IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 14, Issue 10 Ser. IV (October 2020), PP 30-42
www.iosrjournals.org

Application of Biosurfactants in Environmental Remediation

Unaeze, Chizoba Henrietta
Environmental Biotechnology and Bioconservation Department, National Biotechnology Development Agency, Nigeria

Abstract
Background: *Human activities have instigated widespread pollution of the natural global environment. Recalcitrant wastes persist for long periods, are trans-boundary pollutants, travelling large distances from their point of origin in the environment and bioaccumulate in humans and other organism through the food chain. Therefore, environmental remediation is necessary for polluted land to produce safe crops and reduce environment risks. Biosurfactants increase the bioavailability of hydrocarbon resulting in enhanced growth and degradation of contaminants by hydrocarbon-degrading bacteria present in polluted soil. Hence, the presence of surfactants may increase microbial degradation of pollutants. In comparison to their chemically synthesized equivalents they have many advantages.*
Methododology: *This paper reviews various approaches in applying biosurfactants in removing recalcitrant wastes from the environment. They are environmentally friendly, biodegradable, less toxic and non-hazardous.*
Conclusion: *Application of biosurfactant and biosurfactant-producing bacteria in environmental technologies (bioremediation and phytoremediation) has been studied. Both organic and inorganic contaminants can be removed through different processes (physico-chemical and biological) in which biosurfactants are involved. Due to their biodegradability and low toxicity, they are very promising for use in environmental biotechnologies. The commercial success of biosurfactants is still limited by their high production cost.*
Keywords: *Biosurfactants, Wastes, Remediation, Bioavailability, Environment*

Date of Submission: 28-10-2020 Date of Acceptance: 08-11-2020

I. Introduction

With rapid industrial and economic development, human activities have instigated widespread pollution of the natural global environment. Anthropogenic organic pollutants are now dispersed throughout the environment and can be highly recalcitrant to degradation processes. . Recalcitrant wastes persist for long periods, are transboundary pollutants, travelling large distances from their point of origin in the environment and bioaccumulate in humans and other organism through the food chain. Therefore, environmental remediation is necessary for polluted land to produce safe crops and reduce environment risks. (Whang, *et al.* 2008)

Environmental remediation involves providing a remedy for an environmental problem. This can include the removal of pollution or contaminants from environmental media such as soil, groundwater, sediment, or surface water for the general protection of human health and the environment. Depending on the type of damage that is done, this can be a complex and expensive process. Microbes characterized by wide catabolic capabilities, are known to degrade contaminants.

Biosurfactants are amphiphilic compounds produced on living surfaces, mostly microbial cell surfaces, or excreted extracellularly and contain hydrophobic and hydrophilic moieties that reduce surface tension and interfacial tensions between individual molecules at the surface and interface, respectively. Although the type and amount of the microbial surfactants produced depend primarily on the producer organism, factors like carbon and nitrogen, trace elements, temperature, and aeration also affect their production by the organism.

Many properties of microbial surface active compounds such as emulsification/de-emulsification, dispersion, foaming, wetting and coating make them useful in physico-chemical and biological remediation technologies of both organic and metal contaminants. Hydrophobic pollutants present in petroleum hydrocarbons, and soil and water environment require solubilization before being degraded by microbial cells. Surfactants can increase the surface area of hydrophobic materials, such as pesticides in soil and water environment, thereby increasing their water solubility. Biosurfactants increase the bioavailability of hydrocarbon resulting in enhanced growth and degradation of contaminants by hydrocarbon-degrading bacteria present in polluted soil. Hence, the presence of surfactants may increase microbial degradation of pollutants. In heavy-metal polluted soils biosurfactants form complexes with metals at the soil interface, which is followed by desorption of the metal and removal from the soil surface leading to the increase of metal ions concentration and their bioavailability in the soil solution. In comparison to their chemically synthesized equivalents they have many advantages. 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 as well, and can be produced from industrial wastes and from by-products. This last feature makes cheap production of biosurfactants possible and allows utilizing waste substrates and reducing their polluting effect at the same time. (Franzetti *et al.*, 2010).

The 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.

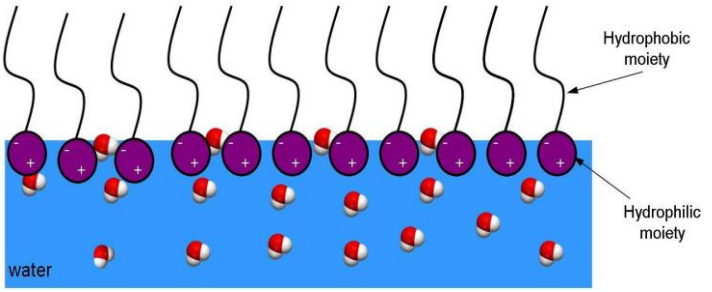

Fig.1: Accumulation of biosurfactants at the interface between liquid and air.

Biosurfactant activities depend on the concentration of the surface-active compounds until the critical micelle concentration (CMC) is obtained. At concentrations above the CMC, biosurfactant molecules associate to form micelles, bilayers and vesicles. Micelle formation enables biosurfactants to reduce the surface and interfacial tension and increase the solubility and bioavailability of hydrophobic organic compounds. The CMC is commonly used to measure the efficiency of surfactant. Efficient biosurfactants have a low CMC, which means that less biosurfactant is required to decrease the surface tension. Micelle formation has a significant role in microemulsion formation. Microemulsions are clear and stable liquid mixtures of water and oil domains separated by monolayer or aggregates of biosurfactants. Microemulsions are formed when one liquid phase is dispersed as droplets in another liquid phase, for example oil dispersed in water (direct microemulsion) or water dispersed in oil (reversed microemulsion).

Biosurfactants also influence the bacterial cell surface hydrophobicity (CSH). This ability has been reported by Al-Tahhan *et al.* (2013) who studied chemical and structural modifications in the CSH of *Pseudomonas aeruginosa* by a rhamnolipid in the presence of hexadecane. Results of their study demonstrated that rhamnolipid, at very low concentration, caused release of lipopolysaccharide (LPS) from the outer membrane resulting in an increase of cell surface hydrophobicity. In contrast, Sotirova *et al.* reported that rhamnolipid at the concentrations below CMC did not affect the LPS component of the bacterial outer membrane but instead changed the composition of outer membrane proteins (OMP). However, all of the changes in the structure of the bacterial cell surface cause increase of accessibility of hydrocarbons to microbial cells.

1.1 Microbiology

Microorganisms utilize a variety of organic compounds as the source of carbon and energy for their growth. When the carbon source is an insoluble substrate like a hydrocarbon (C_xH_y), microorganisms facilitate their diffusion into the cell by producing a variety of substances, the biosurfactants. Some bacteria and yeasts excrete ionic surfactants which emulsify the C_xH_y substrate in the growth medium. Some examples of this group of BS are rhamnolipids which are produced by different *Pseudomonas* sp., or the sophorolipids which are produced by several *Torulopsis* sp. Some other microorganisms are capable of changing the structure of their cell wall, which they achieve by synthesizing lipopolysaccharides or nonionic surfactants in their cell wall. Examples of this group are: *Candida lipolytica* and *C. tropicalis* which produce cell wall-bound lipopolysaccharides when growing on *n*-alkanes; and *Rhodococcus erythropolis,* and many *Mycobacterium* sp. and *Arthrobacter* sp. which synthesize nonionic trehalose corynomycolates. There are lipopolysaccharides, such as Emulsan, synthesized by *Acinetobacter* sp., and lipoproteins or lipopeptides, such as Surfactin and Subtilisin, produced by *Bacillus subtilis*.

II. Classification and chemical nature of biosurfactants

The microbial surfactants (MS) are complex molecules covering a wide range of chemical types including peptides, fatty acids, phospholipids, glycolipids, antibiotics, lipopeptides, etc. Unlike Chemically synthesized surfactants which are usually classified according to the nature of their polar grouping (cation, anion and non-polar type), biosurfactants 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 biosurfactants including glycolipids, phospholipids and lipopeptides and into high-molecular-mass biosurfactants/bioemulsifiers containing amphipathic polysaccharides, proteins, lipopolysaccharides, lipoproteins or complex mixtures of these biopolymers. 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. The yield of MS varies with the nutritional environment of the growing microorganism. Intact microbial cells that have high cell surface hydrophobicity are themselves surfactants. In some cases, surfactants themselves play a natural role in growth of microbial cells on water-insoluble sub-strates like C_xH_y, sulphur, etc. Exocellular surfactants are involved in cell adhesion, emulsification, dispersion, flocculation, cell aggregation, and desorption phenomena. A very brief description of each group is given below.

2.1 Glycolipids

Glycolipids are the most common types of BS. The constituent mono-, di-, tri- and tetrasaccharides include glucose, mannose, galactose, glucuronic acid, rhamnose, and galactose sulphate. The fatty acid component usually has a composition similar to that of the phospholipids of the same microorganism. The glycolipids can be categorized as:

Trehalose lipids: The serpentine growth seen in many members of the genus *Mycobacterium* is due to the presence of trehalose esters on the cell surface. Cord factors from different species of *Mycobacteria*, *Corynebacteria*, *Nocardia*, and *Brevibacteria* differ in size and structure of the mycolic acid esters.

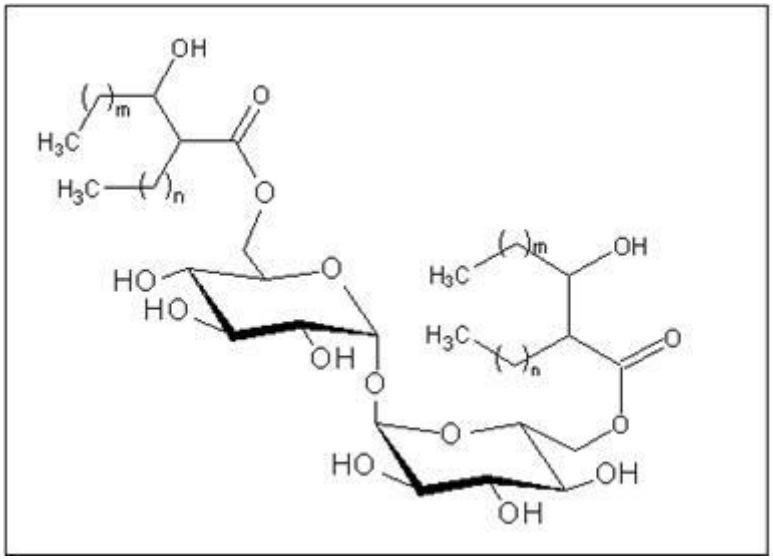

Fig.2: Structure of nonionic trehalose-dicorynomycolates from Rhodococcus erythropolis DSM 43215. n + m = 27 to 30 (Rapp *et al.*, 1979).

Sophorolipids: These are produced by different strains of the yeast, *Torulopsis*. The sugar unit is the disaccharide sophorose which consists of two b -1,2-linked glucose units. The 6 and 6¢ hydroxy groups are generally acetylated. The sophorolipids reduce surface tensions between individual molecules at the surface, although they are effective emulsifying agents. The sophorolipids of *Torulopsis* have been reported to stimulate, inhibit, and have no effect on growth of yeast on water-insoluble substrates.

Rhamnolipids: Some *Pseudomonas* sp. produce large quantities of a glycolipid consisting of two molecules of rhamnose and two molecules of b -hydroxydecanoic acid. While the OH group of one of the acids is involved in glycosidic linkage with the reducing end of the rhamnose disaccharide, the OH group of the second acids is involved in ester formation. Since one of the carboxylic acid is free, the rhamnolipids are anions above pH 4.0. Rhamnolipids are reported to lower surface tension, emulsify C_xH_y, and stimulate growth of *Pseudomonas* on *n*-hexadecane. Formation of rhamnolipids by *Pseudomonas* sp. MVB was greatly increased by nitrogen limitations. The pure rhamnolipid lowered the interfacial tension against *n*-hexadecane in water to about 1 mN/m and had a critical micellar concentration (cmc) of 10 to 30 mg/l depending on the pH and salt conditions. Like sophorolipids, microbially synthesized rhamnolipids are a mixture of a number of analogous molecules. The four most important ones comprise either one or two rhamnose molecules attached to one or two β-hydroxydecanoic acids (Syldatk *et al*., 2005). The structure below gives the structure of a rhamnolipid with two rhamnose and two β-hydroxydecanoic acid units.

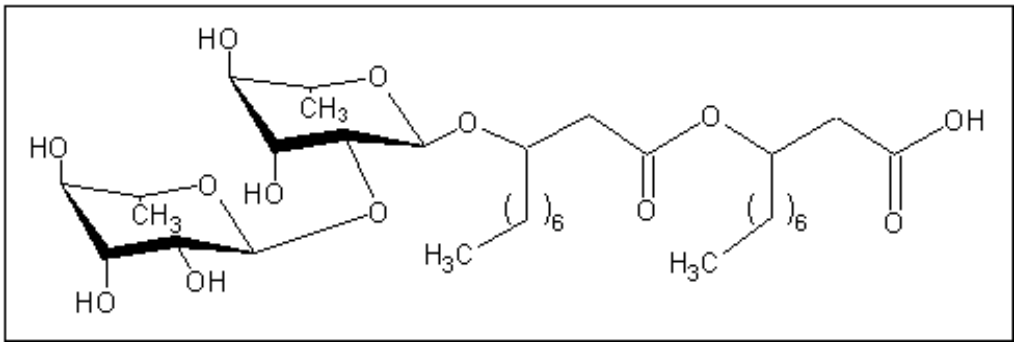

Fig.3: Structure of *rhamnolipid*

The fatty acids produced from alkanes by microbial oxidations have received maximum attention as surfactants. Besides the straight-chain acids, microorganisms produce complex fatty acids containing OH groups and alkyl branches. Some of these complex acids, for example corynomucolic acids, are surfactants.

Mannosylerythritol lipids (MEL) are synthesized by the yeast Pseudozyma antarctica as a mixture of four components; MEL-A and MEL-B as major ones, MEL-C and MEL-D as minor byproducts. The backbone of those molecules is a mannose-erythritol disaccharide on which short (2 to 8 carbon atoms) or long (10 to 18 carbon atoms) fatty acid chains are acetylated (Kitamota *et al*.,2010).

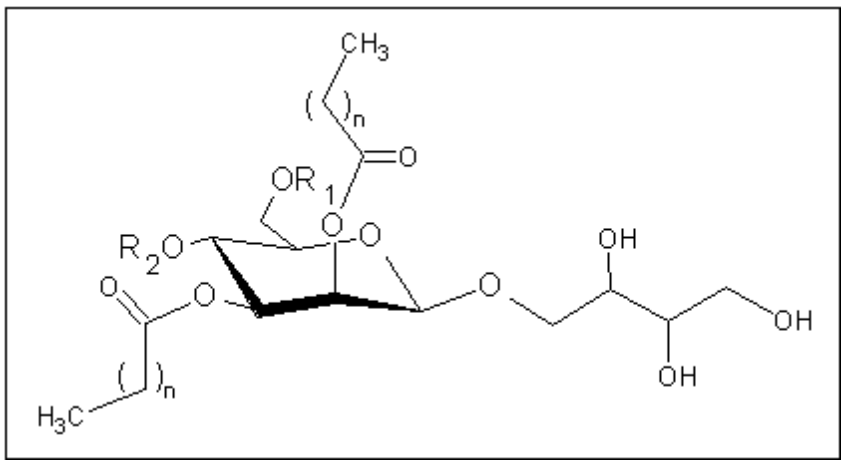

Fig.4: Structure of *Mannosylerythritol lipids*

MEL's can bring down the surface tension of water to 35 mN/m (Lang, 2002) and show versatile biochemical actions, including protein binding toward immunoglobulin G (Im et al., 2003) and lectin (Konishi *et al*., 2007), as well as induction of differentiation with respect to different mammalian cells (Wakamatsu *et al.*, 2001). Because of this interesting biochemical behaviour, the pharmaceutical and medical sectors show al lot of interest in MEL's.

Phospholipids: These are major components of microbial membranes. When certain C_xH_y-degrading bacteria or yeast are grown on alkane substrates, the level of phospholipids increases greatly. Phospholipids from hexadecane-grown *Acinetobacter* sp. have potent surfactant properties. Phospholipids produced by *Thiobacillus thiooxidans* have been reported to be responsible for wetting elemental sulphur, which is necessary for growth.

2.2 Surface active antibiotics

Gramicidin S: Many bacteria produce a cyclosymmetric decapeptide antibiotic, gramicidin S. Spore preparations of *Brevibacterium brevis* contain large amounts of gramicidin S bound strongly to the outer surface of the spores. Mutants lacking gramicidin S germinate rapidly and do not have a lipophilic surface. The antibacterial activity of gramicidin S is due to its high surface activity.

Polymixins: These are a group of antibiotics produced by *Brevibacterium polymyxa* and related bacilli. Polymixin B is a decapeptide in which amino acids 3 through 10 form a cyclic octapeptide. A branched chain fatty acid is connected to the terminal 2,4-diaminobutyric acid (DAB). Polymixins are able to solubilize certain membrane enzymes.

Surfactin (subtilysin): One of the most active biosurfactants produced by *B. subtilis* is a cyclic lipopeptide surfactin. The yield of surfactin produced by *B. subtilis* can be improved to around 0.8 g/l by continuously removing the surfactant by foam fractionation and addition of either iron or manganese salts to the growth medium.

Antibiotic TA: *Myxococcus xanthus* produces antibiotic TA which inhibits peptidoglycan synthesis by interfering with polymerization of the lipid disaccharide pentapeptide. Antibiotic TA has interesting chemotherapeutic applications.

III. Applications Of Biosurfactants

3.1 BIOREMEDIATION

Biosurfactants reduce the surface and interfacial tensions in both water solutions and hydrocarbon mixtures, which makes them potential candidates for Biodegradation, enhancing oil recovery and deemulsification processes (Banat *et al.*, 2010).

Bioremediation is the use of microorganism metabolism to remove pollutants. It involves the chemical dissolution of materials by bacteria or other biological means. Technologies can be generally classified as *in situ* or *ex situ*. *In situ* bioremediation involves treating the contaminated material at the site, while *ex situ* involves the removal of the contaminated material to be treated elsewhere. The term is often used in relation to ecology, waste management, biomedicine, and the natural environment (bioremediation) and is now commonly associated with environmentally friendly products that are capable of decomposing back into natural elements. Organic material can be degraded aerobically with oxygen, or anaerobically, without oxygen. Biosurfactant enhances the biodegradation process.

Bioremediation can occur on its own (natural attenuation or intrinsic bioremediation) or can be spurred on via the addition of fertilizers to increase the bioavailability within the medium (biostimulation). Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants.

3.1.1 Mycoremediation

Mycoremediation is a form of bioremediation in which fungi are used to decontaminate the area. The term *mycoremediation* refers specifically to the use of fungal mycelia in bioremediation.

One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fiber. These are organic compounds composed of long chains of carbon and hydrogen, structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutant. Certain strains have been reported to successfully degrade the nerve gases VX and sarin.

In one conducted experiment, a plot of soil contaminated with diesel oil was inoculated with mycelia of oyster mushrooms; traditional bioremediation techniques (bacteria) were used on control plots. After four weeks, more than 95% of many of the PAH (polycyclic aromatic hydrocarbons) had been reduced to non-toxic components in the mycelial-inoculated plots. It appears that the natural microbial community participates with the fungi to break down contaminants, eventually into carbon dioxide and water. Wood-degrading fungi are particularly effective in breaking down aromatic pollutants (toxic components of petroleum), as well as chlorinated compounds (certain persistent pesticides; Battelle, 2000).

Mycofiltration is a similar process, using fungal mycelia to filter toxic waste and microorganisms from water in soil.

3.1.2 Role of Biosurfactants in Biodegradation Processes

A promising method that can improve bioremediation effectiveness of hydrocarbon contaminated environments is the use of biosurfactants. 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. By reducing surface and interfacial tensions, biosurfactants increase the surface areas of insoluble compounds leading to increased mobility and bioavailability of hydrocarbons. In consequence, biosurfactants enhance biodegradation and removal of hydrocarbons. Addition of biosurfactants can be expected to enhance hydrocarbon biodegradation by mobilization, solubilization or emulsification.

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. In turn, above the biosurfactant CMC

the solubilization process takes place. 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.

3.1.3 Types of bioremediation

There are three types of bioremediation and all are used to remove toxic substances and contaminants from the environment whether they are rivers or crude oils.

3.1.3.1 Biostimulation

Biostimulation is the method in which bacteria are motivated to start the process of bioremediation. In this method, first the experts release nutrients and other important substances in the soil where there is need or removing the contaminants. These are in the form of gas or liquid. It increases the growth of microbes in that area. As a result bacteria and other microorganisms remove the contaminants quickly and efficiently.

3.1.3.2 Bioaugmentation:-

In some processes of bioremediation, there are some special sites where microorganisms are needed to remove the contaminants for example municipal wastewater. Bioaugmentation is used for that purpose. But unfortunately this process is not very successful as it is difficult to control the growth of microorganisms to remove the particular contaminant.

3.1.3.3 Intrinsic bioremediation

The process of intrinsic bioremediation takes place in soil and water because these two places are always full of contaminants and toxins. This process is also called as natural attenuation. It also means use of the microorganisms to remove the harmful substances from soil and water. Especially those sites are treated with this method, which are underground, for example underground petroleum tanks. It is difficult to know if there is a leakage in the petroleum pipes. Contaminants and toxins find their way to enter in these sites and create harmful effects on the petrol. Therefore, only microorganisms can destroy the toxins and clean the tanks. Great care should be taken if some leakage occurs in the petroleum tanks or pipes because it may damage the human health.

3.2 Biosurfactants and hydrocarbon degradation

The extensive production and use of hydrocarbons has resulted in widespread environmental contamination by these chemicals. Due to their toxicity, persistent and negative influence on living organisms, it is important to clean-up the polluted sites. Hydrocarbons, as the hydrophobic organic chemicals, exhibit limited solubility in groundwater and tend to partition to the soil matrix. This partitioning can account for as much as 90–95% or more of the total contaminant mass. As a consequence, the hydrocarbon contaminants exhibit moderate to poor recovery by physico-chemical treatments; limited bioavailability to microorganisms; and limited availability to oxidative and reductive chemicals when applied to *in-situ* and/or *ex-situ* applications.

3.2.1 Hydrocarbon degradation in the soil environment

C_xH_y degradation in soil has been extensively studied. Degradation is dependent on presence in soil of hydrocarbon-degrading species of microorganisms, hydrocarbon composition, oxygen availability, water, temperature, pH, and inorganic nutrients. The physical state of C_xH_y can also affect biodegradation. Addition of synthetic surfactants or MS resulted in increased mobility and solubility of C_xH_y, which is essential for effective microbial degradation.

Use of MS in C_xH_y degradation has produced variable results. In the work of Lindley and Heydeman, the fungus *Cladosporium resiuae*, grown on alkane mixtures, produced extracellular fatty acids and phospholipids, mainly dodecanoic acid and phosphatidylcholine. Supplement of the growth medium with phosphatidylcholine enhanced the alkane degradation rate by 30%. Foght *et al.* reported that the emulsifier, Emulsan, stimulated aromatic mineralization by pure bacterial cultures, but inhibited the degradation process when mixed cultures were used. Oberbremer and Muller-Harting used mixed soil population to assess C_xH_y degradation in model oil. Naphthalene was utilized in the first phase of C_xH_y degradation; other oil components were degraded during the second phase after the surfactants produced by concerned microorganisms lowered the interfacial tension. Addition of biosurfactants, such as some sophorolipids, increased both the extent of degradation and final biomass yield.

Biodetox (Germany) described a process to decontaminate soils, industrial sludges, and waste waters. They also described *in situ* bioreclamation of contaminated surface, deep ground and ground water. Microorganisms were added by means of a biodetox foam that contained bacteria, nutrients and surfactants; and was biodegradable. Another method to remove oil contaminants is to add BS into contaminated soil to increase

C_xH_y mobility. The emulsified C_xH_y could then be recovered by using a production well, and subsequently degrading above ground in a bioreactor. *In situ* washing of soil was studied using two synthetic surfactants, Adsee 799 and Hyonic NP-90. Removal of PCBs and petroleum C_xH_y from soil by adding surfactants to the wash water, has met with some success.

MS can also be used to enhance solubilization of toxic organic chemicals including xenobiotics. Berg *et al.* using the surfactant from *Pseudomonas aeruginosa*, reported an increase in the solubility of hexachlorobiphenyl added to soil slurries, which resulted in a 31% recovery of the compound in the aqueous phase. This was about 3-times higher than that solubilized by the chemical surfactant sodium ligninsulfonate (9.3%). When the *P. aeruginosa* bioemulsifier and sodium ligninsulphonate were used together, additive effect on solubilization (41.5%) was observed. *Pseudomonas ceparia* AC 1100 produced an emulsifier that formed a stable suspension with 2,4,5-T, and also exhibited some emulsifying activity against chlorophenols. Thus, this emulsifier can be used to enhance bacterial degradation of organochlorine compounds.

3.2.3 Hydrocarbon degradation in aquatic environment

When oil is spilled in aquatic environment, the lighter hydrocarbon components volatilize while the polar hydrocarbon components dissolve in water. However, because of low solubility (< 1 ppm) of oil, most of the oil components will remain on the water surface. The primary means of hydrocarbon removal are photooxidation, evaporation, and microbial degradation. Since C_xH_y-degrading organisms are present in seawater, biodegradation may be one of the most efficient methods of removing pollutants. Surfactants enhance degradation by dispersing and emulsifying hydrocarbons. Microorganisms that are able to degrade C_xH_y have been isolated from aquatic environment. These microorganisms which exhibit emulsifying activity as well as the soil microorganisms which produced surfactants may be useful in aquatic environment. Chakrabarty reported that an emulsifier produced by *P. aeruginosa* SB30 was able to quickly disperse oil into fine droplets; therefore it may be useful in removing oil from contaminated beaches. BS produced by oil-degrading bacteria may be useful in cleaning oil tanks. When an oil tanker compartment containing oily ballast water was supplemented with urea and K_2HPO_4 and aerated for 4 days, the tanker was completely free of the thick layer of sludge that remained in the control tanker. Presumably this was owing to the surfactant produced, when growth of the natural bacterial population was enhanced.

Surfactants have been studied for their use in reducing viscosity of heavy oils, thereby facilitating recovery, transportation, and pipelining. Emulsan, a high MW lipopolysacharide produced by *A. calcaoceticus* RAG-1, has been proposed for a number of applications in the petroleum industry such as to clean oil and sludge from barges and tanks, reduce viscosity of heavy oils, enhance oil recovery, and stabilize water-in-oil emulsions in fuels. Specific solubilization of various C_xH_y types during growth of prokaryotic organism was demonstrated by Reddy *et al.* The specific solubilization of C_xH_y was strongly inhibited by EDTA which was overcome by excess Ca^{++}. It was concluded that specific solubilization of C_xH_y is an important mechanism in the microbial uptake of C_xH_y.

3.3 Pesticide-specific biosurfactants

Due to biodegradative property of biosurfactants, they are ideally suited for environmental applications, specially for removal of the pesticides—an important step in bioremediation. Survey of the literature reveals that application of biosurfactants in the field of pesticides is still in its infancy compared to the field of hydrocarbons. In India, a number of laboratories have initiated studies on Biosurfactants. Some of the earlier works are by: (i) Banarjee *et al.* on 2,4,5-tricholoacetic acid, (ii) Patel and Gopinath on Fenthion, and (iii) Anu Appaiah and Karanth on alpha HCH.

3.4 Soil Washing Technology

Soil washing technology is characterized by chemico-physical properties of the biosurfactant and not by their effect on metabolic activities or changes in cell-surface properties of bacteria. However, the processes may enhance the bioavailability for bioremediation. Aqueous solutions of biosurfactants can be also used to release compounds characterized by low solubility from soil and other media in process called washing.

Urum *et al.* (2014) investigated the efficiency of different surfactant solutions in removing crude oil from contaminated soil using a soil washing process. They demonstrated higher crude oil elimination by synthetic surfactant-sodium dodecyl sulfate (SDS) and rhamnolipid biosurfactants (46% and 44%, respectively) than natural surfactants—saponins (27%).

Kang *et al.* (2010) analyzed application of sophorolipid, Tween 80/60/20 and Span 20/80/85 as possible soil washing agents to release 2-methylnapthalene from artificially polluted soil. They observed that sophorolipid had a higher soil washing efficiency that any other tested nonionic surfactants except Tween 80. This could be caused by high hydrophilic-lipophilic balance (HLB) of Tween 80. It appeared that surfactants with a higher HLB resulted in better solubility of 2-methylnapthalene.

Lai *et al.* (2012) studied the ability of removing total petroleum hydrocarbon (TPH) from soil by two biosurfactants: rhamnolipid and surfactin, and two synthetic surfactants: Tween 80 and Triton X-100. The TPH removal efficiency was examined for low TPH-contaminated (LTC) and high TPH-contaminated (HTC) soils (containing 3000 and 9000 $mg \cdot kg^{-1}$ dry soil of TPH, respectively) by washing them with (bio) surfactant solutions. As a result, they observed that addition of 0.2 mass% of rhamnolipid, surfactin, Triton X-100 and Tween 80 to LTC soil resulted in a TPH removal of 23%, 14%, 6% and 4%, respectively, while for HTC soil a significantly higher TPH removal efficiency of 63%, 62%, 40% and 35%, respectively, was observed. These results indicated that among four (bio) surfactants, rhamnolipid and surfactin showed superior performance on TPH removal, compared to synthetic surfactants. The two biosurfactants examined in this work have the potential to be used as biostimulation agents for bioremediation of oil-polluted soils.

Franzetti *et al.* (2009) evaluated the application of surface active compounds produced by *Gordonia* sp. strain BS29 in soil remediation technologies: bioremediation of soils contaminated by aliphatic and aromatic hydrocarbons (microcosm bioremediation experiment), and washing of soils contaminated by crude oil, PAHs, and heavy metals (batch experiment). The work represents the first study on the potential applications of surface-active compounds produced by *Gordonia* sp. in environmental remediation techniques for contaminated soils. In the previous work, surface-active compounds produced by *Gordonia* sp. and their role in the access to hydrocarbons were characterized. The bacterial strain grew on aliphatic hydrocarbons and produced two different types of surface active compounds: extracellular bioemulsan and cell-bound biosurfactant. Bioremediation results showed that the bioemulsans produced by *Gordonia* sp. strain BS29 were able to slightly enhance the biodegradation of recalcitrant branched hydrocarbons. On the other hand, the authors obtained the best results in soil washing of hydrocarbons. The mean of the crude oil removal for bioemulsans was 33%. The study presented by Franzetti *et al.* showed that the BS29 bioemulsans from *Gordonia* sp. are promising washing agents for remediation of hydrocarbon-contaminated soils. The BS29 bioemulsans were also able to remove metals (Cu, Cd, Pb, Zn, Ni), but their potential in the process was lower than rhamnolipids.

3.5 Clean-up Combined Technology

The aim of the research work reported by Kildisas and Baskys (2004) was to develop inexpensive and efficient combined (complex) technology for cleaning up the soil contaminated by oil pollutants in a large scale. The described technology was based on bioremediation or phytoremediation principles and used physical-chemical treatment by washing the contaminated soil. The complex technology consisted of two stages: at the first stage, the migrating fraction of pollutants was separated from soil using biosurfactants; at the second stage, the remaining not migrating fraction was rendered harmless using biodegradation. Phytoremediation was also applied to enhance soil quality. The completed clean up complex technology is presented by Kildisas *et al.* (2004). The presented technology consisted of washing of the migration fraction by application of biosurfactants, separation of water, oil and soil, biodegradation of residual non-migrating oil fraction by use of specific bacteria with potential to degrade the crude oil and oil products, and phytoremediation. The pilot plant for washing the contaminated soil was designed and constructed in a space of 340 m^2 in which 1000 m^3 of contaminated soil was cleaned up. In the beginning of the pilot experiment the concentrations of the oil pollutants were between 180– 270 $g \cdot kg^{-1}$ of soil, and after washing the concentrations were reduced to 34–59 $g \cdot kg^{-1}$ of soil. After degradation, the pollutant concentrations dropped to 3.2–7.3 $g \cdot kg^{-1}$ of soil.

3.6 Microbial Enhanced Oil Recovery (MEOR)

An area of considerable potential for BS application is microbial enhanced oil recovery (MEOR). Microbial enhanced oil recovery (MEOR) methods are used to recover oil remaining in reservoirs after primary (mechanical) and secondary (physical) recovery procedures. It is an important tertiary 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 the 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 the capillary forces preventing oil from moving through rock pores. Biosurfactants 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.

In MEOR, microorganisms in reservoir are stimulated to produce polymers and surfactants which aid MEOR by lowering interfacial tension at the oil–rock interface. To produce MS *in situ*, microorganisms in the reservoir are usually provided with low-cost substrates, such as molasses and inorganic nutrients, to promote growth and surfactant production. To be useful for MEOR *in situ*, bacteria must be able to grow under extreme conditions encountered in oil reservoirs such as high temperature, pressure, salinity, and low oxygen level.

Several aerobic and anaerobic thermophiles tolerant of pressure and moderate salinity have been isolated which are able to mobilize crude oil in the laboratory.

Bordoloi and Konwar (2011) investigated the recovery of crude oil from a saturated column under laboratory conditions. Laboratory studies on MEOR typically utilize core substrates and columns containing the desired substrate, usually sand. This substrate is used to demonstrate the usefulness of biosurfactants in recovery of oil from reservoirs. For this purpose, a glass column is packed with dry sand, then the column is saturated with crude oil and aqueous solution of biosurfactant is poured in the column. The potential of biosurfactants in MEOR is estimated by measuring the amount of oil released from the column after pouring the aqueous solution of biosurfactant in the column. The experiment was carried out in room temperature, 70 and 90 °C to evaluate the influence of temperature on biosurfactant-induced oil recovery. Biosurfactants used in the experiment were produced by bacterial isolates of *P. aeruginosa* strains (MTCC7815, MTCC7814, MTCC7812 and MTCC8165). Biosurfactants of MTCC7815, MTCC7812 and MTCC8165 strains recovered about 49–54% of crude oil from the sand packed column at room temperature; 52–57% at 70 °C and 58–62% at 90 °C. The biosurfactant produced by MTCC7814 was reported to be less efficient. In control samples treated with culture medium, very little recovery of crude oil was obtained.

Jinfeng *et al.* (2005) evaluated the technical feasibility and effectiveness of improving oil recovery by microbial enhanced water-flooding techniques in high temperature petroleum reservoirs. The studies were conducted in Guan 69 Unit in Dagang Oilfield in China by injection of a mixture of *Arthrobacter* sp. (A02), *Pseudomonas* sp. (P15) and *Bacillus* sp. (B24) strain suspension and the nutrient solution through injection wells in an ongoing waterflood reservoir where the temperature reached 73 °C. The pattern of injection "nutrient-suspension-nutrient" was designed based on the knowledge of the reservoir conditions and the mechanism of enhancement of oil recovery by the selected strains in the reservoir. The oil production performance in the unit was periodically monitored before, during and after microbial water-flooding and then compared. Jinfeng *et al.* (2005) observed that the oil production steadily increased after microbial water-flooding. The oil production in the unit before and in the beginning phase of the injection decreased from 55 t/day in January 2000 to 30 t/day in September 2001, which implies a decline rate of 21%. This situation changed markedly six month later and by the end of the July 2004, about 8700 t of additional oil was obtained compared with the predicted oil production. All the seven production wells showed a positive response to the treatment, of which five wells evidently increased in oil production.

Pornsunthorntawee *et al.* (2008) compared the oil recovery activities of the biosurfactants produced by *Bacillus subtilis* PT2 and *Pseudomonas aeruginosa* SP4 with three synthetic surfactants: polyoxyethylene sorbitan monooleate (Tween 80), sodium dodecyl benzene sulfonate (SDBS) and sodium alkyl polypropylene oxidebsulfate (Alfoterra). For this purpose, sand-packed column inoculated with a motor oil complex was used. The surfactant solutions were poured onto the packed column to test their ability to enhanced oil recovery. The results showed that the biosurfactants produced by *Bacillus subtilis* PT2 and *Pseudomonas aeruginosa* SP4 were more efficient in oil recovery, removing about 62% and 57%, respectively, of the tested oil. The biosurfactants produced by *Bacillus subtilis* PT2 could recover oil more effectively than that produced by *Pseudomonas aeruginosa* SP4. In the case of tested synthetic surfactants, the oil recovery was found to be approximately 53–55%.

3.7 BIOSURFACTANTS AND METALS REMEDIATION

Contamination of soil environments with heavy metals is very hazardous for human and other living organisms in the ecosystem. Due to their extremely toxic nature, presence of even low concentrations of heavy metals in the soils has been found to have serious consequences. Nowadays, there are many techniques used to clean up soils contaminated with heavy metals. Biological methods are processes that use plants (phytoremedation) or microorganisms (bioremediation) to remove metals from soil. Application of microorganisms was discovered many years ago to help in reduction of metal contamination. Heavy metals are not biodegradable; they can only be transferred from one chemical state to another, which changes their mobility and toxicity. Microorganisms can influence metals in several ways. Some forms of metals can be transformed either by redox processes or by alkylation. Metals can also be accumulated by microorganisms by metabolism-independent (passive) or by intracellular, metabolism-dependent (active) uptake. Microorganisms can influence metal mobility indirectly by affecting pH or by producing or releasing substances which change mobility of the metals.

The following methods, "soil washing" or "soil flushing", are involved in remediation of metal contaminated soil. The first technique used is *ex situ*—contaminated soil is excavated, put into the glass column and washed with biosurfactant solution. In turn, soil flushing of *in situ* technologies involves use of drain pipes and trenches for introducing and collecting biosurfactant solution to and from the soil. Interestingly, biosurfactants can be used for metal removal from the soil. Biosurfactants can be applied to a small part of contaminated soil in which soil is put in a huge cement mixer, biosurfactant-metal complex is flushed out, soil

deposited back, and biosurfactant-metal complex treated to precipitate out biosurfactant, leaving behind the metal. The bond formed between the positively charged metal and the negatively charged surfactant is so strong that flushing water through soil removes the surfactant metal complex from the soil matrix.

3.7.1 Mechanism of the Process

Using biosurfactants have unquestionable advantages because bacterial strains able to produce surface active compounds do not need to have survival ability in heavy metal-contaminated soil. However, using biosurfactants alone requires continuous addition of new portions of these compounds. The usefulness of biosurfactants for bioremediation of heavy metal contaminated soil is mainly based on their ability to form complexes with metals. The anionic biosurfactants create complexes with metals in a nonionic form by ionic bonds. These bonds are stronger than the metal's bonds with the soil and metal-biosurfactant complexes are desorbed from the soil matrix to the soil solution due to the lowering of the interfacial tension. The cationic biosurfactants can replace the same charged metal ions by competition for some but not all negatively charged surfaces (ion exchange). Metal ions can be removed from soil surfaces also by the biosurfactant micelles. The polar head groups of micelles can bind metals which mobilize the metals in water.

3.7.2 Applications of the Process

Biosurfactants which are used in bioremediation of metal-contaminated soils have been proposed for use in metal removal in recent years. High potential of biosurfactants in mobilization and decontamination of heavy metal contaminated soil was confirmed by Juwarkar *et al.* who used di-rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* BS2 for mobilization of metals from multi-metal contaminated soil. To study the feasibility of di-rhamnolipid to remove chromium, lead, cadmium and copper from soil, a column study was conducted. Heavy metal spiked soil into a glass column was washed with 0.1% di-rhamnolipid biosurfactant solution. The results indicated that di-rhamnolipid selectively removed heavy metals from soil.

In turn, Das *et al.* (2012) investigated the possibility of using the biosurfactant produced by marine bacterium for removal of heavy metals from solutions. The positive role of marine biosurfactant in the remediation of polyaromatic hydrocarbons was reported earlier, however there was no information about the role of this biosurfactant in heavy metal remediation. The study revealed that tested anionic biosurfactant was able to bind the metal ions and the percentage removal of Pb and Cd metals varied with the different concentrations of metals and biosurfactants. The ability of biosurfactant of marine origin to chelate toxic heavy metals and form an insoluble precipitate could be useful in treatment of heavy metal containing wastewater.

Removal of heavy metals from sediments could be enhanced by use of solution containing biosurfactant and inorganic compounds. For example, Dahrazma and Mulligan reported the higher rate of removal of copper and nickel from sediments by adding 1% NaOH to the solution of rhamnolipid. Many metals mostly exist in the environment organic fraction, adding OH^- to the sediment solubilizes this fraction, and thus, more metals are available for removal by a rhamnolipid biosurfactant.

Another effective method for the remediation of heavy metals contaminated soil is biosurfactant foam technology. Wang and Mulligan evaluated the feasibility of using rhamnolipid foam to remove Cd and Ni from a sandy soil. They reported that the use of foam had a significant effect on the mobility of biosurfactant flowing in a porous medium and made a more uniform and efficient contact of biosurfactant with the metals. Application of rhamnolipid foam increases efficiency and allows removal of 73.2% and 68.1% of Cd and Ni, respectively, whereas the rhamnolipid solution flushed only 61.7% and 51% of Cd and Ni, respectively. The system used for the experiment is presented schematically by Wang and Mulligan.

The rate of heavy metal removal from soil strongly depends on its chemical composition. The predominant constituent of the sand and silt fraction in many soils is quartz, thus quartz was chosen for the bioremediation experiment. Aşçi *et al.* (2010) studied recovery of the metal ions from quartz by rhamnolipid. They observed that the best recovery efficiency from quartz, approximately 91.6% of the sorbed Cd and 87.2% of the sorbed Zn, was achieved using 25 mM rhamnolipid concentration.

Biosurfactants were also used to evaluate their potential in arsenic mobilization from the mine tailings. The experimental results showed that introduction of rhamnolipid enhanced As mobilization from the mine tailings significantly. The mobilization increased with the concentration of biosurfactant and became relatively stable when the concentration of rhamnolipid was above 100 $mg \cdot L^{-1}$. It has been reported by Doong *et al.* that the removal of heavy metals increased linearly with increasing surfactant concentration below the CMC and remained relatively constant above the CMC. The CMC of the biosurfactant used by Wang and Mulligan was around 30 $mg \cdot L^{-1}$. The high concentration of rhamnolipid required in this experiment could be due to the sorption of biosurfactant to the mine tailings and the dilution and binding effects of mine tailing particles. The biosurfactant may be enhancing As mobilization by reducing the interfacial tension between As and the mine tailings, by formation of aqueous complexes or micelles and by improving the wettability of the mine tailings.

The results from this research study indicated that biosurfactants have potential to be used in the remediation of As-contaminated mine tailings and they can be also effectively used to remove As from soils.

Besides the mobilization, biosurfactants can be involved in other processes connected with remediation of heavy metals. They are used, for example, in entrapping of trivalent chromium in micelles which provides bacterial tolerance and resistance towards high concentration of Cr (III). Gnanamani *et al.* studied the bioremediation of chromium (VI) by biosurfactant producing, marine isolate *Bacillus* sp. MTCC 5514. The remediation carried out by this strain proceeded via two processes: reduction of Cr (VI) to Cr(III) by extracellular chromium reductase and entrapment of Cr(III) by the biosurfactants. The first process transforms the toxic state of chromium into less-toxic state and the second process prevents the bacterial cells from the exposure of chromium (III). Both reactions keep bacterial cells active all the time and provide tolerance and resistance toward high hexavalent and trivalent chromium concentrations.

3.8 BIOSURFACTANTS AND PHYTOREMEDIATION

Efficiency of phytoremediation of heavy metal contaminated soils can be increased by inoculation of plants by biosurfactant-producing and heavy metal-resistant bacteria. Biosurfactant-producing *Bacillus* sp. J119 strain was investigated for its capability to promote the plant growth and cadmium uptake of rape, maize, sudangrass and tomato in soil contaminated with different levels of Cd. The study demonstrated that the tested strain could colonize the rhizosphere of all studied plants but its application enhanced biomass and Cd uptake only in plant tissue of tomato. This means that root colonization activity of the introduced strain is plant type influenced. However, further analyses of interactions between the plants and biosurfactant-producing bacterial strain J119 may provide a new microbe assisted-phytoremediation strategy for metal-polluted soils. Further work on the applications of biosurfactants and biosurfactants-producing bacteria in phytoremediation, especially in sites co-contaminated with organic and metal pollutants is required.

3.9 BIOSURFACTANTS IN CO-CONTAMINATED SITES REMEDIATION

The presence of toxic metals (lead, cadmium, arsenic) in some cases causes inhibition of organic compound biodegradation. However, a review of the literature shows a number of possible approaches that can lower metal bioavailability and/or increase microbial tolerance to metals. These include inoculation with metal-resistant microorganisms, addition of materials like: clay minerals—kaolinite and montmorillonite, calcium carbonate, phosphate, chelating agents (EDTA), and bio-surfactants. Biosurfactants produced by microorganisms show promise for enhancing organic compound biodegradation in the presence of metals. Application of biosurfactants or microorganism produced biosurfactants in *in situ* co-contaminated sites bioremediation seems to be more environmentally compatible and more economical than using modified clay complexes or metal chelators.

Sandrin *et al.* (2012) showed that metal-complexing rhamnolipids reduced metal toxicity to allow enhanced organic biodegradation by *Burkholderia* sp. under laboratory conditions. This research demonstrated that rhamnolipids induced the release of lipopolisaccharide (LPS) from gram-negative bacteria, *Burkholderia* sp., which does not produce rhamnolipid. The authors suggested that rhamnolipid was able to reduce metal toxicity to microbial consortia in co-contaminated soils through a combination of metal complexation and in the alteration of cell surface properties through the release of lipopolisaccharide (LPS), resulting in enhanced bioremediation effect.

Maslin and Maier (2008) studied the effect of rhamnolipids produced by various *Pseudomonas aeruginosa* strains on the phenanthrene degradation by indigenous populations in two soils co-contaminated with phenanthrene and cadmium. The authors showed that rhamnolipids applied had the ability to complex cationic metals, increasing the phenanthrene bioavailability. The biodegradation of phenanthrene was increased from 7.5 to 35% in one soil, and from 10 to 58% in the second soil, in response to rhamnolipids application. As biosurfactants are degraded by soil populations in 2–3 weeks, Maslin and Maier used a pulsing strategy, in which new portions of rhamnolipids were added to the system to maintain a constant level of biosurfactant during organic contaminant mineralization.

IV. Conclusion

Application of biosurfactant and biosurfactant-producing bacteria in environmental technologies (bioremediation and phytoremediation) has been studied. Both organic and inorganic contaminants can be removed through different processes (physico-chemical and biological) in which biosurfactants are involved. Due to their biodegradability and low toxicity, they are very promising for use in environmental biotechnologies. The commercial success of biosurfactants is still limited by their high production cost. Optimized growth conditions using cheap renewable substrates (agro-industrial wastes) and novel, efficient methods for isolation and purification of biosurfactants could make their production more economically feasible. For lowering the cost of biosurfactant production, commercially viable biological and engineering

solutions are required. One important point in this context is the use of low cost substrates for production of biosurfactants. Careful and controlled use of these interesting surface active molecules will surely help in the enhanced clean up of the toxic environmental pollutants and provide us with a clean environment.

References

[1]. Appanna VD, Finn H, St Pierre M. (2005) Exocellular phosphatidylethanolamine production and multiple-metal tolerance in *Pseudomonas fluorescens* FEMS Microbiol. Lett. 131:53–56.

[2]. Awashti N, Kumar A, Makkar R, Cameotra S. (2009) Enhanced biodegradation of endosulfan, a chlorinated pesticide in presence of a biosurfactant. J. Environ. Sci. Heal B. 34:793–803.

[3]. Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Smyth TJ, Marchant R. Microbial biosurfactants production, applications and future potential. Appl. Microbiol. Biotechnol. 2010;87:427–444.

[4]. Baviere M, Degouy D, Lecourtier J. (2004). Process for washing solid particles comprising a sophoroside solution. 5,326,407. U.S. Patent.

[5]. Calvo C, Manzanera M, Silva-Castro GA, Uad I, González-López J. (2009). Application of bioemulsifiers in soil oil bioremediation processes. Future prospects. Sci. Total Environ.;407:3634–3640.

[6]. Cameron DR, Cooper DG, Neufeld RJ. (2008). The mannoprotein of *accharomyces cerevisiae* is an effective bioemulsifier. Appl. Environ. Microbiol.;54:1420–1425.

[7]. Christofi N, Ivshina IB. (2002). Microbial surfactants and their use in field studies of soil remediation. J. Appl. Microbiol.;93:915–929.

[8]. Das K, Mukherjee AK. (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.

[9]. 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.

[10]. Desai JD, Banat IM. (2007). Microbial production of surfactants and their commercial potential. Microbiol. Mol. Biol. R.;61:47–64.

[11]. Franzetti A, Gandolfi I, Bestetti G, Smyth TJ, Banat IM. (2010). Production and applications of trehalose lipid biosurfactants. Eur. J. Lipid. Sci. Tech.;112:617–627.

[12]. Gunther NW, Solaiman DKY, Fett FW (2007) Processes for the production of rhamnolipids. US patent US7202063

[13]. Herman DC, Artiola JF, Miller RM. (2005). Removal of cadmium, lead, and zinc from soil by a rhamnolipid biosurfactant. Environ. Sci. Technol.;29:2280–2285.

[14]. Hong JJ, Yang SM, Lee CH, Choi YK, Kajiuchi T. (2008) Ultrafiltration of divalent metal cations from aqueous solution using polycarboxylic acid type biosurfactants. J. Colloid Interf. Sci.;202:63–73.

[15]. Ishigami Y, Zhang Y, Ji F. (2000).Spiculisporic acid. Functional development of biosurfactants. Chim Oggi.;18:32–34.

[16]. Jennema GE, McInerney MJ, Knapp RM, Clark JB, Feero JM, Revus DE, Menzie DE. A (2003). Biosurfactants-producing *Bacillus* species potentially useful for enhanced oil recovery. Dev. Ind. Microbiol.;24:485–492.

[17]. Kachholz, T. and Schlingman, M., (2007). *Biosurfactants and Biotechnology*, Marcel Dekker Inc., New York, , 183–210.

[18]. Kamanvalli, C. and Ninnekar, H. Z., (2007). Proceedings of Annual Meetings of Society of Biochemist (India), Andhra University, 22–24 December, Vishakapatnam, , p. 43.

[19]. Kosaric N. (2001).Biosurfactants and their application for soil bioremediation. Food Technol. Biotechnol.;39:295–304.

[20]. Lang S. (2002). Biological amphiphiles (microbial biosurfactants) Curr. Opin. Colloid Inter. Sci.;7:12–20.

[21]. Maier RM, (2000). Soberón-Chávez G. *Pseudomonas aeruginosa* rhamnolipids: biosynthesis and potential applications. Appl. Microbiol. Biotechnol.;54:625–633.

[22]. Nguyen TT, Youssef NH, McInerney MJ, Sabatini DA. (2008). Rhamnolipid biosurfactant mixtures for environmental remediation. Water Res.;42:1735–1743.

[23]. Pesce L. (2002). A biotechnological method for the regeneration of hydrocarbons from dregs and muds, on the base of biosurfactants. 02/062,495. World Patent.

[24]. Rahman KSM, Rahman TJ, Kourkoutas Y, Petsas I, Marchant R, Banat IM. (2003). Enhanced bioremediation of *n*-alkane petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. Bioresour. Technol.;90:159–168.

[25]. Rosenberg E, Ron EZ. (2009). High- and low-molecular-mass microbial surfactants. Appl. Microbiol. Biotechnol.;52:154–162.

[26]. Rosenberg E, Rubinovitz C, Legmann R, Ron EZ. (2008). Purification and chemical properties of *Acinetobacter calcoaceticus* A2 Biodispersan. Appl. Environ. Microbiol.;54:323–326.

[27]. Sasthrulu, G. B. R., Srinivas. P., Rajagopal, S. V. and Reddi, T. G., (2009). Proceedings of Annual Meetings of Society of Biochemist (India), Andhra University, 22–24 December, Vishakapatnam, , p. 48.

[28]. Sifour M, Al-Jilawi MH, Aziz GM. (2007). Emulsification properties of biosurfactant produced from *Pseudomonas aeruginosa* RB 28. Pak. J. Biol. Sci.;10:1331–1335.

[29]. Soberón-Chávez G, Maier RM. (2011). Biosurfactants: a General Overview. In: Soberón-Chávez G, editor. Biosurfactants. Springer-Verlag; Berlin, Germany:. pp. 1–11.

[30]. Srinivas, G. B. R., Saathrulu, Rajagopal, S. V. and Reddi, T. G., (2007). Proceedings of Annual Meetings of Society of Biochemist (India), Andhra University, 22–24 December, Vishakapatnam, p. 49.

[31]. Thomas CP, Duvall ML, Robertson EP, Barrett KB, Bala GA. (2003). Surfactant-based EOR mediated by naturally occurring microorganisms.;11:285–291.

[32]. Toren A, Navon-Venezia S, Ron EZ, Rosenberg E. (2001) .Emulsifying activity of purified alasan proteins from *Acinetobacter radioresistens*. Appl. Environ. Microbiol.;67:1102–1106.

[33]. Veenanadig, N. K., Anu Appaiah, K. A. and Karanth, N. G. K., (2005). Proceedings of the Symposium on Relevance of Biotechnology in Industry, 4–5 March, Cochin University of Science and Technology, Cochin, , p. 28.
[34]. Veenanadig, N. K. and Karanth, N. G. K., (2006). Proceedings of Annual Conference of Association of Microbiology, IIT Chennai, 4–6 December, Chennai, , p.107.
[35]. Veenanadig, N. K. and Karanth, N. G. K., (2007). Proceedings of Annual Meetings of Society of Biochemist (India), Andhra University, 22–24 December, Vishakapatnam, , p. 177.
[36]. Whang LM, Liu PWG, Ma CC, Cheng SS. (2008). Application of biosurfactant, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. J. Hazard. Mater.;151:155–163.

Unaeze, Chizoba Henrietta. "Application of Biosurfactants in Environmental Remediation." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 14(10), (2020): pp 30-42.