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BIOSURFACTANTS ENHANCED REMEDIATION OF CONTAMINATED SOIL

R. JAYASHREE AND R. RIZAM

Department of Environmental Sciences,
Tamil Nadu Agricultural University, Coimbatore 641 003, T.N., India

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Abstract– Biosurfactants are expected to become known as multifunctional materials of the twenty first century as they have applications in different industrial processes as well as potential novel future uses,mostly due to their diverse structures. Microorganisms produce surface active compounds to enhance both the bioavailability of hydrophobic immiscible and mostly inaccessible substrates allowing better survival under low moisture conditions. Biosurfactant production generally requires the presence of miscible hydrophilic and oily/hydrocarbon type carbon source in the culture medium. The process economics and environmental credentials can make it attractive when using waste products as substrates. This mini review aimed to explain the importance of biosurfactants and its application to remediate the heavy metal and oil contaminated soils.

INTRODUCTION

Biosurfactants are a chemically unique class of compounds produced by many bacterial and fungal genera. The molecular structure of biosurfactants comprises of a hydrophilic portion which may consist of mono-, oligo- or polysaccharides, amino acids or peptides or carboxylate or phosphate groups and a hydrophobic portion which is composed of saturated or unsaturated fatty acids or fatty alcohols. Biosurfactants can be classified into several broad groups-glycolipids, lipoaminio acids and lipopeptides, polymers of lipoproteins, lipopolysaccharides, phospolipids, mono and diglycerides, fatty acids and fatty acids/neutral lipids (Mulligan, 2021). In addition, there are species level differences in the chemical structure of biosurfactants. For example, Glycolipid containing rhamnose are called Rhamnolipids and rhamnolipids produced by *Pseudomonas aeruginosa* differ in the member of rhamnose molecules (mono- or dirhamnolipids) (Gaur *et al*., 2019). The different types of biosurfactant produced by microorganisms and structures are presented in Table 1

Properties of Biosurfactants

Biosurfactants are amphiphilic molecules that can modify the properties of a liquid medium at a surface or interface by reducing the surface tension. Biosurfactants reduce surface tension by accumulating at the interface of immiscible fluids and solids, thereby increasing the surface area of insoluble compounds, which leads to increased bioavailability and subsequent biodegradation of hydrocarbons (Kosaric, 2001). The glycolipid produced by *Pseudomonas fluorescens* and the biosurfactant from *Bacillus licheniformis* have been shown to reduce the surface tension of aquous solution to 26-27mN/ m. At low concentration, surfactants are present as individual molecules. However, as the concentration of the surfactant is increased, where no further change in interfacial properties takes place (Das and Kumar, 2018).

The amount of biosurfactant needed to reach the concentration is called the"Critical Micelle Concentration" (CMC). At the CMC, molecules aggregate to form monolater (micelle) or bilayer (vescle and lamella) structures that have the ability to encapsulate hydrocarbon molecule resulting in either solubilisation or emulsification of the hydrocarbons.

Surfactants are amphipathic compounds with both hydrophilic and hydrophobic moieties that preferentially partition at the interface between

(Associate Professor (ENS)

different phases; gas, liquid and solid, and with liquids of different polarities (oil/water and water/oil) and hydrogen bonding. These molecules reduce the surface and interfacial tension, conferring many properties such as detergency, emulsifying, foaming, and dispersing, making them versatile process chemicals (Silva *et al*., 2014).

Biosurfactants are mainly produced by microbial cultures grown on water immiscible substrates, therefore allowing access to these hydrophobic substrates (such as hydrocarbons) and are generally classified into low molecular-mass molecules (lipopeptides, glycolipids) and high molecular-mass polymers (polymeric and particulate surfactants). These molecules offer several advantages over chemical surfactants, such as environmental compatibility, low toxicity, biodegradability, and maintained activity under extreme conditions of temperatures, salinity and pH values (Santos *et al*. 2013; Silva *et al*., 2014). These traits contribute to the relevance of biosurfactants to different industries, especially in the oil industry which has many adverse processes conditions (Silva *et al*. 2014). Most successful biosurfactants applications that managed to reach the market has been mainly driven by economical production process and cost effectiveness. This has been facilitated by the lower purity specifications required for such applications, eliminating the purification downstream processing steps which often represent almost 60.0% of the total production costs (Sarubbo *et al*. 2015). High production cost of biosurfactants has been a major constraining factor that hampers its market growth. Substrate composition accounts for up to 50.0% of the total production costs, the choice of low-cost alternatives therefore is important to the overall economics. Fortunately, biosurfactants can be produced from economical renewable agricultural resources and waste products that can significantly decrease the cost (Rufino *et al*., 2014).

Production and recovery of biosurfactants

Biosurfactants yield and composition are affected growth conditions including carbon sources, culture medium nutrients (N,P and Fe), temperature, pH and aeration (Kosaric, 2001). The carbon source is one of the critical factors affecting the structure and yield of biosurfactant. For example, *Pseudomonas fluorescens* produced an bioemulsifier during the growth on different hydrocarbon substrates and maximum yield was obtained with gasoline as substrate. The biosurfactant production by *Pseudomonas sp.* MR-3 on different carbon sources with maximum yield (6.46g/l) on glucose as sustrate. Nutrients like nitrogen and phosphate etc., could also affect biosurfactant production. For example, biosurfactant production was enhanced when *P.aeruginosa* was grown in nitrate and protease peptone media. The rhamnolipid production by *P.aeruginosa* GLI was stimulated under conditions of nitrogen limitation.

Mechanism of interaction of biosurfactant with microorganism

Biosurfactants enhance the emulsification and solubilization of hydrocarbon substrate and thereby facilitate the growth of microorganisms as hydrocarbons. Biosurfactants produced by microorganism may be cell bound or extracellular, when it is secreted into the growth medium. For example, the *Rhodococcus sp.* produced cell surface associated biosurfactant when grown on

Table 1. Production of different types of biosurfactant by microorganisms

S.No.	Microorganism	Type of biosurfactant
1.	*Pseudomonas aeruginosa P. oleovarans*	Rhamnolipid
2.	*Pseudomonas fluorescens*	Peptidolipid
3.	*Candida lipolytica*	Polysaccharide-protein –lipid complex
4.	*Rhodococcus erythropolis*	Trehalose
5.	*Bacillus licheniformis*	Lipopeptide
6.	*Acinetobacter calcoaceticus*	Emulson (anionic hetero polysaccharide)
7.	*Torulopsis bombicola*	Sophorose lipid
8.	*Acinetobacter sp*	Phospholipid
9.	*Bacillus subtilis*	Surfactin (Lipoprotein)
10.	*Arthrobacter paraffineus*	Sucrose or fructose lipid
11.	*Cornybacterium sp.*	Glycolipid
12.	*Streptospotangium amethytogenes*	Lipopeptide

Banat *et al*., 2000

hydrocarbon *substrates* which exhibited increased cell surface hydrophobicity. The organism that assimilates both solid and liquid alkanes by adhering to the alkane phase. *P. aeruginosa* ATCC 9027 facilitates degradation of hydrocarbons with limited water solubility by producing extracellular rhamnolipids. A mutant of *P. aeruginosa* which lacked extracellular rhamnolipids unable to grow on hexadecane but retained growth upon addition of small amounts of rhamnolipids indicating that rhamnolipids play a major role in hexadecane uptake and utilization by *P. aeruginosa*. A protein like activator is also produced by *P.aeruginosa* and the cooperative action between the activator and rhamnolipid stimulates the growth of the organism on hexadecane. The Rhamnolipid increase the bioavailability by increasing both aqueous dispersion and cell hydrophobicity.

Microbial adhesion to hydrocarbons proceed by many methods. In Acinetobacter *calcoaceticus* RAG-1 it occurs via fimbriae observed two types of adhesion in *Acenetobac tervenetianus* VE-C3. First cell to cell interaction proceeds cell adhesion to n-alkane followed by incorporation of nanodroplets of n-alkanes into the hydropholic capsular polysaccharide to form a more hydrophobic polysaccharide n-alkane matrix surrounding the cell wall. This results in partitioning of the bulk polar phase between the aqueous medium and the outer cell membrane enabling theorganism to grow on diesel oil.

There are 2 mechanisms by which biosurfactants enhance the biodegradation of slightly soluble organic compounds. First, biosurfactants can solubilize hydrophobic compounds in micelle structures, effectively increasing the apparent aqueous of the organic compound and its availability for uptake by a cell. Steps in microbial uptake of hydrocarbons (Miller, 1995)

A- Uptake of hydrocarbons dissolved in the aqueous phase surrounding degradation cells.

B - Uptake via direct contact of degrading cells at the aqueous hydrocarbon

Interface of large oil drops in water.

C- Uptake through direct contact of degrading cells with fine or submicron

Size oil droplets dispersed in the aqueous phase.

D - Enhanced updake as a result of production of biosurfactants.

Second biosurfactants can cause the cell surface to become more hydrophobic thereby bring the association of the cell with the slightly soluble substrate (AI-Tahhan *et al*. 2000).

The mechanism behind biosurfactant-enhanced removal and recovery of oil has been proposed to take place through solubilization, mobilization, or emulsification, increasing the area of contact of hydrocarbons (Santos *et al*., 2016). Solubilization capacity measures a surfactant's ability to increase the solubility of hydrophobic components in an aqueous phase. A significant increase in this capacity occurs when micelles are formed as a result of the partitioning of the hydrocarbon in the hydrophobic part of the micelles. In such a process, higher concentrations of biosurfactants are usually required as hydrocarbon solubility wholly depends on the biosurfactant concentration. Mobilization on the other hand involves both displacement and dispersion. Displacement occurs when hydrocarbon droplets are released from the porous medium as a result of the reduction in interfacial tension. It can also occur when entrapped hydrocarbon undergoes displacement when sufficient reduction of the interfacial tension between the aqueous and oil phases takes overcoming the capillary forces that cause the formation of residual saturation. Displacements therefore are only related to the interfacial tension between aqueous and hydrophobic phases and not emulsion formation. Dispersion in comparison is a process by which hydrocarbons are dispersed into aqueous phases due to emulsions formation and therefore is linked to both the surfactant concentration and interfacial tension (Sarubbo *et al*., 2015; Santos *et al*. 2016).

Application of biosurfactants

Biosurfactants have several advantages over synthetic surfactants such as biosurfactants present surface-active properties differing in some cases from synthetic surfactants, providing new possibilities for industrial applications. Microbial surfactants have been shown to be more effective and specific than many conventional synthetic surfactants in specific applications and they are usually nontoxic and biodegradable.

Recently, most of the studies are focusing on the environmental applications of biosurfactants due to their diverse structure, better physicochemical properties, environment friendly characteristics, suitability for many purposes which include remediation of hydrophobic organic compounds (HOCs) from soil, and removal of heavy metals from contaminated soil. Heavy metals are becoming part of the serious environmental problems. Basically, the most common heavy metals found in contaminated soils are lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), zinc (Zn), copper(Cu), and nickel (Ni) which can create many health issues categorized under inorganic chemical hazards for humans, animals, and plants (Adamuv *et al*., 2015; Hu *et al*., 2017; Li and Qian, 2017; Liu *et al*. 2015; Liu *et al*., 2017; Tang *et al*., 2015). In the past, chemical surfactants had been used to treat heavy metal-contaminated soils and solubilize HOCs. However, the chemical surfactants themselves are known to expose toxic substances and may cause other environmental issues due to its their degradability in the soil (Liu *et al*., 2017; Santos *et al.* 2016). In comparison with chemical surfactants, biosurfactants derived from plants and microorganisms have shown better performance considered suitable in removing heavy metal from contaminated soil (Luna *et al*., 2016; Tang *et al*. 2017; Vijayakumarand Saravanan, 2015). Essentially, there are three main steps involved in the removal of heavy metals from the soil through washing with biosurfactant solution. The heavy metals adsorbed on the surface of soil particles separate through the sorption of biosurfactant molecules at the interfaces between sludge (wet soil) and metal in aqueous solution. Then, the metal will be absorbed by biosurfactants and trapped within the micelle through electrostatic interactions. Finally, the biosurfactant can be recovered through the method of membrane separation

Ni Cd+ + Cd+ Cu+ Zn+ Ni+ Cr+ Cr+ Soil surface Negative charges + OH + H2O + Soil + 2H C O O Me OH O As Hg+ + Hg+ Pb+. Eq. (1) describe the chemical reaction between heavy metal ions and the functional group of biosurfactants. Since soil particles and other organic matters have negative charges on their surfaces, thus, cationic materials can easily be adsorbed to negative charges of the soil surface (Guan *et al*., 2017; Ibrahim *et al*., 2016).

Soil – Men+ + R – (COOH)m + H_2O $\rightarrow$ Soil + R – O – Men+ – (COOH)m + 2H (1) where Men+ represents metal ions and R— (COOH)m is the surfactant molecules. As it can be seen in Eq. (1), surfactants enhanced the extraction of heavy metals from the soil because of the existing carboxylic functional group in biosurfactants which act as organic ligands (Tang *et al*., 2017). The traditional methods of removing heavy metals from contaminated soil such as washing with water, organic and inorganic acids, metal-chelating agents, soil replacement, thermal desorption, and chemical surfactants had been used. However,these methods showed the improperremoval of heavy metals from the soil (Shah *et al*., 2016). Previous studies had reported that the remediation technique using biosurfactants is the best method to eliminate heavy metals from the soil with about 100% efficiency. Guan *et al*. (2017) and Hong *et al*. (2002) studied the efficiency of biosurfactants for removing heavy metals from sludge and soil and achieved the removal rates of 90-100% for Cu, Zn, Cr, and Cd. In addition, natural surfactants are found to be effective in treating contaminated soils with crude oil and diesel (Da *et al*. 2015).

Arab and Mulligan (2020) evaluated the use of sophorolipids for washing mining tailings. Increasing the temperature from 15 to 23%C increased removal of arsenic, copper, and iron, indicating its potential for remediation of mine tailings. In another study, Da Rocha *et al.* (2019) determined that the biosurfactant of *C. tropicalis* was much more effective for Zn and Cu removal than Pb.

The application of surfactants from *Bacillus subtilis 09* on the biodegradation of soils polluted with crude oil was studied by Cubitto *et al*. (2004) reported that *Bacillus subtilis* (09) did not negatively affected the hydrocarbon degrading microbial population and concentration of *Bacillus subtilis* 19 and 19.5 mg stimulated the growth of the population involved in the crude oil degradation and accelerated the biodegradation of the aliphatic hydrocarbons.

The release of surface-active compound promotes an emulsification of the hydrocarbon phase, rendering such lipophilic molecules available to the metabolic pathways of microorganism. The biodegradation of hexadecane by five biosurfactant producing bacterial strains (*Pseudomonas aeruginosa UG2*, *Acinetobacter calcoacticius* RAG 1, *Rhodococcus erythropolis* DSM 43066, R. *erythropolis* ATCC 19538 and strain BCG 112) was determined in the presence and absence of exogeneously added biosurfactants. The degradation of hexadecane by *Pseudomonas aeruginosa UG2* was stimulated only by the rhamnolipid biosurfactant induced by the same organochlorines (Noordman and Janssen, 2002). Different studies had reported that the utilization of microorganisms that can produce biosurfactants are efficiently considered a suitable method for EOR. This method is referred to as microbial-enhanced oil recovery (MEOR) (Banat *et al.,* 2000). In MEOR technique, several microbial species had been applied to generate biosurfactants for oil recovery enhancement such as *Bacillus megaterium*, *Pseudomonas aeruginosa, Bacillus subtilis, Bacillus amyloliquefaciens* Oil reservoir. The rocks surface and porous washed by biosurfactant MEOR Dhanarajan *et al*. 2017; Fernandes *et al*., 2016; Zhao *et al*. 2017b; Zhao *et al*., 2018). The principle behind this technique is that the injection of microbes causes the reduction of oil viscosity and interfacial tension between oil hydrocarbons and surface of rock matrix, which can facilitate the mobilization of oil and further increment of oil recovery (Hosseininoosheri *et al*. 2016). Generally, biosurfactants with high molecular weight are known for their emulsifying properties, therefore they can enhance the heavy oil mobility and oil recovery. However, low-molecular-weight biosurfactants are suitable for reducing surface tension and interfacial tension between oil and water and thus enhancing oil recovery (Banat *et al*. 2010). Basically, there are two ways that microbes can contribute to the generation of biosurfactants for MEOR. They are known as ex-situ and in-situ applications (Geetha *et al*., 2018). In ex-situ applications, the generation of biosurfactants occurs inside a bioreactor through the aerobic fermentation of microbes, and then they can be injected into the oil reservoirs to enhance oil recovery. However, in-situ biosurfactant production is a process where the bacteria and their nutrients inject into the oil reservoir, and the production of biosurfactants happens inside the reservoir which can eventually enhance the oil recovery (Geetha *et al*., 2018). Comparing to ex-situ applications, in-situ biosurfactant production is considered more advantageous for MEOR application due to the low production cost (Cui *et al*., 2017; Youssef *et al*., 2007). To perform a better in-situ application in MEOR, it is necessary to study the properties of microbes suitable for in-situ application under the conditions of oil reservoir such as pressure, temperature, pH, oxygen level, and salinity (Liang *et al*. 2017; Zhao *et al*. 2017a).

CONCLUSION

Biosurfactants are widely known as multi-functional compounds due to their non-harmful properties as compared to the synthetic surfactants. The environmental applications of biosurfactants due to their diverse structure, better physicochemical properties, environmentfriendly characteristics, suitability for many purposes which include remediation of hydrophobic organic compounds (HOCs) from soil, and removal of heavy metals from contaminated soil.Extensive investigation on large scale production of biosurfactant from low-cost substrates were needed to reduce the cost of production and research on the studies of large-scale production of these natural compounds from the novel bacterial stains.

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