metals in soil. There was removal of 95, 90 and 79% Fe, Zn and Pb, respectively. And also removed the 75% Pb and 87% Cd from aqueous solution. Saponin, rhamnolipid, and sophorolipid can effectively enhance heavy metals removal from the sludge in the electrokinetic tests. Biosurfactant rhamnolipid showed the removal of Cu (80.21%), Cd (86.87%), Pb (63.54%) and Cr (63.54%) by 0.8% rhamnolipid after 12 h at pH 7.0.

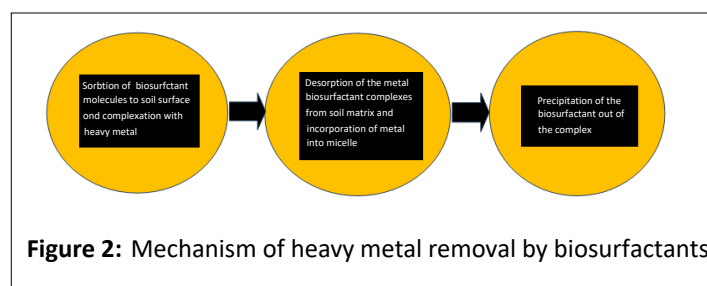


Figure 2: Mechanism of heavy metal removal by biosurfactants.

Advantages of using biosurfactant in bioremediation:

Biosurfactants are readily biodegradable and therefore do not constitute additional pollution threat. Biosurfactants reduce the total time taken for biodegradation of PAHs in contaminated soils. Biosurfactants reduce surface and interfacial tension, and thereby increasing the surface areas of insoluble compounds leading to an increased mobility and bioavailability of hydrocarbons. Surface of active compounds produced by bacterial strains do not need to have survival ability in soils contaminated with heavy metals.

They are environmentally friendly, less toxic and non-hazardous. Their production is potentially less expensive than synthetic surfactants and is achievable *in-situ* at the contaminated by site from inexpensive raw materials [65].

Disadvantages of using biosurfactant in bioremediation:

There is a relatively high production and recovery cost, as well as the difficulty of their mass production. Prolonged exposure of skin to biosurfactants can cause chafing because surfactants (like soaps) disrupt the lipid coating that protects the skin and other cells.

Conclusion

In bioremediation processes the application of biosurfactants is currently an ambiguous topic. Biosurfactants have shown their potential for remediation of contaminated sites by increasing biodegradation rate and reducing contaminant minimum concentration. Soil and water that are contaminated with organic and inorganic pollutants can be effectively treated with biosurfactants. Careful and controlled use of biosurfactants will help to enhance cleanup of toxic environmental pollutants and render the environment clean.

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