Review Article

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

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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 bio surfactants include their unusual structural diversity that may lead to unique properties, the possibility of cost effective production, and their biodegradability. These properties make bio surfactants a promising choice for applications in enhancing hydrocarbon bioremediation.

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 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].

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 the polluted environment and recover contaminated soil and is also an attractive and successful cleaning technique for polluted environment [5].

Biosurfactants are structurally diverse group of surface-active substances produced by microorganisms. All biosurfactants are amphiphiles, they consist of two parts – a polar hydrophilic moiety and a non polar hydrophobic group. A hydrophilic group consists of mono, oligo or polysaccharides, peptides or proteins and a hydrophobic moiety usually contains saturated, unsaturated and hydroxylated fatty acids or fatty alcohols [6].

A characteristic feature of biosurfactants is a hydrophilic-lipophilic balance (HLB) which specifies the portion of hydrophilic and hydrophobic constituents in surface-active substances. Biosurfactants increase the surface area of hydrophobic water-insoluble substances because of their amphiphilic structure and increase the water bioavailability of such substances and change the properties of the bacterial cell surface. Surface activity makes surfactants excellent emulsifiers, foaming and dispersing agents. They are environmentally friendly, biodegradable less toxic and non-hazardous [7].

The hydrophilic moiety of a surfactant is defined as the “head”, while the 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, 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 even when they share the same hydrophobic group. Electrolytes in solution can reduce the CMC by shielding the electrical repulsion among the hydrophilic heads of the molecules; such effect is more pronounced with anionic and cationic surfactants than with nonionic compounds [9]. At concentrations above the CMC, additional quantities of surfactant in solution will promote the formation of more micelles. The formation of micelles leads to a significant increase in the apparent solubility of hydrophobic organic compounds, even above their water solubility limit, as these compounds can partition into the central core of a micelle. The effect of such a process is the 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].

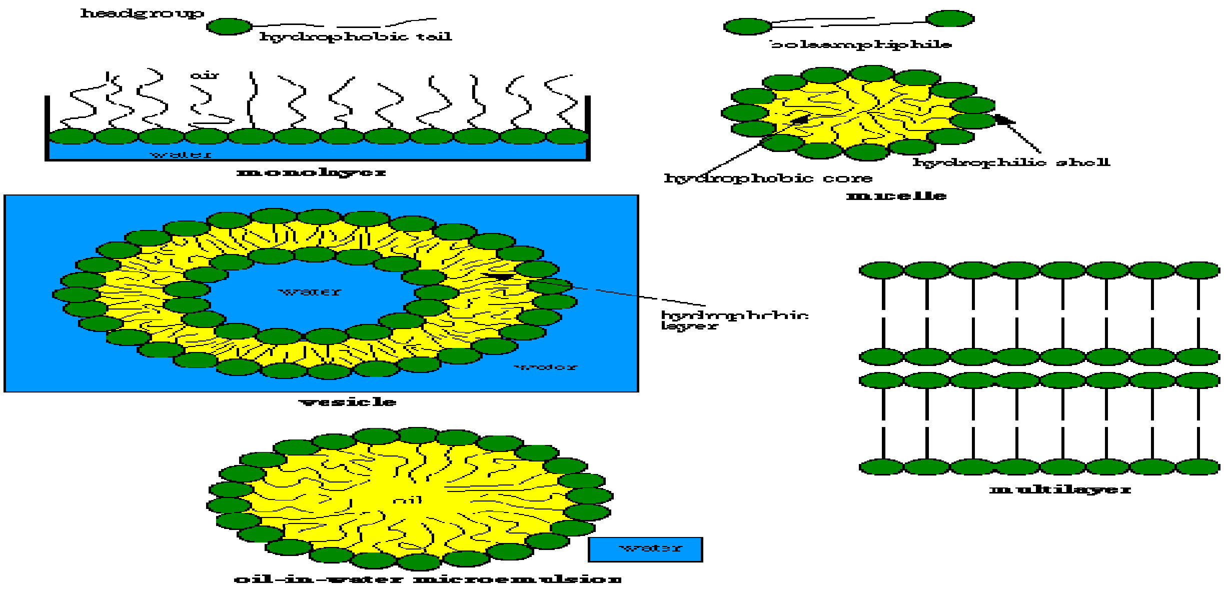
Examples of biosurfactants include: Rhamnolipids produced by *Pseudomonas aeruginosa*[6]**,**Surfactin from *Bacillus subtilis* [20], Mannosylerythritol lipids from

Candida antarticas) [21], Trehalose lipids from *Rodococcuserythropolis*[22], Table 1 shows the different types of biosurfactants.

Table 1: Classification of Biosurfactants and their use in Remediation of heavy metals and hyrocarbon contaminated soils

Biosurfactant		**Microorganism**	**Application in Environmental Biotechnology**
Group	**Class**		
Glycolipids	Rhamnolipids	Pseudomonas aeruginosa pseudomonas sp.	Enhancement of the degradation and dispersion of different classes of hydrocarbons; emulsification of hydrocarbons and vegetable oils; Removal of metal from soil.
	Trehalolipids	Mycobacterium tuberculosis, RhodococcusErythropolis, Arthrobacter sp., Nocardia sp. Corynebacterium sp.	Enhancement of the bioavailability of hydrocarbon
	Sopholipids	TorulopsisBombicola, TorulopsispetrophilumTorulopsisapicola	Recovery of hydrocarbons from dregs and muds; removal of heavy metals from sediments; enhancements of oil recovery
Fatty Acids, Phospholipids and neutral lipids	Corynomycolic acids	Corynebacteriumlepus	Enhancement of Bitumen recovery
	Spiculisporic acid	PenicillumSpiculisporum	Removal of metal ions from aqueous solution; dispersion actions for hydrophilic pigments; preparation of new emulsion-type organogels.
	Phosphati-dyethanolamine	Acinetobacter sp. RhodococcusErythropolis	Increasing the tolerance of bacteria to heavy metals
Lipopeptides	Surfactins	Bacillus Subtillis	Enhancement of the biodegradation of hydrocarbons and chlorinated pesticides. Removal of heavy metals from contaminated soil, sediments and water
	Lychenysin	Bacillus licheniformis	Enhancement of oil recovery

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) [23].



(Source: Soberon-Chavez and Maier 2011)

Figure 1. Accumulation of biosurfactants at the interface between liquid and air

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 ofa givensurfactant. Efficient biosurfactants have a low CMC, which means that less biosurfactant is required to decrease the surface tension [11].

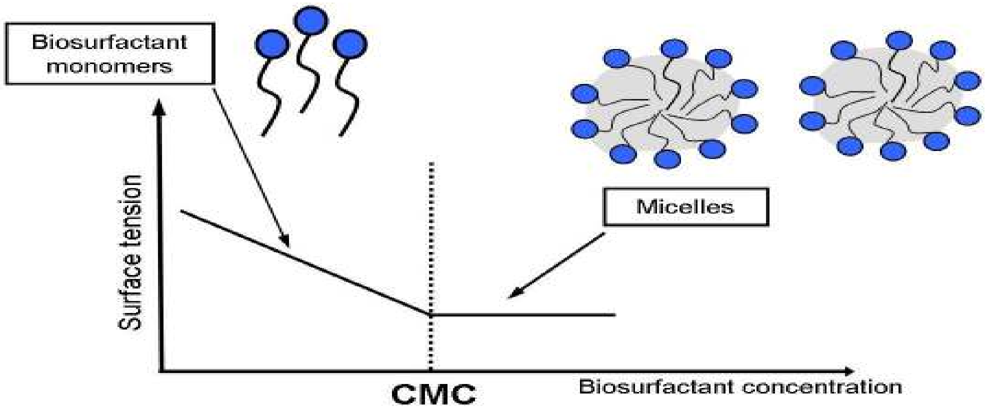


Figure 2. The relationship between biosurfactant concentration, surface tension and formation of micelles

Role of Biosurfactants in Biodegradation Processes

Biosurfactants can enhance hydrocarbon bioremediation by two mechanisms. The first is the increase of substrate bioavailability for microorganisms, while the other involves interaction with the cell surface which increases the hydrophobicity of the surface allowing hydrophobic substrates to associate more easily with bacterial cells [26]. By reducing surface and interfacial tensions, biosurfactants increase the surface areas of insoluble compounds leading to increased mobility and bioavailability of hydrocarbons. Consequently, biosurfactants enhance biodegradation and removal of hydrocarbonsfrom contaminated environments. Addition of biosurfactants is expected to enhance hydrocarbon biodegradation by mobilization, solubilization or emulsification [26,27, 28,14].

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[29].

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

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 [30].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 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 [30].

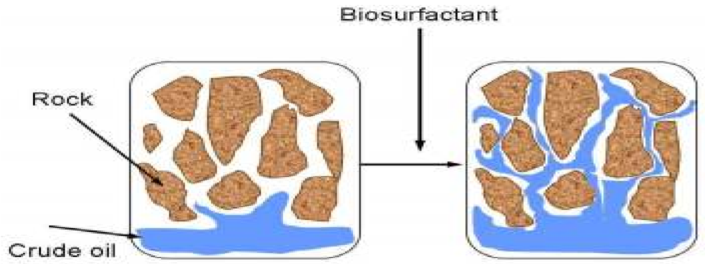
Both organic and inorganic contaminants can be removed through desorption or biodegradation processes. Biosurfactants enhance the desorption of heavy metals or HOCs from soils in two ways: directly by favouring metals or HOCs solubility from soils and indirectly by increasing the metals or HOCs availability 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 [31]. 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} [32]. 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 [33, 34]. 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 [35].



Source: Pacwa-Plociniczak, *et al,* 2011

Fig. 3: Mechanism of enhanced oil recovery by biosurfactants.

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 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[35].

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[36, 37].

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. [39] 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 [40] and maximum removal was obtained by foam produced by 0.5% rhamnolipid solution, after 20 pore volumes. Removal effciency 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 Juwarkar*et al.,* [41] 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.,* [42] 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 usedto 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[43].

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 [44].

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.,* [45]. 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.

Rahman*et 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 [46]. 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 [47]. 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.

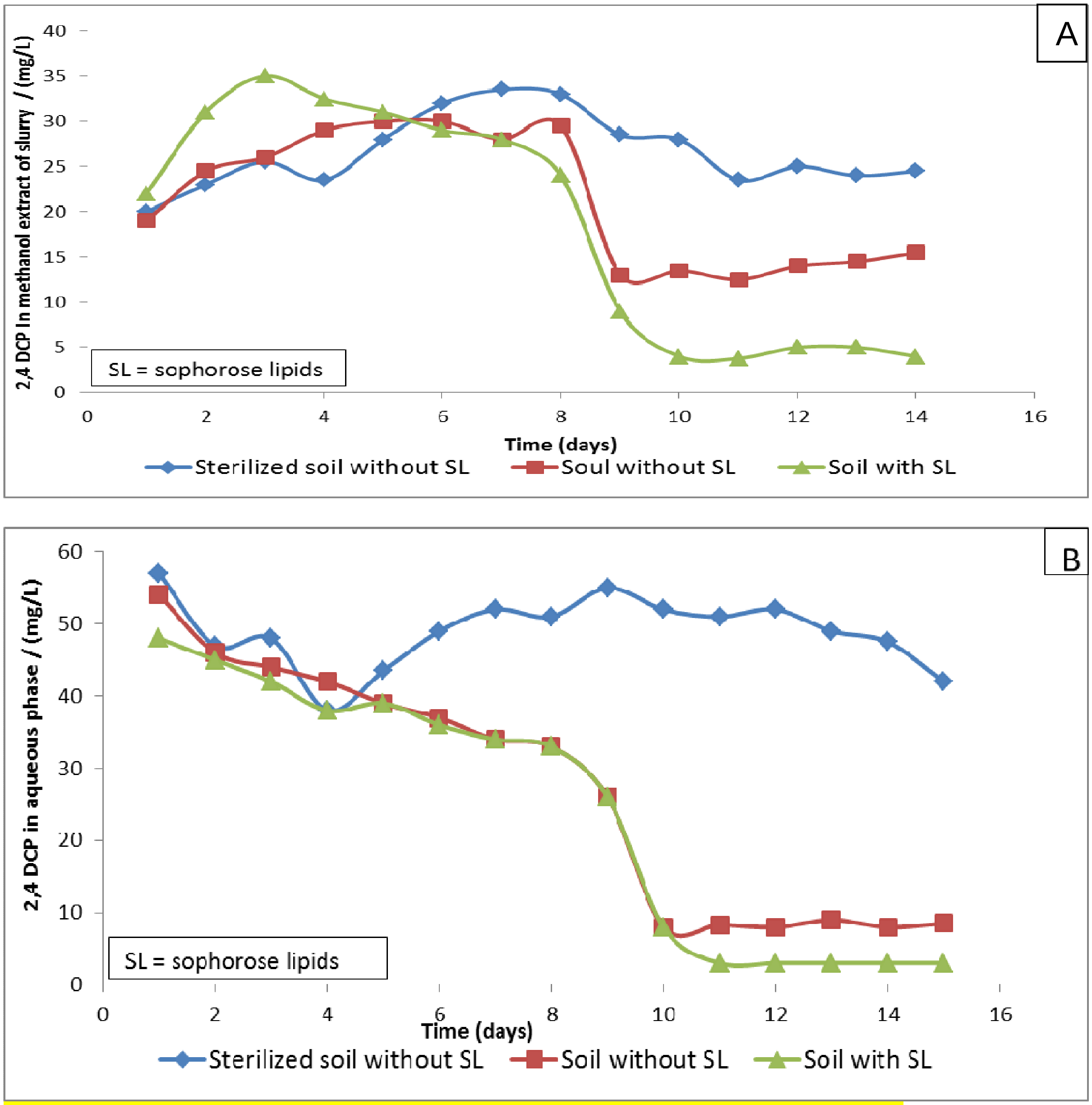


Fig 4: The effect of Sophprose lipids on 2,4 Dichlorophenol in soil suspension: (A) in methanol extract of slurry (B) in Aqueous Phase (soil 40g; water 60 ml;2,4 DCP 6g; sophprose 38mg) **(Kosaric, 2001)**.

Advantages of bioremediation

1. Bioremediation is a natural process and is generally perceived as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded, the

biodegradative population declines. The residues for the treatment are usually harmless products such as carbon dioxide, water and cell biomass.

2. Bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are hazardous can be transformed to harmless products.
3. Instead of transferring contaminants from one environment to another for example land to water or air, the complete destruction of the target pollutants is possible.
4. Bioremediation is less expensive than other technologies that are used for other clean-up of hazardous wastes.

Disadvantages of bioremediation

1. Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.
2. There are some concerns that the products of biodegradation may be more toxic than the parent compound.
3. Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmentally capable microbial populations, suitable environmental growth conditions and appropriate levels of nutrients and contaminants.
4. Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration.
5. Prolonged exposure of skin to biosurfactants can cause chafing because surfactants (like soaps) disrupts 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. This is due to their ability to enhance the pseudo-solubilisation and emulsification of the immiscible fractions of the contaminants, thus enhancing their bioavailability to degrade microorganisms.

Biosurfactants enhance the bioremediation of contaminated soil and water by the reduction of interfacial tension between two immiscible phases.

Surfactants can be used to increase the solubility of dense organic pollutants and is also an effective and relatively inexpensive way of ex-situ remediation of contaminated soils and aquifers.

The introduction of a biosurfactant such as sophoroselipids in a compound like 2,4-DCP, showed a significant drop in the suspension observed after a few days of slow process.

Tertiary oil recovery experiments show that biosurfactants can be used to recovery residual oil from the reservoir

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ABBREVIATIONS

CMC	Critical Micelle Concentration
DCP	Dichlorophenol
HCB	Hexachlorobenzene
HLB	Hydrophilic-Lipophilic Balance
HOC	Hydrophobic Organic Compounds
PAH	Polycyclic aromatic Hydrocarbon
PCP	Pentachlorophenol
PHPA	Hydrolyzed Polyacrylamide
TPH	Total Petroleum Hydrocarbon