Review Article

Potentials for Bio surfactant 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 bio surfactants in remedying contaminated soils and water. Biosurfactants are surfactants produced by bacterial strains 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, Bio surfactants, Contamination, Hydrophobic Organic Compounds

INTRODUCTION

The rate at which microbial cells can convert contaminants during bioremediation depends on the rate of contaminant uptake and metabolism and the rate of transfer to the cell (mass transfer). Increased microbial conversion capacities do not lead to higher biotransformation rates when mass transfer is a limiting factor [1]. This appears to be the case in most contaminated soil and water. Bioavailability of a contaminant is controlled by a number of physico-chemical processes such as sorption and desorption, diffusion, and dissolution. A reduced bioavailability of contaminants in soil is caused by the slow mass transfer to the degrading microbes. Contaminants become unavailable when the rate of mass transfer is zero. The decrease of the bioavailability in the course of time is often referred to as aging or weathering. These bioavailability problems can be overcome by the use of food-grade surfactants [1], which increase the availability of contaminants for microbial degradation.

Surfactants are amphiphilic surface active agents possessing both hydrophilic and hydrophobic moieties that reduce surface and interfacial tensions by accumulating at the interface between two immiscible fluids like oil and water, signifying that surfactants assist the solubility of polar compounds in organic solvents [2]. 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 [3]. 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 [4]. 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 [5]. 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 [3]. The reduction of interfacial tension is particularly relevant when the pollutant is present in soil as a non-aqueous phase liquid. The main surfactant-mediated mechanisms which may potentially enhance hydrophobic organic compound remediation include the reduction of interfacial tension, micellar solubilization and phase transfer between soil particles and the pseudo-aqueous phase.

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 [6]. An important descriptor of chemico-physical properties of surfactants is related to the balance between their hydrophilic and hydrophobic moieties.

Surfactants are of synthetic or biological origin. Due to their properties such as lower toxicity, higher degree of biodegradability, higher foaming capacity and optimal activity at extreme conditions of temperatures, pH levels and salinity, these have increasingly attracted the attention of the scientific and industrial community [2].

Bio surfactants are a group of structurally diverse molecules produced by different microorganisms classified mainly by their chemical structure and microbial origin. Structurally, they contain a hydrophilic moiety, comprising an acid, peptide cations, or anions, mono-, di- or polysaccharides and a hydrophobic moiety of unsaturated or saturated hydrocarbon chains or fatty acids. They are mainly classified into two classes: low-molecular weight surface active agents called bio surfactants (lipopeptide, glycolipids) and bio emulsifiers (high molecular weight surface active agents). They efficiently reduce surface and interfacial tensions [7,8]. Bio surfactants are further divided into six classes: hydroxylated and cross linked fatty acids (mycolic acids), glycolipids, lipopolysaccharides, lipoproteins-lipopeptides, phospholipids and the complete cell surface itself.

All bio surfactants are amphiphiles, they consist of two parts—a polar (hydrophilic) moiety and 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 [9]. A characteristic feature of bio surfactants is a Hydrophilic-Lipophilic Balance (HLB) which specifies the portion of hydrophilic and hydrophobic constituents in surface-active substances. Due to their amphiphilic structure, bio surfactants increase the surface area of hydrophobic water-insoluble substances, 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 [10].

They have many advantages when their chemically synthesized equivalents are compared. They are environmentally friendly, biodegradable, less toxic and non-hazardous. 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 bio surfactants possible and allows utilization of waste substrates and reduction of their polluting effect at the same time [11,12,13,14,15].

Classification and Properties of Bio surfactants

Unlike chemically synthesized surfactants, which are classified according to their dissociation pattern in water, bio surfactants are categorized by their chemical composition, molecular weight, physico-chemical properties and mode of action and microbial origin. Based on molecular weight they are divided into low-molecular-mass bio surfactants which include glycolipids, phospholipids and lipopeptides and into high-molecular-mass bio surfactants/bio emulsifiers containing amphipathic polysaccharides, proteins, lipopolysaccharides, lipoproteins or complex mixtures of these biopolymers. Low-molecular-mass bio surfactants are efficient in lowering surface and interfacial tensions, whereas high-molecular-mass bio surfactants are more effective at stabilizing oil-in-water emulsions [16,17]. The bio surfactants 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 (Figure 1) [18].

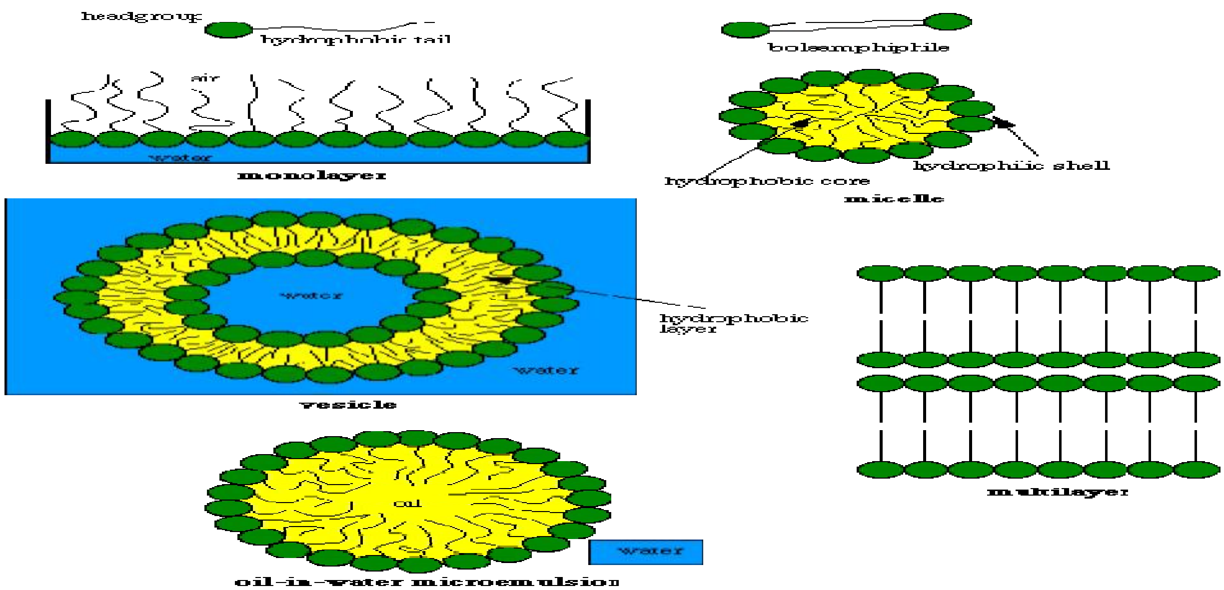

Figure 1. Accumulation of bio surfactants at the interface between liquid and air

Bio surfactant activities depend on the concentration of the surface-active compounds until the critical micelle concentration is obtained. At concentrations above the CMC, bio surfactant molecules associate to form micelles, bilayers and vesicles (Figure 2). Micelle formation enables bio surfactants to reduce the surface and interfacial tension and increase the solubility and bioavailability of hydrophobic organic compounds [19]. The CMC is commonly used to measure the efficiency of surfactant. Efficient bio surfactants have a low CMC, which means that less bio surfactant is required to decrease the surface tension [10].

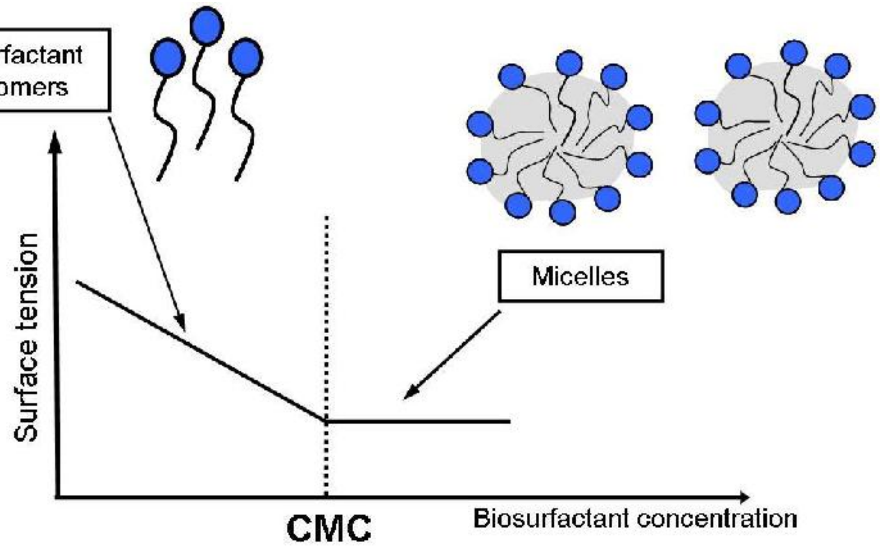

Figure 2. The relationship between bio surfactant concentration, surface tension and formation of micelles

Role of Bio surfactants in Biodegradation Processes

A promising method that can improve bioremediation effectiveness of hydrocarbon contaminated environments is the use of bio surfactants. They can enhance hydrocarbon bioremediation by two mechanisms. The first includes 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 [20]. By reducing surface and interfacial tensions, bio surfactants increase the surface areas of insoluble compounds leading to increased mobility and bioavailability of hydrocarbons. Consequently, bio surfactants enhance biodegradation and removal of hydrocarbons. Addition of bio surfactants is expected to enhance hydrocarbon biodegradation by mobilization, solubilization or emulsification [21,22,23,13,24,25].

The mobilization mechanism occurs at concentrations below the bio surfactant CMC. At such concentrations, bio surfactants reduce the surface and interfacial tension between air/water and soil/water systems. Due to the reduction of the interfacial force, contact of bio surfactants with soil/oil system increases the contact angle and reduces the capillary force holding oil and soil together. And this causes solubilization to take place above the bio surfactant Critical micelle concentration. At these concentrations bio surfactant molecules associate to form micelles, which dramatically increase the solubility of oil. The hydrophobic ends of bio surfactant 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 [24].

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.

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. [26] Timma *et al* evaluated the cleaning efficiency of glycolipid-based anionic bio surfactant with a pH value of 10 critical micelle concentration of 0.1 %, surface tension of 27 mN/m and the hydrophilic-lipophilic balance of 10. Before practical application to remove oil or other hydrophobic substances from soil, the behavior of the bio surfactant across different systems was examined. Process variables, temperature of environment, contact time with dilution of bio surfactant, and concentration of bio surfactant 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 [26].

Response variable and cleaning efficiency were obtained experimentally by a set of laboratory tests. The cleaning efficiency showed various results depending on the initial values of variables. At the upper limit of variables (+35 ºC temperatures of environment, 15 minutes contact time with dilution of bio surfactant, and 0.3 wt% concentration of bio surfactant in washing solution), the cleaning efficiency was 99.32 %. The results of the work of Timma *et al.,*[26] showed that for all variables, there were significant effects on the cleaning efficiency with a confidence level of 95 percent.

CONCLUSION

The use of bio surfactants as an additive in bioremediation applications to soil and groundwater contaminated by insoluble organic pollutants can potentially increase the biodegradation rate and reduce 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 degrading microorganisms.

Bio surfactants 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.

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REFERENCES

1. Boopathy, R., Manning, J. 1998. A laboratory study of the bioremediation of 2,4,6-trinitrotoluene-contaminated soil using aerobic anaerobic soil slurry reactor. *Water Environ. Res.* **70**: 80-86.

2. Saharan B.S., Sahu R.K. and Sharma D. 2011. A Review on Biosurfactants: Fermentation, Current Developments and Perspectives. *Genetic Engineering and Biotechnology Journal GEBJ*-29. http://astonjournals.com/gebj
3. Christofi, N., and Ivshina, I.B. 2002. Microbial surfactants and their use in field studies of soil remediation. *Journal of Applied Microbiology*. **93**(6):(915).
4. Haigh, S. D. 1996. A review of the interaction of surfactants with organic contaminants in soil. *Sci. Total Environ*., **185**: 161-170.
5. 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 ed Timmis, K.N. Chapter 47, Volume 2 part 7 pp.1501- 1512, Springer-Verlag, Berlin Heidelberg
6. Volkering, F., Breure, A. M., and Rulkens W. H. 1998. Microbiological aspects of surfactant use for biological soil remediation. *Biodegradation.* 8(6):401-17.
7. Smyth T. J. P., Perfumo A., Marchant R. and Banat I. M. 2010a. Isolation and analysis of low molecular weight microbial glycolipids. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin.
8. Smyth T. J. P., Perfumo A., McClean S., Marchant R., Banat I. M. , 2010b. Isolation and analysis of lipopeptides and high molecular weight biosurfactants. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin.
9. Lang, S. 2002. Biological amphiphiles (microbial biosurfactants). *Curr. Opin. Colloid Inter. Sci.* ***7***: 12–20.
10. Desai, J.D., Banat, I.M. 1997. Microbial production of surfactants and their commercial potential. *Microbiol. Mol. Biol. R.* **61**: 47–64
11. Kosaric, N. 1992. Biosurfactants in industry. *Pure Appl. Chem.*, **64**: 1731–1737.
12. Kosaric, N. 2001. Biosurfactants and their application for soil bioremediation. *Food Technol. Biotechnol.* **39**: 295–304.
13. Rahman, K.S.M., Rahman, T.J., Kourkoutas, Y., Petsas, I., Marchant, R. and Banat, I.M. 2003. Enhanced bioremediation of *n*-alkane petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. *Bioresour. Technol.*, **90**: 159–168.
14. Das, K.; Mukherjee, A.K. 2007. Comparison of lipopeptide biosurfactants production by *Bacillus subtilis* strains in submerged and solid state fermentation systems using a cheap carbon source: some industrial applications of biosurfactants. *Process Biochem.*, **42**: 1191–1199.
15. Das, P.; Mukherjee, S.; Sen, R. 2008. Improved bioavailability and biodegradation of a model polyaromatic hydrocarbon by a biosurfactant producing bacterium of marine origin. *Chemosphere* **72**: 1229–1234.

16. Rosenberg, E. and Ron, E.Z. 1999. High- and low-molecular-mass microbial surfactants. *Appl. Microbiol. Biotechnol.*, **52**: 154–162.
17. Calvo, C.; Manzanera, M.; Silva-Castro, G.A.; Uad, I.; González-López, J. 2009. Application of bioemulsifiers in soil oil bioremediation processes. Future prospects. *Sci. Total Environ.* **407**: 3634–3640.

18. Soberón-Chávez, G.; Maier, R.M. 2011. Biosurfactants: a General Overview. In *Biosurfactants*; Soberón-Chávez, G., Ed.; Springer-Verlag: Berlin, Germany, pp. 1–11.
19. Whang, L. M., Liu, P.W.G., Ma, C.C. and Cheng, S.S. 2008. Application of biosurfactant, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. *J. Hazard. Mater.* **151**: 155–163.
20. Mulligan, C. N. and Gibbs, B. F. 2004. Types, production and applications of biosurfactants. *Proc. Indian Nat. Sci. Acad.*, **1**: 31–55.
21. Nguyen, T. T., Youssef, N. H., McInerney, M. J. and Sabatini, D. A. 2008. Rhamnolipid biosurfactant mixtures for environmental remediation. *Water Res.*, **42**: 1735–1743.
22. Déziel, E., Paquette, G., Villemur, R., Lépine, F., Bisaillon, J.G. 1996. Biosurfactant production by a soil *Pseudomonas* strain growing on polycyclic aromatic hydrocarbons. *Appl. Environ. Microbiol.* **62**: 1908–1912
23. 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.
24. Urum, K. Pekdemir, T. 2004. Evaluation of biosurfactants for crude oil contaminated soil washing. *Chemosphere*, **57**: 1139–1150.
25. Nievas, M.L., Commendatore, M.G., Estevas, J.L., Bucalá, V. 2008. Biodegradation pattern of hydrocarbons from a fuel oil-type complex residue by an emulsifier-producing microbial consortium. *J. Hazard. Mater.* **154**: 96–104.
26. Timma, L, . Sams, K., Valtere, S., Vilgerts, J. and Blumberga, D. (*2014)* Full Factorial Design of Screening Experiments for Biosurfactant Enhanced Remediation of Hydrophobic Substances in Soil *Journal of Clean Energy Technologies, 2(1) pg 52-56.*