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

## ABSTRACT

1

2

3

4

11 12

> 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.

13

14 Keywords: Bioavailability, Biosurfactants, Contamination, Hydrophobic Organic Compounds

### 15 **INTRODUCTION**

16

17 Soil and water contamination are serious challenges which are caused by inorganic 18 pollutants such as heavy metals and organic pollutants like hydrophobic organic compounds. Heavy metals such as lead (Pb), Cadmium (Cd) Copper (Cu), 19 Chromium (Cr), Zinc (Zn) and Nickel (Ni) are known environmental pollutants and 20 21 accumulation of these toxic metals in soil and water constitute potential health 22 hazard for man and the ecosystem. HOCs such as Pentachlorophenol (PCP), 23 Hexachlorobenzene (HCB), Polychlorinated biphenyls (PCBs) and Dichlorophenol 24 (DCP) are known environmental pollutants and their removal from the contaminated 25 sites is a major environmental concern [1]. Bioremediation is any process that uses microorganisms or their enzymes to return 26 27 the environment altered by contaminants to its original condition. It can also be

28 defined as the use of biological processes to degrade or break down contaminants

29 from soil and water. It is a natural process which relies on bacteria, fungi and plants

30 to alter contaminants as these organisms carry out their normal life functions[2].

31 During bioremediation, microbes utilize chemical contaminants in the soil as an energy source and through oxidation-reduction reactions, metabolize the target 32 contaminant into useable energy for microbes. By-products which are called 33 metabolites released back into the environment are usually less toxic than the 34 parent contaminants. For example, petroleum hydrocarbons can be degraded by 35 36 microorganisms in the presence of oxygen through aerobic respiration. The hydrocarbon loses electrons and is oxidized while oxygen gains electrons and is 37 38 reduced. The end result of this reaction is the formation of carbon dioxide and water. 39 When oxygen is limited in supply or absent, as in saturated or anaerobic soils or lake sediment, anaerobic respiration occurs [3]. 40

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

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].

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

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.

98

#### 99 Classification and Properties of Biosurfactants

Biosurfactants are categorized by their chemical composition, molecular weight, 100 physico-chemical properties, mode of action and microbial origin, unlike chemically 101 synthesized surfactants, which are classified according to their dissociation pattern 102 103 in water. Based on molecular weight they are divided into low-molecular-mass biosurfactants which include glycolipids, phospholipids and lipopeptides (as seen in 104 Table 1) and into high-molecular-mass biosurfactants which are polymeric 105 amphiphiles. Low-molecular-mass biosurfactants are efficient in lowering surface 106 107 and interfacial tensions, whereas high-molecular-mass biosurfactants are more effective at stabilizing oil-in-water emulsions [17, 18, 19]. 108

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

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

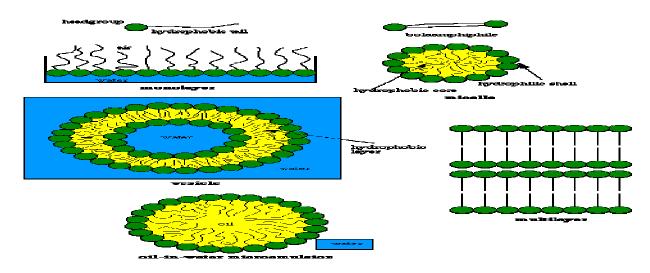
## 112 shows the different types of biosurfactants.

4 contaminated soils Biosurfactant			Application in Environmental	Refs.
Group	Class	Microorganism	Biotechnology	Reis.
	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		27
		tuberculosis,		
Glycolipids		RhodococcusErythropolis	Enhancement of the bioavailability of	
		, Arthrobacter sp.,	hydrocarbon	
		Nocardia sp.		
		Corynebacterium sp.		
	Sophololipids	TorulopsisBombicola, TorulopsispetrophilumTor ulopsisapicola	Recovery of hydrocarbons from dregs	[24,28,29]
			and muds; removal of heavy metals	
			from sediments; enhancements of oil	
			recovery	
	Corynomycolic acids	Corynebacteriumlepus	Enhancement of Bitumen recovery	30
Fatty Acids,	Spiculisporic acid	PenicillumSpiculisporum	Removal of metal ions from aqueous	[31-33]
Phospholipids and neutral lipids			solution; dispersion actions for	
			hydrophilic pigments; preparation of	
			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	[35-37]
			of hydrocarbons and chlorinated	
			pesticides. Removal of heavy metals	
			from contaminated soil, sediments	
			and water	
	Lychenysin	Bacillus lichenifornis	Enhancement of oil recovery	38

# Table 1: Classification of Biosurfactants and their use in Remediation of heavy metals and hyrocarboncontaminated soils

115 Biosurfactants accumulate at the interface between two immiscible fluids or between 116 a fluid and a solid. By reducing surface (liquid-air) and interfacial (liquid-liquid) 117 tension; they reduce the repulsive forces between two dissimilar phases and allow 118 these two phases to mix and interact more easily (see Fig. 1) [39].

119



120 121

122 (Source: Soberon-Chavez and Maier 2011)

Biosurfactant

monomers

Surface tension

Figure 1. Accumulation of biosurfactants at the interface between liquid and air 123

124 Biosurfactant activities depend on the concentration of the surface-active 125 compounds until the critical micelle concentration is obtained. At concentrations 126 127 above the CMC, biosurfactant molecules associate to form micelles, bilayers and 128 vesicles (Figure 2). Micelle formation enables biosurfactants to reduce the surface 129 and interfacial tension and increase the solubility and bioavailability of hydrophobic 130 organic compounds [24]. The CMC is commonly used to measure the efficiency of 131 givensurfactant. Efficient biosurfactants have a low CMC, which means that less biosurfactant is required to decrease the surface tension [11]. 132

133

134

- 135
- 136 137
- 138



смс Figure 2. The relationship between biosurfactant concentration, surface tension and 140 formation of micelles 141

Micelle

Biosurfactant concentration

142

#### 143 Role of Biosurfactants in Biodegradation Processes

145 Biosurfactants can enhance hydrocarbon bioremediation by two mechanisms. The 146 first is the increase of substrate bioavailability for microorganisms, while the other involves interaction with the cell surface which increases the hydrophobicity of the 147 148 surface allowing hydrophobic substrates to associate more easily with bacterial cells [40]. By reducing surface and interfacial tensions, biosurfactants increase the 149 150 surface areas of insoluble compounds leading to increased mobility and 151 bioavailability of hydrocarbons. Consequently, biosurfactants enhance 152 biodegradation and removal of hydrocarbonsfrom contaminated environments. 153 Addition of biosurfactants is expected to enhance hydrocarbon biodegradation by 154 mobilization, solubilization or emulsification [41,42, 43,14].

155

144

156 The mobilization mechanism occurs at concentrations below the biosurfactant CMC. At such concentrations, biosurfactants reduce the surface and interfacial tension 157 between air/water and soil/water systems. Due to the reduction of the interfacial 158 159 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 160 take place above the biosurfactant CMC. At these concentrations biosurfactant 161 162 molecules associate to form micelles, which dramatically increase the solubility of 163 oil. The hydrophobic ends of biosurfactant molecules connect together inside the micelle while the hydrophilic ends are exposed to the aqueous phase on the 164 165 exterior. Consequently, the interior of a micelle creates an environment compatible 166 for hydrophobic organic molecules. The process of incorporation of these molecules 167 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].

172

#### 173 Biosurfactant Enhanced Remediation of Hydrophobic Substances in Soil

174 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 factorial design.

Many physical properties used to characterize surfactants depend on the CMC. 185 emulsion, oil solubilization, foaming and detergency, interfacial and surface 186 tensions. These properties may be used to assess the suitability of surfactant for 187 environmental bioremediation, such as soil washing. They used the Photo 188 189 colorimetric method in their research to determine the cleaning efficiency instead of the generally gravimetric assessment. The experimental tests showed high reliability 190 191 for the assessment of degreasing and therefore are especially suited for exploration 192 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].

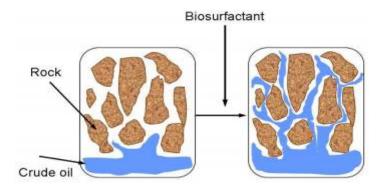
197

#### 198 Other uses of Biosurfactants

Biosurfactants are used in various industries such as food, pharmaceutical and 199 cosmetic, as they are biodegradable and less toxic than the synthetic surfactants 200 currently used [46]. The most important surface-active properties evaluated in 201 202 screening microorganisms for biosurfactants with potential industrial applications are surface tension reduction, emulsion forming and stabilizing capacity. The criterion 203 used for selection of biosurfactant producers is the ability to reduce the surface 204 tension below 40 mN m<sup>-1</sup> [47]. Sophorose lipids produced by certain strains of yeast 205 have been formulated for anti-dandruff solutions, hair gels, deodorant sticks, after-206 207 shave lotions, and hair and body shampoos.

## 208 Microbial Enhanced Oil Recovery (MEOR)

209 Biosurfactants can also be involved in microbial enhanced oil recovery. This 210 method is used to recover oil remaining in reservoirs after primary and secondary 211 recovery procedures [48, 49]. It is an important tertiary recovery process where 212 microorganisms or their metabolites, including biosurfactants, biopolymers, biomass, acids, solvents, gases and also enzymes, are used to increase recovery of 213 oil from depleted reservoirs. Application of biosurfactants in enhanced oil recovery is 214 215 one of the most promising advanced methods to recover a significant proportion of 216 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. 217 Biosurfactants reduce interfacial tension between oil/water and oil/rock. This 218 reduces capillary forces preventing oil from moving through the rock pores (Fig. 3). 219 Surfactants can also bind tightly to the oil-water interface and form emulsion. This 220 stabilizes the desorbed oil in water and allows removal of oil along with the injection 221 222 water [50].



223

224 Source: Pacwa-Plociniczak, et al, 2011

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

226

Tertiary oil recovery experiments showed that biosurfactant solutions with 227 228 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 229 residual oil present in Berea sandstone cores. When PHPA was used alone, about 230 231 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 232 233 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 234 recovered at biosurfactant concentrations below the CMC (about 10 mg/l). Below 235

this concentration, the IFT values were high. At biosurfactant concentrations 236 237 between 10 and 40 mg/l, the IFT was 1 mN/m. When the CMC is greater than 40 mg/I, IFT decreased to around 0.1 mN/m. At biosurfactant concentrations above the 238 CMC, residual oil recovery is a linear function of biosurfactant concentration[50]. 239 240 241 242 **Biosurfactants and Metals Remediation** 243 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 244 245 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 246 247 clean up soils contaminated with heavy metals. Remediation of these soils includes non-biological methods such as excavation and disposal of contaminated soil to 248 landfill sites or biological techniques[51, 52]. 249 250 251 A promising approach seems to be the application of inoculants of biosurfactant 252 producing bacteria in phytoremediation of hydrocarbon polluted soil to improve the efficiency of this technology. Application of the biosurfactants in phytoremediation 253 254 on a large scale requires studies to identify their potential toxic effect on plants. Although the biosurfactants are thought to be ecofriendly, some experiments 255 indicated that under certain circumstances they can be toxic to the environment[38]. 256 Nevertheless, careful and controlled use of these interesting surface active 257 258 molecules will surely help in the enhanced cleanup of the toxic environmental 259 pollutants and provide us with a clean environment [2]. 260 261 Application of Biosurfactant Technology on Contaminated Soils 262 **Remediation of heavy metals pollution** 263 Hong et al. [54] examined the removal of cadmium and zinc, by saponin, (a plant-264 265 derived biosurfactant) from three types of soils - AndosolCambisol, and Regosolsoils. Rates of removal of 90-100% for cadmium and 85-98% for zinc were 266 267 obtained. Saponin concentrations of 3% were optimal for metal removal within 6 268 hours and maximal removal was obtained from Regosol soil. 269 Rhamnolipidproduced by Pseudomonas bacteria which has a strong affinity for 270 271 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 272 273 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. 274 275 Removal effciency for the biosurfactant foam was 73.2% of Cd and 68.1% of Ni 276 while removal efficiency for the biosurfactant liquid solution was 61.7% Cd and 277 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 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.

284

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.

290 291

292

## 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[58].

299

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].

306

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.

315

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 [61]. The
 rhamnolipidssorbed onto the solids, thus increasing the amount of solid phase
 PAHs.

323

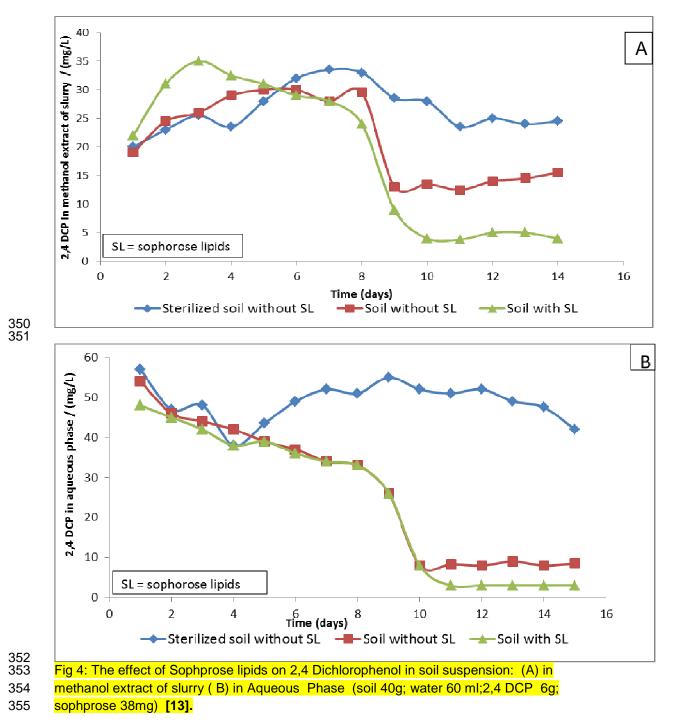
The study of sophorolipids effects on phenanthrene biodegradation showed that the 324 325 concentration of phenanthrene with initial concentration of 80 mg/L within 36 hour decreased to 0.5 mg/L in the presence of 500 mg/L of the surfactant compared to 326 327 2.3 mg/L without surfactant in a 10% soil suspension [62]. The maximal degradation 328 by Sphingomonasyanoikuyae was 1.3 mg/L/h with the sophorolipid instead of 0.8 329 mg/L/h. The sophorolipids seem to enhance the phenanthrene concentration as shown by fluorescence measurements instead of increasing biomass concentration. 330 In addition, toxicity of the sophorolipid was low for concentrations up to 1 g/L. The 331 CMC of the sophorolipid in water was 4 mg/L but this increased to 10 mg/L in the 332 presence of 10% soil suspensions indicating adsorption of the surfactant onto the 333 soil. 334

335

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.

349



356 Advantages of bioremediation

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

360		contaminant is present; when the contaminant is degraded, the
361		biodegradative population declines. The residues for the treatment are
362		usually harmless products such as carbon dioxide, water and cell biomass.
363	2.	Bioremediation is useful for the complete destruction of a wide variety of
364		contaminants. Many compounds that are hazardous can be transformed to
365		harmless products.
366	3.	Instead of transferring contaminants from one environment to another for
367		example land to water or air, the complete destruction of the target pollutants
368		is possible.

369
 369
 370
 370
 369
 and the second s

## 371 **Disadvantages of bioremediation**

- Bioremediation is limited to those compounds that are biodegradable. Not all
   compounds are susceptible to rapid and complete degradation.
- 374
   2. There are some concerns that the products of biodegradation may be more
   375 toxic than the parent compound.
- 376
   378
   378
   378
   379
   379
   380
   380
   376
   377
   378
   379
   379
   380
   380
   380
   370
   371
   372
   373
   374
   375
   375
   376
   376
   376
   377
   378
   379
   379
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
   380
- 381
  381
  4. Bioremediation often takes longer than other treatment options, such as exc382
  avation and removal of soil or incineration.
- 383 5. Prolonged exposure of skin to biosurfactants can cause chafing because
   384 surfactants (like soaps) disrupt the lipid coating that protects the skin and
   385 other cells.

## 386 CONCLUSION

387 Biosurfactants have shown their potential for remediation of contaminated soils by increasing 388 biodegradation rate and reducing contaminant minimum concentration.

Soil and water that are contaminated with organic and inorganic pollutants can be effectivelytreated with biosurfactants.

Bioremediation is a biological process that degrades or breaks down contaminants from soiland water naturally with the help of bacteria, fungi and plants.

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.

- Bioremediation is often a longer treatment option and prolonged exposure of biosurfactants
   to skin can be very harmful.
- 398 Biosurfactants have several applications in various industries such as agriculture, petroleum
- 399 sector microbial enhanced oil recovery, medicine, pharmaceutical and cosmetic industries.

## 400 ACKNOWLEDGMENTS

401

The Authors wish to thank the staff of Biochemistry department, University of Port
 Harcourt and the staff of Petroleum Engineering department, University of Ibadan
 for their support during the preparation of this paper.

## 406 **REFERENCES**

407

405

- Xia, H and Yan, Z.: 2010 Effects of biosurfactant on the remediation of contaminated soils. *College of Environmental Science and Engineering,* Zhejiang Gongshang University Hangzhou, China. *IEEE*
- Pacwa-Plociniczak, M., Plaza, G. A., Piotrowska-Seget, Z. andCameotra, S. S.
  Environmental Applications of Biosurfactants: Recent Advances. *Int. Journ. Mol. Sci.* 2011, 12, 633-654.
- And S. Nester, Eugene W., Denise G. Anderson, C. Evans Roberts Jr., Nancy N. Pearsall, and Martha T. Nester. 2001. Microbiology: A Human Perspective. 3rd Ed. New York: McGraw-Hill.
- 417 4. State of Mississippi.Department of Environmental Quality. 1998. Fundamental 418 Bioremediation. April 1998. 27 Nov Principles of 2006 <http://www.deg.state.ms.us/MDEQ.nsf/pdf/GARD Bioremediation/\$File/Bioremedia 419 420 -tion.pdf? Verified 12/15/2006.
- 4215.Kumar, A., Bisht, B.S, Joshi, V.D, Dhewa, T.: Review on Bioremediation of Polluted422Environment:A423Tool. International Journal of Environ. Sciences Volume 1 No.6, 2011 pp1079-1093.
- 424
  425
  426
  427
  428
  428
  429
  429
  429
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
  420
- 426 7. Desai, J.D., Banat, I.M.. Microbial production of surfactants and their commercial 427 potential. *Microbiol. Mol. Biol. R.* 199761: 47–64
- 428 8. Christofi, N., and Ivshina, I.B. Microbial surfactants and their use in field studies of soil remediation. *Journal of Applied Microbiology*.2002.93(6):(915).
- 430 9. Haigh, S. D. A review of the interaction of surfactants with organic contaminants in soil. *Sci. Total Environ*.1996. 185: 161-170.
- Perfumo, A., Smyth, T. J. P., Marchant, R. and Banat, I.M. 2010. 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
- 436 11. Volkering, F., Breure, A. M., and Rulkens W. H. Microbiological aspects of 437 surfactant use for biological soil remediation. *Biodegradation*.19988(6):401-17.
- 438 12. Kosaric, N..Biosurfactants in industry. *Pure Appl. Chem.*, 199264: 1731–1737.
- 439 13. Kosaric, N.:.Biosurfactants and Their Application for Soil Bioremediation *Food*440 *Technol. Biotechnol.*2001 39 (4) 295–304.
- Rahman, K.S.M., Rahman, T.J., Kourkoutas, Y., Petsas, I., Marchant, R. and Banat,
  I.M. Enhanced bioremediation of *n*-alkane petroleum sludge using bacterial
  consortium amended with rhamnolipid and micronutrients. *Bioresour. Technol.*,200390: 159–168.
- 445 15. Das, K.; Mukherjee, A.K. Comparison of lipopeptidebiosurfactants production by
  446 *Bacillus subtilis*strains in submerged and solid state fermentation systems using a
  447 cheap carbon source: some industrial applications of biosurfactants. *Process*448 *Biochem.*,200742: 1191–1199.

- 449 16. Das, P.; Mukherjee, S.; Sen, R.Improved bioavailability and biodegradation of a
  450 model polyaromatic hydrocarbon by a biosurfactant producing bacterium of marine
  451 origin. *Chemosphere*200872 1229–1234.
- 452 17. Rosenberg, E. and Ron, E.Z..High- and low-molecular-mass microbial surfactants.
  453 *Appl. Microbiol. Biotechnol.*,199952: 154–162.
- 454 18. Calvo, C.; Manzanera, M.; Silva-Castro, G.A.; Uad, I.; González-López,
  455 J.Application of bioemulsifiers in soil oil bioremediation processes. Future prospects.
  456 Sci. Total Environ.2009407: 3634–3640.
- 457 19. Groboillot, A., Portet-Koltalo, F., Derf, F.L, Feuilloley, MG. Orange, N
  458 DuclairoirPoc,, CD. Novel Application of CyclolipopeptideAmphisin: Feasibility Study
  459 as Additive to Remediate Polycyclic Aromatic Hydrocarbon (PAH) Contaminated
  460 Sediments. Int J Mol Sci. 2011; 12(3): 1787–1806.
- 461 20. Bognolo, G., Biosurfactants as emulsifying agents for hydrocarbons. Colloids Surf. A
  462 Physicochem. Eng. Asp., 1999 152: 41-52.
- 463 21. Kitamoto, D., H. Yanagishita, T. Shinbo, T. Makane, C. Kamisawa and T. Nakahara,
  464 Surface active properties and antimicrobial activities of mannosylerythritol lipids as
  465 biosurfactant produced by *Candida antractica*. J. Biotechnol., 1993.29: 91-96.
- 466 22. Singer, M.E., W.R. Finnerty and A. Tunelid, Physical and chemical properties of a biosurfactant synthesized by *Rhodococcus* species H13A. Can. J. Microbiol., 199036: 746-750.
- 469 23. Sifour, M. Al-Jilawi, M. H, Aziz, GM. Emulsification Properties of Biosurfactant
  470 produced from *Pseudomonas aeruginosa*RB 28 *Pak, J. Biol. Sci.* 2007, 10, 1331471 1335.
- Whang, L. M., Liu, P.W.G., Ma, C.C. and Cheng, S.S. Application of biosurfactant,
  rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated
  water and soil. *J. Hazard. Mater.*2008 151: 155–163.
- 475 25. Herman, D.C. Artiola, J. F.; Miller, R. M. Removal of Cadmium, lead, and zinc from
  476 soil by a rhamnolipidBiosurfactant*Environ. Sci. Technol.* 1995, 29, 2280-2285.
- 477 26. Maier, R.M. Soberon-Chavez, G. *Pseudomonas aeruginosa*rhamnolipids;
  478 Biosythesis and potential applications *Appl. Microbiol. Biotechnol.* 2000, 54, 625479 633.
- 480 27. Franzetti, A. Gandolfi, I. Bestetti, G. Smyth, T. J. Banat, I.M. Production and 481 applications of trehalose lipid biosurfactants Eur. Journ. Lipid. Sci. Tech. 2010, 112, 482 617-627.
- 483 28. Pesce, L. A. Biotechnological method for the regeneration of hydrocarbons from
  484 dregs and muds on the base of biosurfactants. World Patent 02/062,,2002495.
- 485 29. Baviere, M. Degouy, D. Lecourtier, J. Process for washing solid particles comprising
  486 a sophoroside solution U. S. Patent 5, 326, 406, 1994.
- 487 30. Gerson, O.F. Zajic, J.E. Surfactant production from hydrocarbon
  488 byCorynebacteriumlepus, sp.nov.andPseudomonas asphaltenicus, spnov. Dev. Ind.
  489 Microbiol. 1978, 19, 577-599.
- 490 *31.* Ishigami, Y. Yamazaki, S. Gama, Y. Surface active properties of biosoap from
  491 spiculisporic acid *J. ColliodInterf. Sci.* 1983, 94, 131-139.
- 492 32. Ishigami, Y. Zhang, Y. Ji, F. Spiculisporic acid. Functional development of
  493 biosurfactantsChimOggi, 2000, 18, 32-34.
- 494 33. Hong, J.J. Yang, S.M. Lee, C.H. Choi, Y.K. Kajiuchi, T. Ultrafiltration of divalent
  495 metal cations from aqueous solution using polycarboxylic acid type biosurfactants J.
  496 Colloid Interf. Sci. 1998, 202, 63-73.

- 497 34. Appanna, V.D. Finn, H. Pierre, M. St. ExocellularPhosphatidylethanolamine
  498 production and multiple-metal tolerance in Pseudomonas flourescens. FEMS
  499 Microbiol. Lett. 1995. 131, 53-56.
- Jennema, G.E. McInerney, M.J. Knapp, R.M. Clark, J.B. Feerp, J.M. Revus, D. E.
  Menzie, D.E. A halotolerantbiosurfactants-producing Bacillus species potentially useful for enhanced oil recovery Dev. Ind. Microbiol. 1983, 24, 485-492.
- 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.
- 50637.Arima K Kakinuma, A. Tamura, G. Surfactin a crystalline peptide lipid surfactant507produced by Bacillus subtillis isolation characterization and its inhibition of fibrin clot508formation Biochem. Biophys, Res. Commun. 1968, 31, 488-494.
- 50938.Thomas, C. P., Duvall, ML.Robertson, E.P. Barrett, K.B. Bala, G.A. Surfactant-510based EOR mediated by naturally occurring microorganism. 1993, 11, 285-291.
- 51139.Soberón-Chávez, G.; Maier, R.M. 2011. Biosurfactants: a General Overview. In512Biosurfactants; Soberón-Chávez, G., Ed.; Springer-Verlag: Berlin, Germany, pp. 1–51311.
- 514 40. Mulligan, C. N. and Gibbs, B. F. 2004. Types, production and applications of 515 biosurfactants. *Proc. Indian Nat. Sci. Acad.*, 1: 31–55.
- 516 41. Nguyen, T. T., Youssef, N. H., McInerney, M. J. and Sabatini, D. A. 2008.
  517 Rhamnolipidbiosurfactant mixtures for environmental remediation. *Water Res.*, 42:
  518 1735–1743.
- 519 42. Déziel, E., Paquette, G., Villemur, R., Lépine, F., Bisaillon, J.G. 1996. Biosurfactant
  520 production by a soil *Pseudomonas* strain growing on polycyclic aromatic
  521 hydrocarbons. *Appl. Environ. Microbiol.*62: 1908–1912
- 43. Bai, G.Y., Brusseau, M.L., and Miller, R.M. 1997. Biosurfactant enhanced removal of residual hydrocarbon from soil. *J. Cont. Hydrol.*25: 157–170.
- 44. Urum, K.Pekdemir, T. 2004. Evaluation of biosurfactants for crude oil contaminated soil washing. *Chemosphere*, 57: 1139–1150.
- 526 45. Timma, L, Sams, K., Valtere, S., Vilgerts, J. and Blumberga, D. (2014) Full Factorial
  527 Design of Screening Experiments for Biosurfactant Enhanced Remediation of
  528 Hydrophobic Substances in Soil Journal of Clean Energy Technologies, 2(1) pg 52529 56.
- 530 46. Tabatabaee, A., M..Mazaheri, Noohi A.A. and Sajadian, V.A. 2005. Isolation of
  531 biosurfactant producing bacteria from oil reservoirs. *Iran. J. Environ. Health Sci.*532 *Eng.*, 2: 6-12.
- 533 47. Banat, I, M, Makkar, R. S. Cameotra, S. S. Potential commercial applications of 534 microbial surfactants *Appl. Environ. Microbiol*2000, 53, 495-508.
- 535 48. Sen, R. Biotechnology in Petroleum recovery: the microbial EOR. *Prog. Energ.*536 *Combust.* 2008, 34, 714-724.
- 53749.Suthar, H; Hingurao, K.; Desai, A. Nerurkar, A. Evaluation of bioemulsifier mediated538microbial enhanced oil recovery using sand pack column. J. Microbiol. Methods5392008, 75, 225-230.
- 540 50. McInerney, M. J. Maudgalya, S. K. Knapp, R. and Folmsbee, M. Development of
  541 Biosurfactant-mediated oil recovery in Model Porous Systems and Computer
  542 Simulations of Biosurfactant-Mediated Oil Recovery A Topical Report of the
  543 Department of Petroleum Engineering and Department of Botany and Microbiology
  544 University of Oklahoma 2004.

- 545 51. Hubálek, T. Vosáhlová, S.Matějů, V., Kováčová, N., Novotný, C.: Ecotoxicity
  546 Monitoring of Hydrocarbon-Contaminated Soil During Bioremediation: A Case Study,
  547 Archives of Environmental Contamination and Toxicology January 2007, Volume 52,
  548 Issue 1, pp 1-7.
- 549 52. Asci, Y. Nurbas, M Acikel, Y. S. Investigation of sorption/desorption equilibria of heavy metal ions on/from quartz using rhamnolipidbiosurfactant. *J. Environ. Manage.* 2010 91, 724-731
- 552 53. Shin, K. Kim, J. and Kim, K.: Effect of Biosurfactant Addition on the Biodegradation 553 of Phenanthrene in Soil-water System, *Environ. Eng. Res.* Vol. 13, No. 1, pp. 8-13, 554 2008.
- 555 54. Hong, K.J., Tokunaga, S. and Kajiuchi, T "Evaluation of remediation process with plant-derived biosurfactant for recovery of heavy metals from contaminated soils,"
  557 Chemosphere, vol. 49 (4), pp. 379–387, 2002.
- 558 55. Mulligan, C.N., and Wang, S. "Remediation of a heavy metal contaminated soil by a
  rhamnolipid foam," In: Yangt, R.N.,Thomas, H.R. (Eds.), Geoenvironmental
  Engineering. Integrated Management of groundwater and contaminated land.
  Thomas Telford Pub., London,UK, pp. 544–551, 2004.
- 562 56. Juwarkar, A. A. Nair, A., Dubey, K. V., Singh, S. K. and Devotta, S., Biosurfactant 563 technology for remediation of cadmium and lead contaminated soils, Chemosphere, 564 vol. 68, pp.1996–2002, 2007.
- 565 57. Mulligan, C. N. Yong, C.N., and Gibbs, B.F., Heavy metal removal from sediments 566 by biosurfactants, J. Hazard Mat, vol.85, pp.111–125, 2001.
- 567 58. Mulligan, C.N., and Eftekhari, F. "Remediation with surfactant foam of PCPcontaminated soil," Engineering Geology, vol. 70, pp. 269–279, 2003.
- 569 59. Kommalapati, R.R,Valsaraj, KT., Constant, W.D., and Roy, **D.**"Aqueous solubility 570 enhancement and desorption of hexachlorobenzene from soil using a plant-based 571 surfactant," Wat. Res. vol. 31 (9), pp. 2161-2170, 1997.
- 572 60. Lai, C. C.,Huanga, Y.C., Wei, Y.H., and Chang, J.S. "Biosurfactant enhanced 573 removal of total petroleum hydrocarbons from contaminated soil" Journal of 574 Hazardous Materials, vol. 167, pp. 609– 614, 2009.
- 575 61. Burd, G. and Ward, O. P. "Bacterial degradation of polycyclic aromatic hydrocarbons on agar plates: the role of biosurfactants," Biotechnology Techniques, vol. 10, pp. 371–374, 1996.
- 578 62. Schippers, C. Gebner, K. Muller, T.Scheper, T. "Microbial degradation of phenanthrene by addition of a sophorolipid mixture," Journal of Biotechnology, vol. 83, pp. 189–198, 2000.
- 581 582

## 583 ABBREVIATIONS

- 584
- 585 **CMC** Critical Micelle Concentration
- 586 **DCP** Dichlorophenol
- 587 **HCB**Hexachlorobenzene
- 588 **HLB** Hydrophilic-Lipophilic Balance
- 589 **HOC** Hydrophobic Organic Compounds
- 590 **PAH** Polycyclic aromatic Hydrocarbon
- 591 **PCP** Pentachlorophenol
- 592 **PHPA**Hydrolyzed Polyacrylamide
- 593 **TPH** Total Petroleum Hydrocarbon