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Bioremediation of Hydrocarbon Contaminated Soil and Water – a Review

Potentials for Biosurfactant Enhanced

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 the components of petroleum products. They are non-toxic, nontransform 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.

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].

During bioremediation, microbes utilize chemical contaminants in the soil as an energy source and through oxidation-reduction reactions, metabolize the target contaminant into useable energy for microbes. By-products which are called metabolites released back into the environment are usually less toxic than the parent contaminants. For example, petroleum hydrocarbons can be degraded by microorganisms in the presence of oxygen through aerobic respiration. The hydrocarbon loses electrons and is oxidized while oxygen gains electrons and is reduced. The end result of this reaction is the formation of carbon dioxide and water. When oxygen is limited in supply or absent, as in saturated or anaerobic soils or lake sediment, anaerobic respiration occurs [3].

Three primary ingredients for bioremediation are: presence of a contaminant, electron acceptor and presence of microorganisms that are capable of degrading the specific contaminant. Generally, a contaminant is more easily degraded if it is a naturally occurring compound in the environment, or chemically similar to a naturally occurring compound, because microorganisms capable of its biodegradation are more likely to have evolved [4].

48 toxic waste from environment. Bioremediation is the most effective management 49 tool to manage the polluted environment and recover contaminated soil and is also an attractive and successful cleaning technique for polluted environment [5]. 50 51 Biosurfactants are structurally diverse group of surface-active substances produced by microorganisms. All biosurfactants are amphiphiles, they consist of two parts - a 52 53 polar hydrophilic moiety and a non-polar hydrophobic group. A hydrophilic group consists of mono, oligo or polysaccharides, peptides or proteins and a hydrophobic 54 moiety usually contains saturated, unsaturated and hydroxylated fatty acids or fatty 55 alcohols [6]. 56 A characteristic feature of biosurfactants is a hydrophilic-lipophilic balance (HLB) 57 which specifies the portion of hydrophilic and hydrophobic constituents in surface-58 active substances. Biosurfactants increase the surface area of hydrophobic water-59 60 insoluble substances because of their amphiphilic structure and increase the water bioavailability of such substances and change the properties of the bacterial cell 61 62 surface. Surface activity makes surfactants excellent emulsifiers, foaming and 63 dispersing agents. They are environmentally friendly, biodegradable, less toxic and 64 non-hazardous [7]. 65 The hydrophilic moiety of a surfactant is defined as the "head", while the 66 hydrophobic one is referred to as the "tail" of the molecule which generally consists of a hydrocarbon chain of varying length. Surfactants are classified as anionic, 67 68 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 69 Concentrations (CMCs) than nonionic surfactants even when they share the same 70 71 hydrophobic group. Electrolytes in solution can reduce the CMC by shielding the electrical repulsion among the hydrophilic heads of the molecules; such effect is 72 more pronounced with anionic and cationic surfactants than with nonionic 73 compounds [9]. At concentrations above the CMC, additional quantities of surfactant 74 in solution will promote the formation of more micelles. The formation of micelles 75 leads to a significant increase in the apparent solubility of hydrophobic organic 76 compounds, even above their water solubility limit, as these compounds can 77 partition into the central core of a micelle. The effect of such a process is the 78

The term bioremediation describes the process of using biological agents to remove

enhancement of mobilization of organic compounds and of their dispersion in solution [10]. This effect is also achieved by the lowering of the interfacial tension between immiscible phases. In fact, this contributes to the creation of additional surfaces, thus improving the contact between different phases [4]. The reduction of interfacial tension is particularly relevant when the pollutant is present in soil as a non-aqueous phase liquid.

The most common hydrophobic parts of chemically synthesized surfactants are paraffins, olefins, alkylbenzenes, alkylphenols and alcohols. The hydrophilic part is usually a sulphate, sulphonate or a carboxylate group in anionic surfactants, a quaternary ammonium group in cationic surfactants and polyoxyethylene, sucrose or a polypeptide in nonionic surfactants [11].

They have many advantages when their chemically synthesized equivalents are compared. They have better foaming properties and higher selectivity. They are active at extreme temperatures, pH and salinity and can be produced from industrial wastes and from by-products. This last feature makes cheap production of biosurfactants possible and allows utilization of waste substrates and reduction of their polluting effect at the same time [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 categorized by their chemical composition, molecular weight, physico-chemical properties, mode of action and microbial origin, unlike chemically synthesized surfactants, which are classified according to their dissociation pattern in water. Based on molecular weight they are divided into low-molecular-mass biosurfactants which include glycolipids, phospholipids and lipopeptides (as seen in Table 1) and into high-molecular-mass biosurfactants which are polymeric amphiphiles. Low-molecular-mass biosurfactants are efficient in lowering surface and interfacial tensions, whereas high-molecular-mass biosurfactants are more effective at stabilizing oil-in-water emulsions [17, 18, 19].

Examples of biosurfactants include: Rhamnolipids produced by <u>Pseudomonas</u> aeruginosa[6],Surfactin from <u>Bacillus subtilis</u> [20], Mannosylerythritol lipids from

Candida antarticas) [21], Trehalose lipids from Rodococcuserythropolis[22], Table 1 111

shows the different types of biosurfactants. 112

Table 1. Classification of Biosurfactants and their use in Remediation of heavy metals and hydrocarbon contaminated soils

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4 and hydrocarbon contaminat Biosurfactant			Application in Environmental	Defe
Group	Class	Microorganism	Biotechnology	Refs.
Glycolipids	Rhamnolipids		Enhancement of the degradation and	[23-26]
		Pseudomonas	dispersion of different classes of	
		aeruginosa	hydrocarbons; emulsification of	
		Pseudomonas sp.	hydrocarbons and vegetable oils;	
			Removal of metal from soil.	
	Trehalolipids	Mycobacterium		0.7
		tuberculosis,		
		RhodococcusErythropolis	Enhancement of the bioavailability of	
		, Arthrobacter sp.,	hydrocarbon	27
		Nocardia sp.		
		Corynebacterium sp.		
	Sophololipids	Tarulanajahamhiaala	Recovery of hydrocarbons from dregs	val of heavy metals enhancements of oil [24,28,29]
		Torulopsisbombicola,	and muds; removal of heavy metals	
		TorulopsispetrophilumTor	from sediments; enhancements of oil	
		ulopsisapicola	recovery	
Fatty Acids, Phospholipids	Corynomycolic acids	Corynebacteriumlepus	Enhancement of Bitumen recovery	30
	Spiculisporic acid	Penicillumspiculisporum	Removal of metal ions from aqueous	[31-33]
			solution; dispersion actions for	
and neutral			hydrophilic pigments; preparation of	
lipids			new emulsion-type organogels.	
	Phosphati- dyethanolamine	Acinetobacter sp.	Increasing the tolerance of bacteria to	34
		RhodococcusErythropolis	heavy metals	
Lipopeptides	Surfactins	Bacillus subtillis	Enhancement of the biodegradation	
			of hydrocarbons and chlorinated	[35-37]
			pesticides. Removal of heavy metals	
			from contaminated soil, sediments	
			and water	
		Bacillus lichenifornis	Enhancement of oil recovery	38

Biosurfactants accumulate at the interface between two immiscible fluids or between a fluid and a solid. By reducing surface (liquid-air) and interfacial (liquid-liquid) tension; they reduce the repulsive forces between two dissimilar phases and allow these two phases to mix and interact more easily (see Fig. 1) [39].



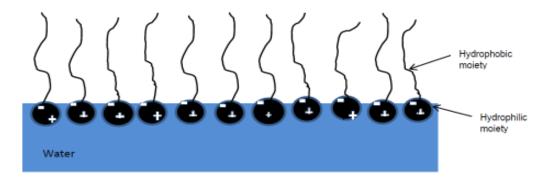


Fig. 1. Accumulation of biosurfactants at the interface between liquid and air [63]

Biosurfactant activities depend on the concentration of the surface-active compounds until the critical micelle concentration is obtained. At concentrations above the CMC, biosurfactant molecules associate to form micelles, bilayers and vesicles (Figure 2). Micelle formation enables biosurfactants to reduce the surface and interfacial tension and increase the solubility and bioavailability of hydrophobic organic compounds [24]. The CMC is commonly used to measure the efficiency of given surfactant. Efficient biosurfactants have a low CMC, which means that less biosurfactant is required to decrease the surface tension [11].

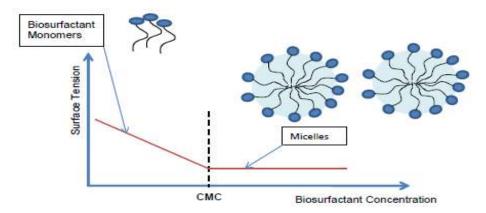


Fig. 2.The relationship between biosurfactant concentration, surface tension and formation of micelles [24]

Role of Biosurfactants in Biodegradation Processes

135 136 Biosurfactants can enhance hydrocarbon bioremediation by two mechanisms. The first is the increase of substrate bioavailability for microorganisms, while the other 137 involves interaction with the cell surface which increases the hydrophobicity of the 138 139 surface allowing hydrophobic substrates to associate more easily with bacterial cells [40]. By reducing surface and interfacial tensions, biosurfactants increase the 140 141 surface areas of insoluble compounds leading to increased mobility and 142 bioavailability of hydrocarbons. Consequently, biosurfactants enhance 143 biodegradation and removal of hydrocarbonsfrom contaminated environments. Addition of biosurfactants is expected to enhance hydrocarbon biodegradation by 144 mobilization, solubilization or emulsification [41,42, 43,14]. 145

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The mobilization mechanism occurs at concentrations below the biosurfactant CMC. At such concentrations, biosurfactants reduce the surface and interfacial tension between air/water and soil/water systems. Due to the reduction of the interfacial force, contact of biosurfactants with soil/oil system increases the contact angle and reduces the capillary force holding oil and soil together. This causes solubilization to take place above the biosurfactant CMC. At these concentrations biosurfactant molecules associate to form micelles, which dramatically increase the solubility of oil. The hydrophobic ends of biosurfactant molecules connect together inside the micelle while the hydrophilic ends are exposed to the aqueous phase on the exterior. Consequently, the interior of a micelle creates an environment compatible for hydrophobic organic molecules. The process of incorporation of these molecules into a micelle is known as solubilization[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].

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Biosurfactant Enhanced Remediation of Hydrophobic Substances in Soil

Wide application and improper disposal of oil products and other hazardous wastes, as well as accidents related to them result in long-lasting contamination of soil and

subsurface environment. Contamination inevitably will affect ecosystems and human health [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 oil or other hydrophobic substances from soil, the behaviour of the biosurfactant across different systems was examined. Process variables, temperature of environment, contact time with dilution of biosurfactant.

and concentration of biosurfactant in washing solution were modeled by applying full

175 factorial design.

Many physical properties used to characterize surfactants depend on the CMC, emulsion, oil solubilization, foaming and detergency, interfacial and surface tensions. These properties may be used to assess the suitability of surfactant for environmental bioremediation, such as soil washing. They used the Photo colorimetric method in their research to determine the cleaning efficiency instead of the generally gravimetric assessment. The experimental tests showed high reliability for the assessment of degreasing and therefore are especially suited for exploration and optimization of different surfactants and their mixes [45].

Both organic and inorganic contaminants can be removed through desorption or biodegradation processes. Biosurfactants enhance the desorption of heavy metals or HOCs from soils in two ways: directly by favouring metals or HOCs solubility from soils and indirectly by increasing the metals or HOCs availability 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)

Biosurfactants can also be involved in microbial enhanced oil recovery. This method is used to recover oil remaining in reservoirs after primary and secondary recovery procedures [48, 49]. It is an important tertiary recovery process where microorganisms or their metabolites, including biosurfactants, biopolymers, biomass, acids, solvents, gases and also enzymes, are used to increase recovery of oil from depleted reservoirs. Application of biosurfactants in enhanced oil recovery is one of the most promising advanced methods to recover a significant proportion of residual oil. The remaining oil is often located in regions of the reservoir that are difficult to access and the oil is trapped in the pores by capillary pressure. Biosurfactants reduce interfacial tension between oil/water and oil/rock. This reduces capillary forces preventing oil from moving through the rock pores (Fig. 3). Surfactants can also bind tightly to the oil-water interface and form emulsion. This stabilizes the desorbed oil in water and allows removal of oil along with the injection water [50, 64].

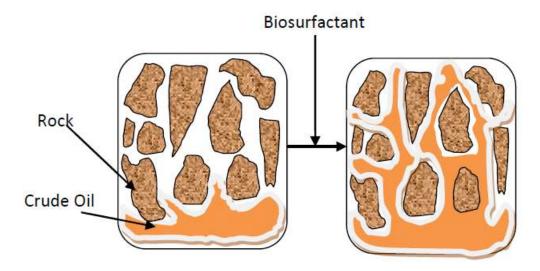


Fig. 3. Mechanism of enhanced oil recovery by biosurfactants [2]

Tertiary oil recovery experiments showed that biosurfactant solutions with concentrations ranging from 10 to 60 mg/l in the presence of 0.1 mM 2,3-butanediol and 1 g/l of partially hydrolyzed polyacrylamide (PHPA) recovered 10-40% of the residual oil present in Berea sandstone cores. When PHPA was used alone, about

10% of the residual oil was recovered. Thus, about 10% of the residual oil recovered in these experiments was due to the increase in viscosity of the displacing fluid. The remainder of the recovered oil was due to the effect of the JF-2 biosurfactant on interfacial tension between oil and the displacing aqueous phase. Little or no oil was recovered at biosurfactant concentrations below the CMC (about 10 mg/l). Below this concentration, the IFT (Interfacial Tension) values were high. At biosurfactant concentrations between 10 and 40 mg/l, the IFT was 1 mN/m. When the CMC is greater than 40 mg/l, IFT decreased to around 0.1 mN/m. At biosurfactant concentrations above the CMC, residual oil recovery is a linear function of biosurfactant concentration[50].

Biosurfactants and Metals Remediation

Contamination of soil environments with heavy metals is very hazardous for human and other living organisms in the ecosystem. As a result of their extremely toxic nature, presence of even low concentrations of heavy metals in the soils has been found to have serious consequences. Currently, there are many techniques used to clean up soils contaminated with heavy metals. Remediation of these soils includes non-biological methods such as excavation and disposal of contaminated soil to landfill sites or biological techniques[51, 52].

A promising approach seems to be the application of inoculants of biosurfactant producing bacteria in phytoremediation of hydrocarbon polluted soil to improve the efficiency of this technology. Application of the biosurfactants in phytoremediation on a large scale requires studies to identify their potential toxic effect on plants. Although the biosurfactants are thought to be ecofriendly, some experiments indicated that under certain circumstances they can be toxic to the environment[38]. Nevertheless, careful and controlled use of these interesting surface active molecules will surely help in the enhanced cleanup of the toxic environmental pollutants and provide us with a clean environment [2].

Application of Biosurfactant Technology on Contaminated Soils

Remediation of heavy metals pollution

Hong *et al.* [54] examined the removal of cadmium and zinc, by saponin, (a plant-derived biosurfactant) from three types of soils - AndosolCambisol, and Regosolsoils. Rates of removal of 90–100% for cadmium and 85–98% for zinc were obtained. Saponin concentrations of 3% were optimal for metal removal within 6 hours and maximal removal was obtained from Regosol soil.

Rhamnolipidproduced by Pseudomonas bacteria which has a strong affinity for metals such as Cd, Zn and Pb has been used in soil washing in recent years. Heavy metal removal from a sandy soil contaminated with 1710 mg/kg of Cd and 2010

mg/kg of Ni was studied by Mulligan and Wang [55] and maximum removal was obtained by foam produced by 0.5% rhamnolipid solution, after 20 pore volumes. Removal efficiency for the biosurfactant foam was 73.2% of Cd and 68.1% of Ni while removal efficiency for the biosurfactant liquid solution was 61.7% Cd and 51.0% Ni.

Rhamnolipidbiosurfactant produced by *Pseudomonas eruginosa*was also evaluated by Juwarkaret al., [56] to know the potential of removing Cd and Pb from contaminated soil. Their results showed that di-rhamnolipid removed not only the leachable or available fraction of Cd and Pb but also the bound metals as compared to tap water which removed the mobile fraction only.

Mulligan *et al.*, [57] evaluated the possibility of using surfactin, rhamnolipid, and sophorolipid for the removal of Cu and Zn, from sediments. A single washing with 0.5% rhamnolipid removed 65% of the copper and 18% of the zinc, 4% sophorolipid removed 25% of the copper and 60% of the zinc while surfactin was the least effective, removing 15% of the copper and 6% of the zinc.

Remediation of Hydrophobic Organic Compounds

Pentachlorophenol (PCP) is a toxic, suspected carcinogenic compound which can be used to preserve wood. A rhamnolipidbiosurfactant was used to investigate the removal efficiency in soils contaminated with PCP; 1% of the rhamnolipid removed 60% and 61% of PCP from fine sand soil and sandy-silt soil respectively, contaminated with 1000 mg/kg PCP. Of this, 36% and 44% of the PCP was removed by volatilization by the biosurfactant[58].

A plant-based surfactant extracted from fruit pericarps of *Sapindusmukorossi*was proposed for remediation of hexachlorobenzene (HCB) contaminated soil. HCB concentration in the natural surfactant solution approached up to 90% of the HCB solubility in the respective solutions in batch desorption studies for soils contaminated to high levels, and the HCB recoveries were up to 90% of the total HCB for soils contaminated to lower levels [59].

Biosurfactants exhibited much higher Total Petroleum Hydrocarbon (TPH) removal efficiency than the tested synthetic ones as observed by the experimental results from Lai *et al.*, [60]. By using 0.2 mass% of rhamnolipids, surfactin, Tween 80, and Triton X-100, the TPH removal from the soil contaminated with ca. 3,000 mg TPH/kg dry soil was 23%, 14%, 6%, and 4%, respectively, while removal efficiency increased to 63%, 62%, 40%, and 35%, respectively, for the soil contaminated with ca. 9000 mgTPH/kg dry soil.

Rahmanet al. [14] demonstrated that addition of rhamnolipid produced by *Pseudomonas sp.* along with poultry waste and coir pith enhanced ex situ bioremediation of a gasoline-contaminated soil. Research with another strain, *Pseudomonas marginalis*, also indicated that the produced biosurfactants solubilized (PAHs) such as phenanthrene and enhanced biodegradation [61]. The rhamnolipidssorbed onto the solids, thus increasing the amount of solid phase PAHs.

The study ofsophorolipidseffects on phenanthrene biodegradation showed that the concentration of phenanthrene with initial concentration of 80 mg/L within 36 hour decreased to 0.5 mg/L in the presence of 500 mg/L of the surfactant compared to 2.3 mg/L without surfactant in a 10% soil suspension [62]. The maximal degradation by *Sphingomonasyanoikuyae* was 1.3 mg/L/h with the sophorolipid instead of 0.8 mg/L/h. The sophorolipids seem to enhance the phenanthrene concentration as shown by fluorescence measurements instead of increasing biomass concentration. In addition, toxicity of the sophorolipid was low for concentrations up to 1 g/L. The CMC of the sophorolipid in water was 4 mg/L but this increased to 10 mg/L in the presence of 10% soil suspensions indicating adsorption of the surfactant onto the soil.

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.



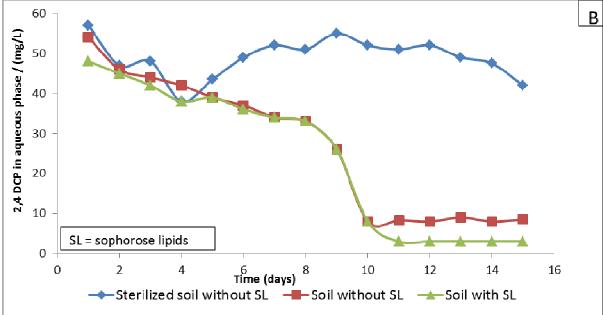


Fig. 4. The effect of Sophprose lipids on 2,4 Dichlorophenol in soil suspension: (A) in methanol extract of slurry (B) in Aqueous Phase (soil 40g; water 60 ml;2,4 DCP 6g; sophprose 38mg) [13].

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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 the contaminated 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.
- 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

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- Biosurfactants have shown their potential for remediation of contaminated soils by increasing biodegradation rate and reducing contaminant minimum concentration.
- 367 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.
- 372 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.
- Biosurfactants have 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.
- 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, University of 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

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- 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.
- 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.
- 400 4. State of Mississippi.Department of Environmental Quality. Fundamental Principles of 401 Bioremediation. April 1998. http://www.deq.state.ms.us/MDEQ.nsf/pdf/GARD_Bioremediation/\$File/Bioremedia 403 -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.
- 407 6. Lang S. Biological amphiphiles (microbial biosurfactants). Curr. Opin. Colloid Inter. 408 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.
- 413 9. Haigh SD. A review of the interaction of surfactants with organic contaminants in soil. Sci. Total Environ.1996; 185:161-170.
- 415 10. Perfumo A, Smyth TJP, Marchant R, Banat IM. Production and roles of 416 biosurfactants and bioemulsifiers in accessing hydrophobic substrates. *In:* 417 Handbook of Hydrocarbon and Lipid Microbiology edTimmis, K.N. Chapter 47, 418 Volume 2 part 7 pp.1501- 1512, Springer-Verlag, Berlin Heidelberg. 2010.
- Volkering F, BreureAM, Rulkens WH. Microbiological aspects of surfactant use for biological soil remediation. Biodegradation. 1998;8(6):401-17.
- 421 12. Kosaric N.Biosurfactants in industry. Pure Appl. Chem., 1992;64: 1731–1737.
- 422 13. Kosaric N.Biosurfactants and their Application for Soil Bioremediation Food Technol.
 423 Biotechnol. 2001;39(4):295–304.
- 424 14. Rahman KSM, Rahman TJ, Kourkoutas Y, Petsas I, Marchant R, Banat IM.
 425 Enhanced bioremediation of *n*-alkane petroleum sludge using bacterial consortium
 426 amended with rhamnolipid and micronutrients.Bioresour. Technol.,2003;90:159–
 427 168.
- 428 15. Das K, Mukherjee AK. Comparison of lipopeptidebiosurfactants production by
 429 *Bacillus subtilis*strains in submerged and solid state fermentation systems using a
 430 cheap carbon source: some industrial applications of biosurfactants. Process
 431 Biochem.,2007;42: 1191–1199.
- 432 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.
- 435 17. Rosenberg E, Ron EZ.High- and low-molecular-mass microbial surfactants. Appl. 436 Microbiol. Biotechnol.,1999;52:154–162.
- 437 18. Calvo C,ManzaneraM, Silva-Castro GA, Uad I, González-López J.Application of 438 bioemulsifiers in soil oil bioremediation processes. Future prospects. Sci. Total 439 Environ.2009;407: 3634–3640.

- 440 19. Groboillot A, Portet-Koltalo F, Derf FL, Feuilloley MJG, Orange N,PocCD. Novel 441 Application of CyclolipopeptideAmphisin: Feasibility Study as Additive to Remediate 442 Polycyclic Aromatic Hydrocarbon (PAH) Contaminated Sediments. Int J Mol Sci. 443 2011;12(3): 1787–1806.
- 444 20. Bognolo G. Biosurfactants as emulsifying agents for hydrocarbons. Colloids Surf. A Physicochem. Eng. Asp., 1999;152: 41-52.
- 446 21. Kitamoto D, Yanagishita H,Shinbo T, Makane T, Kamisawa C, Nakahara, T. Surface
 447 active properties and antimicrobial activities of mannosylerythritol lipids as
 448 biosurfactant produced by *Candida antractica*. J. Biotechnol., 1993;29:91-96.
- 449 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.
- 452 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.
- 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.
- 457 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.
- 459 26. Maier RM, Soberon-Chavez G. *Pseudomonas aeruginosa*rhamnolipids; Biosythesis and potential applications Appl. Microbiol. Biotechnol. 2000;54;625-633.
- 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.
- Pesce LA. Biotechnological method for the regeneration of hydrocarbons from dregs and muds on the base of Biosurfactant. 02/062,495. World Patent 2002.
- Baviere M, Degouy D,Lecourtier J. BiosurfactantsProcess for washing solid particles comprising a sophoroside solution U. S. Patent 5, 326, 406, 1994.
- 467 30. GersonOF,Zajic JE, Surfactant production from hydrocarbon 468 by*Corynebacteriumlepus*, sp. nov.and*Pseudomonas asphaltenicus*, *spnov*.Dev. Ind. 469 Microbiol. 1978;19:577-599.
- 470 31. Ishigami Y, Yamazaki S, Gama Y. Surface active properties of biosoap from spiculisporic acid J. ColliodInterf. Sci. 1983;94:131-139.
- 472 32. Ishigami Y, Zhang Y, Ji F. Spiculisporic acid. Functional development of biosurfactantsChimOggi, 2000;18:32-34.
- 474 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.
- 477 34. Appanna VD, Finn H,St. Pierre M. ExocellularPhosphatidylethanolamine production 478 and multiple-metal tolerance in *Pseudomonas flourescens*. FEMS Microbiol. Lett. 479 1995;131:53-56.
- 480 35. Jennema GE,McInerney MJ, Knapp RM, Clark JB,Feerp JM,Revus DE. *et al.*, A
 481 halotolerantbiosurfactants-producing *Bacillus* species potentially useful for enhanced
 482 oil recovery. Dev. Ind. Microbiol. 1983;24:485-492.
- 483 36. Awashti N, Kumar A, Makkar R,Cameotra S. Enhanced biodegradation of endosulfan, a chlorinated pesticide in presence of a biosurfactant. J. Environ. Sci. Heal. B. 1999;34:793-803.

- 486 37. Arima K,Kakinuma A, Tamura G. Surfactin, a crystalline peptide lipid surfactant 487 produced by *Bacillus subtillis* isolation characterization and its inhibition of fibrin clot 488 formation Biochem. Biophys, Res. Commun. 1968;31:488-494.
- Thomas CP, Duvall ML,Robertson EP, Barrett KB, Bala GA. Surfactant-based EOR mediated by naturally occurring microorganism. 1993;11:285-291.
- 491 39. Soberon-Chavez G, Maier RM. Biosurfactants: a General Overview. In 492 *Biosurfactants*; Soberon-Chavez, G., Ed.; Springer-Verlag: Berlin, Germany, pp. 1– 493 11. 2011.
- 494 40. Mulligan CN, Gibbs BF. Types, production and applications of biosurfactants. Proc. 495 Indian Nat. Sci. *Acad.*,2004;1:31–55.
- 496 41. Nguyen TT, Youssef NH, McInerney MJ, Sabatini DA. Rhamnolipidbiosurfactant mixtures for environmental remediation. Water Res., 2008;42: 1735–1743.
- 498 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.
- 501 43. Bai GY, Brusseau ML, Miller RM. Biosurfactant enhanced removal of residual hydrocarbon from soil. J. Cont. Hydrol.1997;25:157–170.
- 503 44. Urum K,Pekdemir T, Evaluation of biosurfactants for crude oil contaminated soil washing. Chemosphere,2004;57:1139–150.
- 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.
- 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.
- 511 47. Banat IM, Makkar RS, Cameotra SS. Potential commercial applications of microbial surfactants. Appl. Environ. Microbiol2000;53:495-508.
- 513 48. Sen R. Biotechnology in Petroleum recovery: the microbial EOR. Prog. Energ. 514 Combust. 2008;34: 714-724.
- 515 49. Suthar H,Hingurao K, Desai A,Nerurkar A. Evaluation of bioemulsifier mediated 516 microbial enhanced oil recovery using sand pack column. J. Microbiol. Methods 517 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.
- 523 51. Hubalek T,Vosahlova S,Mataju V, Kovacova N, Novotny C. Ecotoxicity Monitoring 524 of Hydrocarbon-Contaminated Soil During Bioremediation: A Case Study, Archives 525 of Environmental Contamination and Toxicology2007;52(1): 1-7.
- 526 52. Asci Y, Nurbas M, Acikel YS. Investigation of sorption/desorption equilibria of heavy 527 metal ions on/from quartz using rhamnolipidbiosurfactant. J. Environ. Manage. 528 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.
- 531 54. Hong KJ, Tokunaga S,Kajiuchi T. "Evaluation of remediation process with plant-532 derivdbiosurfactant for recovery of heavy metals from contaminated soils," 533 Chemosphere, 2002;49(4):379–387.
- 534 55. Mulligan CN,Wang S.Remediation of a heavy metal contaminated soil by a rhamnolipid foam, In: Yangt RN, Thomas H. (Eds.), Geoenvironmental Engineering.

- 536 Integrated Management of groundwater and contaminated land. Thomas Telford 537 Pub., London, UK, 2004; pp. 544-551.
- 538 56. Juwarkar AA, Nair A, Dubey KV, Singh SK, DevottaS. Biosurfactant technology for 539 remediation of cadmium and lead contaminated soils, Chemosphere, 2007;68:1996-540
- 541 Mulligan CN, Yong CN, Gibbs BF. Heavy metal removal from sediments by 57. biosurfactants, J. Hazard Mat, 2001;85:111-125. 542
- 543 58. MulliganCN, Eftekhari F. Remediation with surfactant foam of PCP-contaminated soil. 544 Engineering Geology, 2003;70: 269-279.
- Kommalapati RR, Valsaraj KT, ConstantWD, RoyD. Aqueous solubility enhancement 545 59. 546 and desorption of hexachlorobenzene from soil using a plant-based surfactant, Wat. 547 Res. 1997; 31(9):2161-2170.
- Lai CC. Huanga YC, WeiYH, Chang JS. Biosurfactant enhanced removal of total 548 60. 549 petroleum hydrocarbons from contaminated soil. Journal of Hazardous Materials, 2009; 167:609–614. 550
- BurdG, WardOP. Bacterial degradation of polycyclic aromatic hydrocarbons on agar 551 61. 552 plates: the role of biosurfactants, Biotechnology Techniques. 1996;10: 371-553
 - 62.

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561 562

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578

- 554 63. SchippersC, GebnerK, MullerT, Scheper T. Microbial degradation of phenanthrene by 555 addition of asophorolipid mixture, Journal of Biotechnology, 2000; 83:189-198.
 - 64. MatvyeyevaOL, Vasylchenko OA, AliievaOR. Microbial Biosurfactants Role in Oil Products Biodegradation International Journal of Environmental Bioremediation & Biodegradation, 2014; 2(2): 69-74.
 - Qazi MA, Subhan M, Fatima N, Ali MI, Ahmed S. Role of Biosurfactant Produced By 65. 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.

ABBREVIATIONS

TPH

568	CMC	Critical Micelle Concentration
569	DCP	Dichlorophenol
570	HCB	Hexachlorobenzene
571	HLB	Hydrophilic-Lipophilic Balance
572	HOC	Hydrophobic Organic Compounds
573	IFT	Interfacial Tension
574	MEOR	Microbial Enhanced Oil Recovery
575	PAH	Polycyclic aromatic Hydrocarbon
576	PCP	Pentachlorophenol
577	PHPA	Hydrolyzed Polyacrylamide

Total Petroleum Hydrocarbon