•Review Article

Potentials for Biosurfactant Enhanced Bioremediation of Hydrocarbon Contaminated Soil and Water – a Review

.

ABSTRACT

Bioavailability of Hydrophobic Organic Compounds (HOCs) to microorganisms could be a limiting factor during the biodegradation process. Application of surfactants to contaminated soil and water, at concentrations above their Critical Micelle Concentration (CMC) values, can potentially reduce the interfacial tension, increase the solubility and bioavailability of HOCs, and thus, facilitate their biodegradation. Studies with respect to enhanced bioremediation by surfactant addition have greatly focused on chemically synthetic surfactants. This paper reviews the potentials of biosurfactants in remedying contaminated soils and water. Biosurfactants are surface-active substances produced by microorganisms that can degrade or transform the components of petroleum products. They are non-toxic, non-hazardous, biodegradable and environmentally friendly compounds which may be cost effectively produced under ex-situ conditions; in-situ production may be stimulated at the site of contamination and can be recovered and recycled. Their application in bioremediation processes may be more acceptable from a social point of view due to their naturally occurring property. Potential advantages of biosurfactants include their unusual structural diversity that may lead to unique properties, the possibility of cost effective production, and their biodegradability. These properties make biosurfactants a promising choice for applications in enhancing hydrocarbon bioremediation.Biosurfactants have many other applications in various industries such as agriculture, medicine, petroleum, pharmaceutical and cosmetics.

Keywords: Bioavailability, Biosurfactants, Contamination, Hydrophobic Organic Compounds

INTRODUCTION

Soil and water contamination are serious challenges which are caused by inorganic pollutants such as heavy metals and organic pollutants like hydrophobic organic compounds. Heavy metals such as lead (Pb), Cadmium (Cd) Copper (Cu), Chromium (Cr), Zinc (Zn) and

Nickel (Ni) are known environmental pollutants and accumulation of these toxic metals in soil and water constitute potential health hazard for man and the ecosystem. HOCs (Hydrophobic Organic Compounds) such as Pentachlorophenol (PCP), Hexachlorobenzene (HCB), Polychlorinated biphenyls (PCBs) andDichlorophenol (DCP) are known environmental pollutants and their removal from the contaminated sites is a major environmental concern [1].

Bioremediation is 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 relies on bacteria, fungi and plants to alter contaminants as these organisms carry out their normal life functions[2].

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 environment 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). The end product of this reaction is usually carbon dioxide and water [3].

Three main conditions required for bioremediation are: availability of contaminant, electron acceptor and microorganisms with the ability to degradecontaminants. Degradation of contaminants are made easy if the microorganisms to be used, occur naturally in the environment [4].

The term bioremediation describes the process of using biological agents to remove toxic waste from environment. Bioremediation is the most effective management tool to manage polluted environments and restore contaminated soil to its original state and is also an attractive and successful cleaning technique for polluted environments [5].

Microorganisms produce different groups of surface-active substances called Biosurfactants. These biosurfactants are amphiphiles which consist of two parts: a polar hydrophilic group and a non-polar hydrophobic group. Examples of hydrophilic group are the mono, polysaccharides, peptides or proteins and the hydrophobic group are the saturated, unsaturated and hydroxylated fatty acids or fatty alcohols [6].

A notable feature of biosurfactants is a Hydrophilic-Lipophilic Balance (HLB) which specifies the part of the compound that is hydrophilic and the one that is hydrophobic.The amphiphilic structure of biosurfactants, enables them to increase the surface area of hydrophobic water-insoluble substances, increase the water bioavailability and alter the properties of the bacterial cell surface. These characteristics make biosurfactants to be used as emulsifiers, foaming and dispersing agents. They are ecofriendly, easily biodegradable,harmless andnon-hazardous [7].

The hydrophilic part of the surfactant is called the “head”, while the hydrophobic part is the “tail” of the molecule, which generally consists of hydrocarbon chain of varyinglength. Surfactants are classified as anionic, cationic, non-ionic and zwitter-ionic, according to the ionic charge of the hydrophilic head of the molecule[8]. Anionic surfactants have higher Critical Micelle Concentrations (CMCs) than nonionic surfactants. The CMC can be reduced by shielding the electrical repulsion among the hydrophilic heads of the molecules especially in anionic and cationic surfactants[9]. Additionalquantities of surfactant in solution will result in the formation of more micelles above the CMC, which leads to significant increase in the apparent solubility of hydrophobic organic compounds. Such process will cause mobilization improvement in organic compounds and their spread in solution [10]. This process also causes reduction of interfacial tension between immiscible phases [4]. When a pollutant occurs in the soil as a non-aqueous phase liquid, it makes the interfacial tension reduction imperative.

Common hydrophobic parts of synthesized surfactants are paraffins, olefins, alkylbenzenes, alkylphenols and alcohols and common hydrophilic parts aresulphates, sulphonates or carboxylate groups in anionic surfactants. The hydrophilic part in cationic surfactants is the quaternary ammonium group and in the nonionic surfactants the polyoxyethylene, sucrose or polypeptide [11].

They have many advantages when their chemically synthesized equivalents are compared.They possess better foaming characteristics, higher selectivity and they work well in extreme temperatures, pH and salinity andcan be produced from industrial wastes. Cheap production of biosurfactants is possible because of this last feature as well as utilization of waste substrates and reduction of their polluting effects [12,13,14,15,16].

Biosurfactants produced from microbes such as rhamnolipids, sophorolipid and surfactin and from plants such as saponin have been considered in this paper.

Classification and Properties of Biosurfactants

Biosurfactants are classified based on their chemical composition, molecular weight, and physico-chemical properties unlike the chemically synthesized surfactants, which are classifedbased on theirpattern of dissociation in water. In terms of molecular weight there are low-molecular-mass biosurfactants which include glycolipids, phospholipids and lipopeptides; (see Table 1) and high-molecular-mass biosurfactants which are polymeric amphiphiles. Low-molecular-mass biosurfactants are good in surface and interfacial tensions reduction while,high-molecular-mass biosurfactants are good in oil-in-water emulsionsstabilization [17, 18, 19].

The following are examples of biosurfactants: *Pseudomonas aeruginosa*microorganisms whichproduce Rhamnolipids[6]**,***Bacillus subtilis* produce Surfactin [20], *Candida antarticas* produce Mannosylerythritol lipids [21], *Rodococcuserythropolis*produce Trehalose lipids [22]. Table 1 shows the different types of low-molecular-massbiosurfactants.

Table 1.BiosurfactantsClassificationsand their Application in Biotechnology

Biosurfactant		**Microorganism**	**Application in Environmental Biotechnology**	**Refs.**
Group	**Class**			
Glycolipids	Rhamnolipids	*Pseudomonas aeruginosa* *Pseudomonas sp.*	Degradation improvement and hydrocarbon dispersion in solution; hydrocarbon and vegetable oils emulsification; metal removal from contaminated soil.	[23-26]
	Trehalolipids	*Mycobacterium tuberculosis,* *Rhodococcuserythropolis,* *Arthrobacter sp.,* *Nocardia sp.* *Corynebacterium sp.*	Improvement of the hydrocarbon bioavailability	27
	Sophololipids	*Torulopsisbombicola,* *TorulopsispetrophilumTorulopsisapicola*	Hydrocarbon recovery from dregs and muds; heavy metal removal from sediments; oil recovery improvement	[24,28,29]
Fatty Acids, Phospholipids and neutral lipids	Corynomycolic acids	*Corynebacteriumlepus*	Improvement of Bitumen recovery	30
	Spiculisporic acid	*Penicillumspiculisporum*	Metal recovery from aqueous solution; hydrophilic pigmentsdispersion in solution; enhances formation of new emulsion-type organogels.	[31-33]
	Phosphati-dyethanolamine	*Acinetobacter sp.* *Rhodococcuserythropolis*	Allows the bacteria in the heavy metals	34
Lipopeptides	Surfactins	*Bacillus subtillis*	Improves the biodegradation of	[35-37]

			hydrocarbons and chlorinated pesticides. Removal of heavy metals from contaminated soil, sediments and water	
	Lychenysin	*Bacillus licheniformis*	Enhancement of oil recovery	38

Biosurfactantsgathers over a period of time at the interface between two phases; either two immiscible fluids ora fluid and a solid. The reduction of the repulsive forces between two dissimilar phases allows them to mix and interact more easily (see Fig.1) [39]. This ultimately reduces surface (liquid/air) and interfacial (liquid/liquid) tensions.

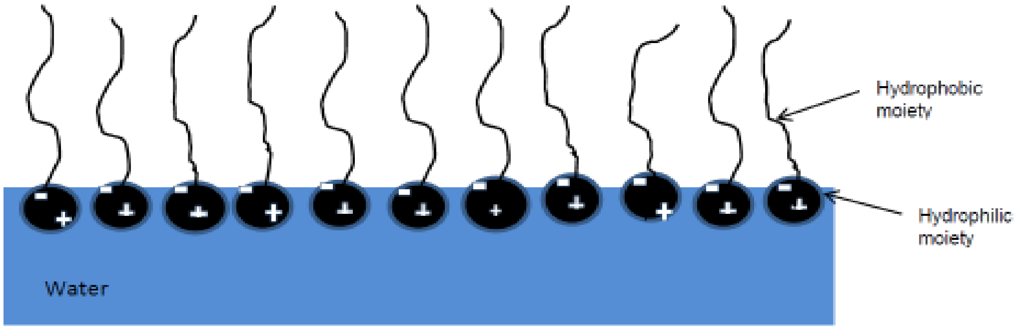

Fig. 1: Biosurfactant accumulation at the liquid/air interface [63]

The activities of biosurfactant depend on the surface-active compounds concentration until the CMC is reached. Above the CMC, micelles, bilayers and vesicles are formed by biosurfactant compounds (Figure 2). The formation of micelles allows biosurfactants to reduce the surface and interfacial tension and increase the solubility and bioavailability of HOCs [24]. The CMC is a means of measuring a givenbiosurfactant efficiency. When the value of CMC is low, it means that less biosurfactant is needed for surface tension tension reduction [11].

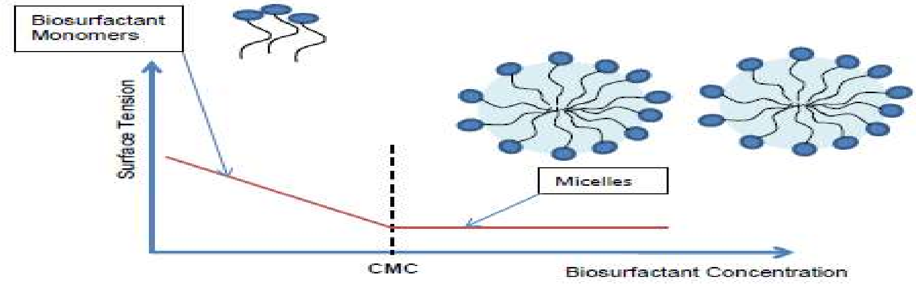

Fig. 2. Relationship between biosurfactant concentration, surface tension and formation of micelles [24]

Role of Biosurfactants in Biodegradation Processes

Biosurfactantsimproves hydrocarbon bioremediation by two methods:

(i) Substrate bioavailability increase for microorganisms and

(ii) Cell surface interaction which increases the hydrophobicity of surface and allows the hydrophobic substrates to associate more easily with bacterial cells [40].

Biosurfactant increase the surface areas of insoluble compounds by surface tension reduction causing increase in mobility and bioavailability of hydrocarbons. Consequently, biosurfactants improves biodegradation and hydrocarbon removal from contaminated environments. Three processes are used by biosurfactants to improve biodegradation of hydrocarbon: mobilization, solubilization or emulsification [41,42, 43,14].

Mobilization occurs below the CMC, where the surface and interfacial tension is reduced between air/water and soil/water systems. When this happens the biosurfactant increase the contact angle in the soil/oil system and reduces the capillary forces holding the oil and soil together.Solubilization occurs above the CMC where the micelles are formed and the oil solubility is increased. The hydrophobic ends of biosurfactant molecules join together inside the micelle while the hydrophilic ends are exposed on the exterior to the aqueous phase. Consequently, a compatible environment for the hydrophobic organic molecule is created by the interior of a micelle[44].

Interest in microbial surfactants has been progressively escalating in recent years due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under intense circumstances and their impending applications in environmental fortification [7].

Biosurfactant Enhanced Remediation of Hydrophobic Substances in Soil

Long-lasting contamination of soil and subsurface environment is caused by indiscriminate disposal of oil products and other hazardous wastes, which adversely affect the ecosystems and man [45].Blend of glycolipid-based anionic biosurfactant was used to investigate this. The biosurfactant was purchased as 5 wt % solution with pH value of 10, CMC was 0.1 % (surface tension-27 mN/m) and the HLB was 10.

Before practical application to remove contaminants from soil, the biosurfactantbehaviour in different systems was examined. Three process variables were modeled with full factorial to study the practical application of biosurfactants: temperature of the environment, contact time with dilution of biosurfactant and biosurfactant concentration in washing solution.

Boisurfactants can be characterized by some physical properties depending on the CMC, emulsion, oil solubilization, foaming and detergency, interfacial and surface tensions. These properties may be used to know if the biosurfactants are suitable for environmental bioremediation. Results from experiments have shown that they are useful for degreasing and can therefore be suitable for exploration and optimization of various kinds of surfactants [45].

Both organic and inorganic contaminants can be removed through desorption or biodegradation processes. Biosurfactantscan be used to increase the desorption of heavy metals and HOCs from soils in two ways:

i. Enhancing the solubility of the metals and the HOCs in soils
ii. Increasing the availability of the metals and HOCs to the plants [1].

Other uses of Biosurfactants

Biosurfactants are used in various industries such as food, pharmaceutical and cosmetic, as they are biodegradable and less toxic than the synthetic surfactants currently used [46]. The most important surface-active properties evaluated in screening microorganisms for biosurfactants with potential industrial applications are surface tension reduction, emulsion forming and stabilizing capacity. The criterion used for selection of biosurfactant producers is the ability to reduce the surface tension below 40 mN m^{-1} [47]. Sophorose lipids produced by certain strains of yeast have been formulated for anti-dandruff solutions, hair gels, deodorant sticks, after-shave lotions, and hair and body shampoos.

Microbial Enhanced Oil Recovery (MEOR)

Microbial enhanced oil recovery (MEOR) is the use of biosurfactant to improve oil recovery from depleted reservoirs. Enhanced oil recovery is also call tertiary recovery process which is any other process used to recover the remaining oil in the reservoir after the primary and secondary recovery processes[48, 49]. The primary recovery process involves the natural energy of the reservoir while the secondary recovery process is usually water or gas flooding operations. In MEOR, microorganisms or other metabolites which include biosurfactants, biopolymers, biomass, acids, solvents, gases and enzymes, are applied to increase oil recovery from depleted reservoirs. The use ofbiosurfactants in enhanced oil recovery is one of the modern techniques for recovery of substantial amount of residual oil. The residual oil is usually trapped in the pores of rocks by capillary pressure; and application of biosurfactants, reduces the interfacial tensions between the oil/water and oil/rock interface. Consequently, the capillary forces that prevent oil from moving through the rock are reduced considerably and allow the oil to flow upwards. This process also causes wettability alteration in the rock. See Fig. 3. Surfactants also forms emulsion when they bind tightly to the oil-water interface. When emulsion occurs, the desorbed oil in water is stabilized and oil is recovered along with the injection water [50, 64].

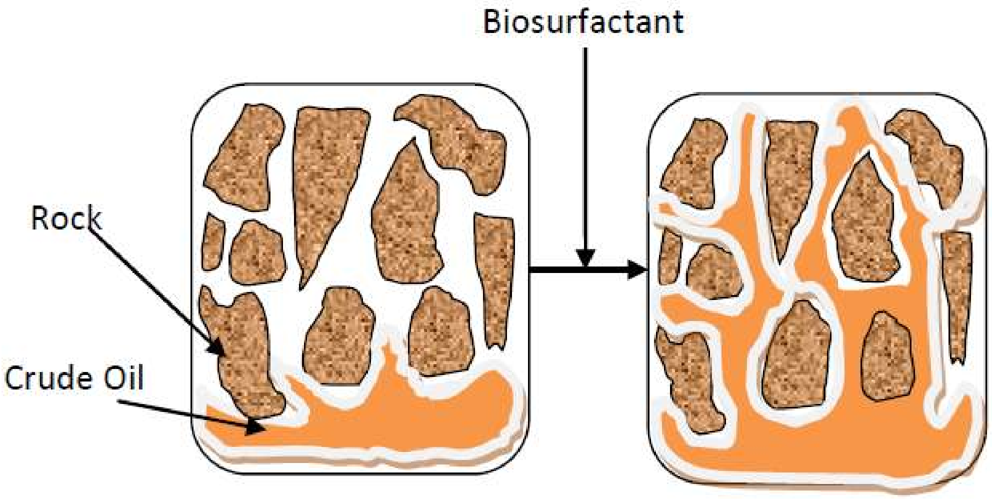

Fig. 3. Application ofbiosurfactants in Microbial Enhanced Oil Recovery [2]

Berea sandstone cores were used in MEOR experiments and it was found that biosurfactant solutions with 10 to 60 mg/l concentration range in the presence of 0.1 mM 2,3-butanediol and 1 g/l of partially hydrolyzed polyacrylamide (PHPA) could recover 10-40% of the residual oil. When PHPA was used, the residual oil recovered was about 10%. It can be inferred that the 10% residual oil recovered was because of the viscosity increase of the displacing fluid while the rest of the recovered oil was due to the effect of JF-2 biosurfactant interfacial tension reduction.Below CMC concentration, (about 10 mg/l) little or no oil was recovered. Below CMC concentration the Interfacial Tension (IFT)values were high. Between 10 to 40 mg/l, of biosurfactant concentration, the IFT was 1 mN/m while above 40 mg/l, the IFT decreased to around 0.1 mN/m. Thus, at biosurfactant concentrations above the CMC, residual oil recovery is a linear function of biosurfactant concentration[50].

Biosurfactants and Metals Remediation

Man and other living organisms in the ecosystem have been affected by soils contaminated with heavy metals. The presence of even low concentrations of heavy metals in the soil, have proven to have serious consequences because of its toxic nature. Currently,many methods are adopted to remove heavy metals from contaminated soils. These methods include non-biological techniques like excavation and disposal to landfill sites and biological techniques[51, 52]. In biological techniques, plants or microorganisms are used to remove metals from soils.

The application of inoculants of biosurfactant-producing bacteria in phytoremediation is a new technique to enhance the efficiency of this technology. In phytoremediation, biosurfactants are applied to identify their toxic effects on plants. Usually, biosurfactants are environmentally friendly, but some experiments under certain conditions have shown them to exhibit some toxicity in the environment[38].However, a cautious use of these compounds will help to improve cleanup of the toxic pollutant in the environment and ensure a clean environment [2].

Application of Biosurfactant Technology on Contaminated Soils

Remediation of heavy metals pollution

Hong *et al.* [54] examined the use of saponin, (a plant-derived biosurfactant) to remove cadmium and zinc, from three soil types. The soils used were Andosol, Cambisol, and Regosol. For Cd the rates of removal was 90–100%, while the rate of removal for Zn was 85–98%. An optimum concentration of 3% saponin was required for metal removal within 6 hours and the soil with maximum efficiency was Regosol.

Pseudomonas bacteria producesRhamnolipidbiosurfactantswhich has been used in soil washing for metals like Cd, Znand Pb in recent years. Mulligan and Wang [55] studied the removal of heavy metal from sandy soil contaminated with 1710 mg/kg of Cd and 2010 mg/kg of Ni. 0.5% foam solution of rhamnolipid, after 20 pore volumes was able to achieve maximum metal removal efficiency of 73.2% Cd and 68.1% Ni while the removal efficiency for theliquid solution was 61.7% Cd and 51.0% Ni.

Rhamnolipidbiosurfactant produced by *Pseudomonas eruginosa*was also evaluated by Juwarkar*et al.,* [56] to know the potential of removing Cd and Pb from contaminated soil. Their results showedthat while tap water only removedthe mobile fraction from contaminated soil, di-rhamnolipid removed both available fraction of Cd andPb and the bound metals.

The possibility of using surfactin, rhamnolipid, and sophorolipid for Cu, Pb and Zn removal from sediments was studied by Mulligan *et al.,* [57].0.5% rhamnolipid was able to remove 65% of the copper and 18% of the zinc in a single wash; 4% sophorolipid removed 25% of the copper and 60% of the zinc while,surfactinremoved only 15% of the copper and 6% of the zinc.

Remediation of Hydrophobic Organic Compounds

The capacity of a rhamnolipidbiosurfactant to remove a contaminant called Pentachlorophenol (PCP) (a highly toxic, wood preservative) from soil was investigated by Mulligan et al [58]. 1000 mg/kg contamination of PCP in fine sand soil and sandy-silt soil, was treated with 1% rhamnolipid; 60% and 61% of PCP was removed from the soils respectively. The biosurfactant removed 36% and 44% of the PCP by volatilization while the rest was removed by other means.

The remediation of hexachlorobenzene (HCB) contaminated soil was proposed using a plant-based surfactant extracted from fruit pericarps of a microorganism called *Sapindusmukorossi.* 90% of the HCB solubility was realized in batch desorption studies for high level soil contaminations; alsotheHCB recoveries for low level soil contaminations were up to 90% of the total HCB[59].

Biosurfactantshave been found to be more efficient in Total Petroleum Hydrocarbon (TPH) removal than syntheticbiosurfactants as observed by the experimental results of Lai *et al.*, [60]. When 0.2 mass% of rhamnolipids, surfactin, Tween 80, and Triton X-100, were applied to ca. 3,000 mg TPH/kg contaminated dry soil; 23%, 14%, 6%, and 4%, respectively of TPH was realized. When applied to ca. 9000 mgTPH/kg dry soil, the removal efficiency increased to 63%, 62%, 40%, and 35%, respectively.

The removal of a gasoline contaminated soil can be achieved by addition of rhamnolipid produced by *Pseudomonas sp*. along with poultry litter [14]. Research withother kinds of microorganisms like *Pseudomonas marginalis*, also showed that the produced biosurfactantsalso solubilized (PAHs) such as phenanthrene and improved biodegradation [61].

The study ofsophorolipidseffects on phenanthrene biodegradation showed that the concentration of phenanthrene with initial concentration of 80 mg/L within 36 hour in the presence of 500 mg/L reduced to 0.5 mg/l of the surfactant compared to 2.3 mg/L without surfactant in a 10% soil suspension [62]. The microorganism *Sphingomonasyanoikuyae*obtained a maximal degradation of 1.3 mg/L/h with the sophorolipid instead of 0.8 mg/L/h from fluorescence measurements; the sophorolipids showed improved phenanthrene concentration instead of increased biomass concentration. For concentrations up to 1 g/L the toxicity of the sophorolipid was low. Adsorption of the surfactant to the soil was observed as CMC of the sophorolipid in waterincreased from 4 mg/L to 10 mg/L in the presence of 10% soil suspensions.

Biosurfactants have been shown to promote biodegradation of hydrocarbons and a pilot plant and large scale bioremediations of soil contaminated with polycyclic aromatic hydrocarbons (PAH) and heavy oil were performed. In the presence of selected biosurfactants, a preferential and significant removal of PAHs was observed after only 22 days of bioremediation. These results show a significant reduction of the time required to bioremediate contaminated sites bearing in mind that bioremediation is generally a slow process[13].

The compound used for the experiment, 2,4-dichlorophenol (2,4-DCP), was considerably more degraded when sophorose lipids were added to the soil slurry (Fig. 4). Decrease of 2,4 –DCP in the suspensions was found to be generally a slow process for the first 8 days. However, a sharp drop in the suspension was observed afterwards (Fig. 4a, b). In the presence of sophorose lipids, this drop was significantly deeper as compared to the sample without sophorose lipids.

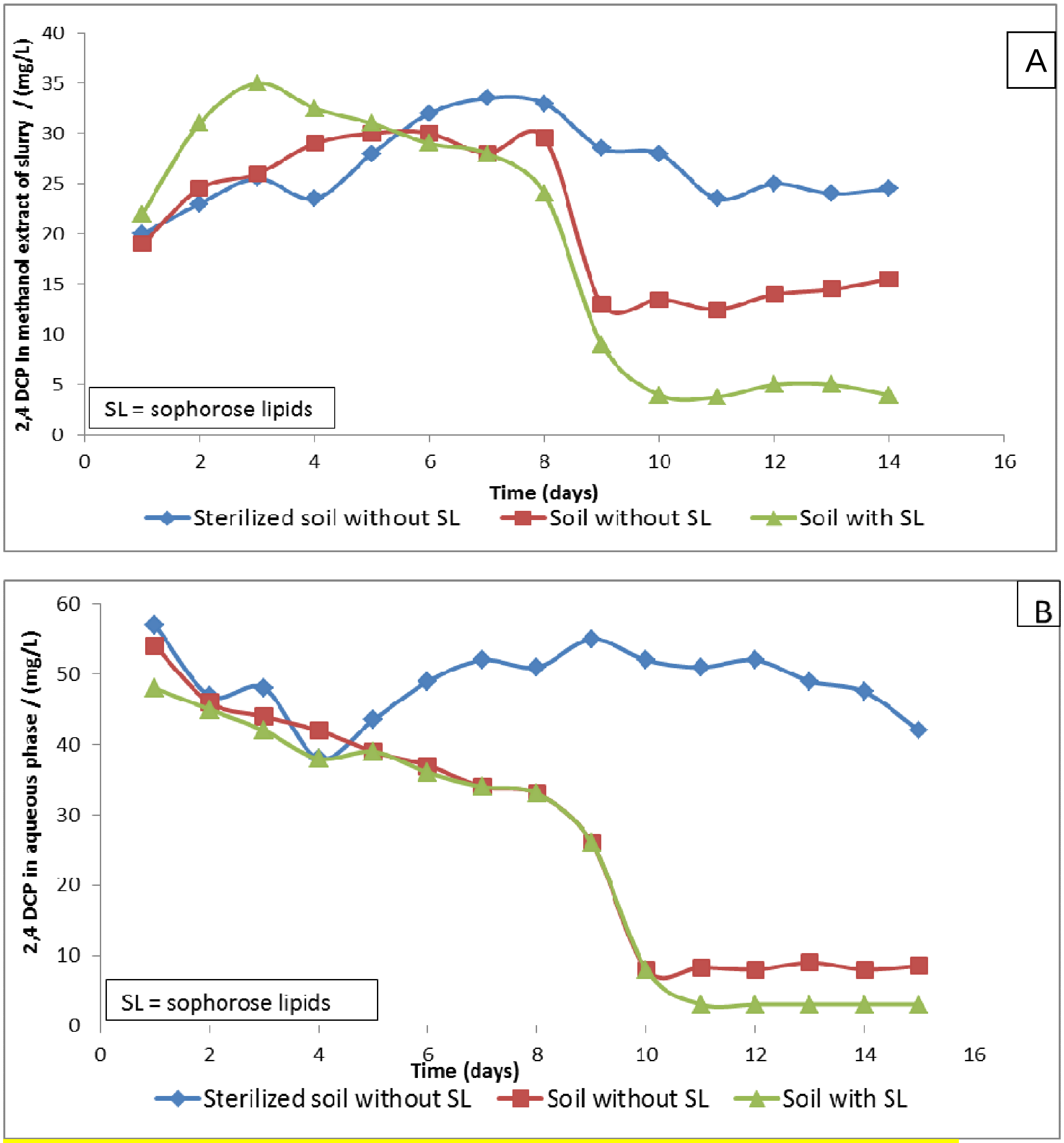

Fig. 4. The effect of Sophprose lipids on 2,4 Dichlorophenol in soil suspension: (A) in methanol extract of slurry (B) in Aqueous Phase (soil 40g; water 60 ml;2,4 DCP 6g; sophprose 38mg) [13].

Plants or microorganisms used as biosurfactants

Natural biosurfactants can be produced extracellularly or as part of the cell membrane by a wide variety of microorganisms such as bacteria, fungi, and yeast. Some examples include *Pseudomonas aeruginosa* which produces rhamnoplids, *Bacillus subtilis* which produces surfactin [65, 66], *Nocardiaamarae* [67], and *Saccharomyces lipolytica* CCT-0913 [68]. The hydrophobic part is based on long chain fatty acids, hydroxyl fatty acids or α-alkyl-β-hydroxy fatty acids. The hydrophilic group can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol [69, 70].

Production of rhamnolipid from bacterial isolates

Biosurfactants are produced by many different bacterial genera. Isolated bacterium was used for the production of biosurfactant by growing the organism in a specific medium. In the study of detoxification of heavy metals by Sinha and Paul [71], biosurfactant production was carried out in water-insoluble medium containing 1.5% (V/V) cooked vegetable as substrate along with $MgSO_4.7H_2O$, KH_2PO_4, $NaNO_3$, yeast extract and peptone. The cultures were taken in 500 ml Laboratory flasks with 100 ml of medium. Filter-sterilized trace element solution was added to the medium which was autoclaved and allowed to cool. Then 2 ml of the culture was added to the medium and incubated at 30^{o}C for 48 – 72 hours[71].

Advantages of using biosurfactant in bioremediation

1. Biosurfactants are readily biodegradable and therefore do not constitute additional pollution threat.
2. Biosurfactants reduce the total time taken for biodegradation of PAHs in contaminated soils.
3. Biosurfactants reduce surface and interfacial tension, thereby increasing the surface areas of insoluble compounds leading to increased mobility and bioavailability of hydrocarbons.
4. Surface active compounds produced by bacterial strains do not need to have survival ability in soils contaminated with heavy metals.
5. They are environmentally friendly, less toxic and non-hazardous.
6. Their production is potentially less expensive than synthetic surfactants and is achievable in situ at thecontaminated sites from inexpensive raw materials.

Disadvantages of using biosurfactant in bioremediation

1. There is a relatively high production and recovery cost, as well as the difficulty of their mass production.
2. 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

Biosurfactants have shown their potential for remediation of contaminated soils 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.

The value of CMC determines the ability of biosurfactants to reduce the surface tension and interfacial tension and increase the solubility and bioavailability of hydrophobic organic compounds.

Careful and controlled use of biosurfactants will help to enhance cleanup of toxic environmental pollutants and render the environment clean.

Soils contaminated with heavy metals like Cadmium, lead and zinc have been effectively treated with Rhamnolipids which is produced by *Pdeudomonasaeruginosa..*

Biosurfactants possess higher TPH removal efficiency than other substances as observed by experimental results.

Biosurfactantshave been shown to enhance biodegradation of hydrocarbons as 2,4-DCP in soil suspension was considerably more degraded when sophorose lipids was introduced into the soil slurry.

Bioremediation is often a longer treatment option and prolonged exposure of biosurfactants to skin can be very harmful.

Biosurfactants have several applications in various industries such as agriculture, petroleum sector – microbial enhanced oil recovery, medicine, pharmaceutical and cosmetic industries.

ACKNOWLEDGMENTS

The Authors wish to thank the department of Biochemistry, Universityof Port Harcourt, Esther B. Olanisebe and the staff of the Shell Professorial Chair in Petroleum Engineering, University of Ibadan for their support during the preparation of this paper.

REFERENCES

1. Xia H, Yan Z. Effects of biosurfactant on the remediation of contaminated soils. College of Environmental Science and Engineering*,* Zhejiang Gongshang University Hangzhou, China. *IEEE.* 2010.
2. Pacwa-Plociniczak M, Plaza GA, Piotrowska-Seget Z, Cameotra SS. Environmental Applications of Biosurfactants: Recent AdvancesInt. Journ. Mol. Sci*.* 2011;12:633-654.
3. Nester EW, AndersonDG, Roberts CE, Jr., Pearsall NN, Nester MT. Microbiology: A Human Perspective. 3rd Ed. New York: McGraw-Hill. 2001.
4. State of Mississippi.Department of Environmental Quality. Fundamental Principles of Bioremediation. April 1998. <http://www.deq.state.ms.us/MDEQ.nsf/pdf/GARD_Bioremediation/$File/Bioremedia-tion.pdf? Verified 12/15/2006.
5. Kumar A, Bisht BS, Joshi VD, Dhewa T. Review on Bioremediation of Polluted Environment: A Management Tool.International Journal of EnvironmentalSciences. 2011;1(6):1079-1093.
6. Lang S. Biological amphiphiles (microbial biosurfactants). Curr. Opin. Colloid Inter. Sci*.* 2002;*7*:12–20.
7. Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiol. Mol. Biol. R.1997;61:47–64.
8. Christofi N, Ivshina IB.Microbial surfactants and their use in field studies of soil remediation. Journal of Applied Microbiology.2002;93(6):915.
9. Haigh SD. A review of the interaction of surfactants with organic contaminants in soil. Sci. Total Environ.1996; 185:161-170.
10. Perfumo A, Smyth TJP, Marchant R, Banat IM. Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates. *In:* Handbook of Hydrocarbon and Lipid Microbiology edTimmis, K.N. Chapter 47, Volume 2 part 7 pp.1501- 1512, Springer-Verlag, Berlin Heidelberg. 2010.

11. Volkering F, BreureAM, Rulkens WH. Microbiological aspects of surfactant use for biological soil remediation. Biodegradation*.* 1998*;*8(6):401-17.

12. Kosaric N.Biosurfactants in industry. *Pure Appl. Chem.*, 1992;64: 1731–1737.

13. Kosaric N.Biosurfactants and their Application for Soil Bioremediation Food Technol. Biotechnol. 2001;39(4):295–304.

14. Rahman KSM, Rahman TJ, Kourkoutas Y, Petsas I, Marchant R, Banat IM. Enhanced bioremediation of *n*-alkane petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients.Bioresource. Technol.,2003;90:159–168.

15. Das K, Mukherjee AK. Comparison of lipopeptidebiosurfactants production by *Bacillus subtilis*strains in submerged and solid state fermentation systems using a cheap carbon source: some industrial applications of biosurfactants. Process Biochem.,2007;42: 1191–1199.

16. Das P, Mukherjee S, Sen R.Improved bioavailability and biodegradation of a model polyaromatic hydrocarbon by a biosurfactant producing bacterium of marine origin. Chemosphere*.*2008;72:1229–1234.

17. Rosenberg E, Ron EZ.High- and low-molecular-mass microbial surfactants. Appl. Microbiol. Biotechnol.,1999;52:154–162.

18. Calvo C,ManzaneraM, Silva-Castro GA, Uad I, González-López J.Application of bioemulsifiers in soil oil bioremediation processes. Future prospects. Sci. Total Environ.2009;407: 3634–3640.

19. Groboillot A, Portet-Koltalo F, Derf FL, Feuilloley MJG, Orange N,PocCD. Novel Application of CyclolipopeptideAmphisin: Feasibility Study as Additive to Remediate Polycyclic Aromatic Hydrocarbon (PAH) Contaminated Sediments. Int J Mol Sci. 2011;12(3): 1787–1806.

20. Bognolo G. Biosurfactants as emulsifying agents for hydrocarbons. Colloids Surf. A Physicochem. Eng. Asp., 1999;152: 41-52.

21. Kitamoto D, Yanagishita H,Shinbo T, Makane T, Kamisawa C, Nakahara, T. Surface active properties and antimicrobial activities of mannosylerythritol lipids as biosurfactant produced by *Candida antractica*. J. Biotechnol., 1993;29:91-96.

22. Singer ME, Finnerty WR,Tunelid, A. Physical and chemical properties of a biosurfactant synthesized by *Rhodococcus* species H13A. Can. J. Microbiol., 1990;36: 746-750.

23. Sifour M, Al-Jilawi MH, Aziz GM.Emulsification Properties of Biosurfactant produced from *Pseudomonas aeruginosa*RB 28Pak, Journal of Biol. Sci. 2007;10:1331-1335.

24. Whang LM, Liu PWG, Ma CC, Cheng SS. Application of biosurfactant, rhamnolipid and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. J. Hazard. Mater*.*2008; 151:155–163.

25. Herman DC,Artiola JF, Miller RM. Removal of Cadmium, lead, and zinc from soil by a rhamnolipidBiosurfactant. Environ. Sci. Technol. 1995;29:2280-2285.

26. Maier RM, Soberon-Chavez G. *Pseudomonas aeruginosa*rhamnolipids; Biosynthesis and potential applications Appl. Microbiol. Biotechnol*.* 2000;54;625-633.

27. Franzetti A,Gandolfi I,Bestetti G, Smyth TJ, Banat IM. Production and applications of trehalose lipid biosurfactants Eur. Journ. Lipid. Sci. Tech. 2010;112: 617-627.

28. Pesce LA. Biotechnological method for the regeneration of hydrocarbons from dregs and muds on the base ofBiosurfactant. 02/062,495. World Patent 2002.

29. Baviere M, Degouy D,Lecourtier J. BiosurfactantsProcess for washing solid particles comprising a sophoroside solution U. S. Patent 5, 326, 406, 1994.

30. GersonOF,Zajic JE, Surfactant production from hydrocarbon by*Corynebacteriumlepus,* sp. nov.and*Pseudomonas asphaltenicus, spnov.*Dev. Ind. Microbiol*.* 1978;19:577-599.

31. Ishigami Y, Yamazaki S, Gama Y. Surface active properties of biosoap from spiculisporic acid J. ColliodInterf. Sci*.* 1983;94:131-139.

32. Ishigami Y, Zhang Y, Ji F. Spiculisporic acid. Functional development of biosurfactantsChimOggi, 2000;18:32-34.

33. Hong JJ, Yang SM, Lee CH, Choi YK,Kajiuchi T. Ultrafiltration of divalent metal cations from aqueous solution using polycarboxylic acid type biosurfactants J. Colloid Interf. Sci*.* 1998;202:63-73.

34. Appanna VD, Finn H,St. Pierre M. ExocellularPhosphatidylethanolamine production and multiple-metal tolerance in *Pseudomonas flourescens*. FEMS Microbiol. Lett. 1995;131:53-56.

35. JennemaGE,Mclnerney MJ, Knapp RM, ClarkJB,FeerpJM,Revus DE. *et al*., A halotolerantbiosurfactants-producing *Bacillus* species potentially useful for enhanced oil recovery. Dev. Ind. Microbiol. 1983;24:485-492.

36. Awashti N, Kumar A, MakkarR,Cameotra S. Enhanced biodegradation of endosulfan, a chlorinated pesticide in presence of a biosurfactant. J. Environ. Sci. Heal. B*.* 1999;34:793-803.

37. ArimaK,Kakinuma A, Tamura G. Surfactin, a crystalline peptide lipid surfactant produced by *Bacillus subtillis* isolation characterization and its inhibition of fibrin clot formation Biochem. Biophys, Res. Commun. 1968;31:488-494.

38. Thomas CP, DuvallML,Robertson EP, Barrett KB, Bala GA. Surfactant-based EOR mediated by naturally occurring microorganism. 1993;11:285-291.

39. Soberon-Chavez G, Maier RM. Biosurfactants: a General Overview. In *Biosurfactants*; Soberon-Chavez, G., Ed.; Springer-Verlag: Berlin, Germany, pp. 1–11. 2011.

40. Mulligan CN, Gibbs BF. Types, production and applications of biosurfactants. Proc. Indian Nat. Sci. *Acad.*,2004;1:31–55.

41. Nguyen TT, Youssef NH, McInerney MJ, Sabatini DA. Rhamnolipidbiosurfactant mixtures for environmental remediation. Water Res*.*, 2008;42: 1735–1743.

42. Déziel E, Paquette G, Villemur R, Lépine F, Bisaillon JG.Biosurfactant production by a soil *Pseudomonas* strain growing on polycyclic aromatic hydrocarbons. Appl. Environ. Microbiol. 1996;62: 1908–1912.

43. Bai GY, Brusseau ML, Miller RM. Biosurfactant enhanced removal of residual hydrocarbon from soil. J. Cont. Hydrol.1997;25:157–170.

44. Urum K,Pekdemir T, Evaluation of biosurfactants for crude oil contaminated soil washing. Chemosphere,2004;57:1139–1150.

45. Timma L, Sams K, Valtere S, Vilgerts J, Blumberga D. Full Factorial Design of Screening Experiments for Biosurfactant Enhanced Remediation of Hydrophobic Substances in SoilJ. Clean Energy Technologies*,*2014;2(1):52-56.

46. Tabatabaee A,Mazaheri MA,Noohi AA, SajadianVA. Isolation of Biosurfactant producing bacteria from oil reservoirs. Iran. J. Environ. Health Sci. Eng*.*, 2005;2: 6-12.

47. Banat IM, Makkar RS,Cameotra SS. Potential commercial applications of microbial surfactants. Appl. Environ. Microbiol2000;53:495-508.

48. Sen R. Biotechnology in Petroleum recovery: the microbial EOR. Prog. Energ. Combust. 2008;34: 714-724.

49. SutharH,Hingurao K, Desai A,Nerurkar A. Evaluation of bioemulsifier mediated microbial enhanced oil recovery using sand pack column. J. Microbiol. Methods 2008;75: 225-230.

50. McInerney MJ,Maudgalya SK, Knapp R, Folmsbee M. Development of Biosurfactant-mediated oil recovery in Model Porous Systems and Computer Simulations of Biosurfactant-Mediated Oil Recovery. A Topical Report of the Department of Petroleum Engineering and Department of Botany and Microbiology University of Oklahoma 2004.

51. Hubalek T,Vosahlova S,Mataju V, Kovacova N, Novotny C. Ecotoxicity Monitoring of Hydrocarbon-Contaminated Soil During Bioremediation: A Case Study, Archives of Environmental Contamination and Toxicology2007;52(1): 1-7.

52. Asci Y, Nurbas M, Acikel YS. Investigation of sorption/desorption equilibria of heavy metal ions on/from quartz using rhamnolipidbiosurfactant. J. Environ. Manage. 2010;91: 724-731.

53. Shin K, Kim J,Kim K. Effect of Biosurfactant Addition on the Biodegradation of Phenanthrene in Soil-water System*, Environ. Eng. Res.*2008;13(1): 8-13.

54. Hong KJ, Tokunaga S,Kajiuchi T. “Evaluation of remediation process with plant-derivedbiosurfactant for recovery of heavy metals from contaminated soils,” Chemosphere, 2002;49(4):379–387.

55. Mulligan CN,Wang S.Remediation of a heavy metal contaminated soil by a rhamnolipid foam, In: Yangt RN, Thomas H. (Eds.), Geoenvironmental Engineering. Integrated Management of groundwater and contaminated land. Thomas Telford Pub., London, UK, 2004; pp. 544–551.

56. Juwarkar AA, Nair A, Dubey KV, Singh SK,DevottaS. Biosurfactant technology for remediation of cadmium and lead contaminated soils, Chemosphere, 2007;68:1996–2002.

57. Mulligan CN, Yong CN, Gibbs BF. Heavy metal removal from sediments by biosurfactants, J. Hazard Mat*,* 2001;85:111–125.

58. MulliganCN,Eftekhari F.Remediation with surfactant foam of PCP-contaminated soil. Engineering Geology*,* 2003;70: 269–279.

59. Kommalapati RR,Valsaraj KT, ConstantWD, RoyD**.**Aqueous solubility enhancement and desorption of hexachlorobenzene from soil using a plant-based surfactant, Wat. Res. 1997; 31(9):2161-2170.

60. Lai CC,Huanga YC, WeiYH, Chang JS.Biosurfactant enhanced removal of total petroleum hydrocarbons from contaminated soil. Journal of Hazardous Materials, 2009; 167:609–614.

61. BurdG, WardOP. Bacterial degradation of polycyclic aromatic hydrocarbons on agar plates: the role of biosurfactants, Biotechnology Techniques. 1996;10: 371–374.

62. SchippersC,GebnerK,MullerT, Scheper T. Microbial degradation of phenanthrene by addition of asophorolipid mixture, Journal of Biotechnology, 2000; 83:189–198.

63. MatvyeyevaOL,Vasylchenko OA,AliievaOR. Microbial Biosurfactants Role in Oil Products Biodegradation International Journal of Environmental Bioremediation & Biodegradation,2014;2(2): 69-74.

64. Qazi MA, Subhan M, Fatima N, Ali MI, Ahmed S. Role of Biosurfactant Produced By *Fusarium sp.* BS-8 in Enhanced Oil Recovery (EOR) Through Sand Pack ColumnInternational Journal of Bioscience, Biochemistry and Bioinformatics*,* November 2013;3(6):598-604*.*

65. Ron EZ, Rosenberg E, Minireview: Natural roles of biosurfactants. Environ. Microbiol. 2001;3:229-236.

66. Mata-Sandoval JC, Karns J, Torrents A. Influence of rhamnolipids and Triton X-100 on the desorption of pesticides from soils. Environ. Sci. Technol. 2002;36:4669-4675.

67. Moussa TAA, Ahmed GM, Abdelhamid SMS. Optimization of cultural conditions for biosurfactants production from *Nocardiaamarae.* J. Appl. Sci. Res. 2006; 2:844-850.

68. Lima AS, Alegre RM, Evaluation of emulsifier stability of biosurfactant produced by *Saccha-romyceslipolytica* CCT-0913. Braz. Arch. Biol.Technol. 2009;5:285-290.

69. Mulligan CN, Yong RN, Gibbs BF. Surfactant-enhanced remediation of contaminated soil: a review. Eng. Geol. 2001;60:371-380.

70. Bustamante M, Duran N, DiezMC.Biosurfactantsare useful tools for the bioremediation of contaminated soils: a Review *Journal of Soil Science and Plant Nutrition* 2012;12(4):667-687

71. Sinha SN, Paul D. Detoxification of Heavy Metals by Biosurfactants Bulletin of Environmental and Scientific Research 2012; 1(4):1-3. Available online at http://www.besr.org.in.

ABBREVIATIONS

CMC	Critical Micelle Concentration
DCP	Dichlorophenol
HCB	Hexachlorobenzene
HLB	Hydrophilic-Lipophilic Balance
HOC	Hydrophobic Organic Compounds
IFT	Interfacial Tension
MEOR	Microbial Enhanced Oil Recovery
PAH	Polycyclic aromatic Hydrocarbon
PCP	Pentachlorophenol
PHPA	Partially Hydrolyzed Polyacrylamide
TPH	Total Petroleum Hydrocarbon