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Biosurfactant-enhanced bioremediation of hydrophobic pollutants*

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Abstract: Biosurfactants are surface-active compounds synthesized by a wide variety of microorganisms. They are molecules that have both hydrophobic and -philic domains and are capable of lowering the surface tension and the interfacial tension of the growth medium. Biosurfactants possess different chemical structures—lipopeptides, glycolipids, neutral lipids, and fatty acids. They are nontoxic biomolecules that are biodegradable. Biosurfactants also exhibit strong emulsification of hydrophobic compounds and form stable emulsions. Polycyclic aromatic hydrocarbons (PAHs), crude oil sludge, and pesticides can be toxic, mutagenic, and carcinogenic compounds that pollute the environment. They are released into the environment as a result of oil spillage and by-products of coal treatment processes. The low water solubility of these compounds limits their availability to microorganisms, which is a potential problem for bioremediation of contaminated sites. Microbially produced surfactants enhance the bioavailability of these hydrophobic compounds for bioremediation. Therefore, biosurfactant-enhanced solubility of pollutants has potential bioremediation applications.

Keywords: analytical chemistry; particle synthesis; recommendations; sampling; soil.

INTRODUCTION

The scientific discoveries arising from the study of microorganisms over the last several centuries have never failed to alter our perception of life on the planet we inhabit and our relationship with it. The historical record is rich with revelations concerning our battle with infectious diseases, ensuring crop protection, securing adequate clean food and water production and clean environment. From the earliest days of barely recognizing that microbial life can exist, we moved toward the realization that such organisms play essential roles in geochemical cycles and agriculture, are the prime cause of serious diseases, and guide our social behavior and provision of basic needs from rural communities to megacities. Human activities have profound effects on the Earth and have influenced its subsurface with interrelated geochemical, biochemical, physiological, population, and community characteristics. Moderate to very high pollution due to human activities is relatively widespread in all the ecosystems, from coastal and estuarine ecosystems to ecosystems in rivers and on land. Pollutant influences have changed and will probably continue to change on time scales of decades. Environmental contamination

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by organic compounds is one of the most ubiquitous and costly environmental concerns facing society today.

The major environmental threats include organic aqueous waste (pesticides), organic liquids (solvents from dry-cleaning), oils (lubricating, automotive, hydraulic, and fuel oils) and organic sludge/solids (painting operations, tars from dyestuffs intermediates). Most soil contamination is the result of accidental spills and leaks; it originates from cleaning of equipment, residues left in used containers and outdated materials, use of excessive pesticides in agriculture, landfill leachate, which contains mixtures of organic [e.g., benzene, toluene, ethylbenzene, and xylene (BTEX), chlorinated hydrocarbons, pesticides, medicals] compounds and inorganic (e.g., heavy metals, macrocomponents) compounds [1,2]. Other sources of chemical contaminants include improperly managed landfills, automobile service and maintenance establishments, photographic film processors, and household wastes, which includes pesticides, paint products, household cleaners, and automotive products [3].

REMEDIATION OF POLLUTANTS

Improved detection methods in recent years added to our ability to measure or detect organic chemical contaminants in the range of parts per billion levels of contaminants in the soil and groundwater. As discussed above, toxic inorganic and organic chemicals are major contributors to environment contamination and pose a major health risk to the human population. Containing and avoiding future contamination from these compounds presents an immense technical challenge [4]. Remediation techniques involving physical and chemical intervention are quite widespread and include disposal in a landfill, incineration of the wastes, and direct injection of chemical oxidants into contaminated soil and groundwater, thereby altering native aquatic chemistry. Because of an increased interest in eco-friendliness, bio-based treatments commonly known as "bioremediation" are a cost-effective and ecologically adaptable method.

Bioremediation relies on improved detoxification and degradation of toxic pollutants either through intracellular accumulation or via enzymatic transformation to less toxic or nontoxic compounds [5]. Many microorganisms naturally possess the ability to degrade, transform, or chelate various toxic chemicals, but these natural transformations are relatively slow and require information on feasibility, verification, and capability of the process. The principal mediators of the bioremediation are microbes and their products [2] as they transform or mineralize pollutants, thereby decreasing their masses and toxicities in contrast to most other components of environment. Bioremediation uses natural as well as recombinant microorganisms to break down toxic and hazardous substances by aerobic and anaerobic means. They can be applied on site (in situ) or off site (ex situ), mediated by mixed microbial consortia and/or pure microbial strains and. plants (phytoremediation) or even natural attenuation. They include several processes—bioventing, biosparging, biostimulation, bioaugmentation, bioleaching, and biosorption.

The success of bioremediation is governed by three important factors: availability of microbes, accessibility of contaminants, and a conducive environment. The efficiency of the bioremediation is dependent upon the microbial ability to degrade these complex mixtures and their rate-limiting kinetics [6,7]. The intensity of biodegradation is influenced by several factors, such as nutrients, oxygen, pH value, composition, concentration, and bioavailability of the contaminants, chemical and physical characteristics, and the pollution history of the contaminated environment. [7]. As described by Rittmann et al., microbial communities need to be characterized in terms of structure, phenotypic potential, function, and interactions with the environment [8]. For a successful application, any bioremediation process should demonstrate that removal of contaminants is the primary effect of biodegradation, and it has a better degradation rate than the natural rate of decontamination [9].

A typical bioremediation process will consist of the application of nitrogenous and phosphorous fertilizers, adjusting the pH and water content, addition of emulsifiers and surface-active agents in two processes, and biostimulation and bioaugmentation to enhance the biodegradation rate by increasing the

bioavailability of the pollutant. Biostimulation refers to the addition of specific nutrients to a contaminated site with emphasis on the naturally indigenous microbes presumably present in sufficient numbers and types to break down the waste effectively. This assumes that every organism needed to accomplish the desired treatment results is present. An alternative approach is to use bioaugmentation, which is the scientific approach to achieve controlled, predictable, and programmed biodegradation.

Bioaugmentation involves the addition of specifically formulated microorganisms to a contaminated site. It is done in conjunction with the development and monitoring of an ideal growth environment, in which these selected bacteria can live and work. The basic premise for this intervention is that the metabolic capacities of the indigenous microbial community already present in the contaminated site will be increased by an exogenously enhanced genetic diversity, thus leading to a wider repertoire of productive biodegradation reactions. Bioaugmentation has been a subject of several reviews in the literature. However, bioaugmentation remains debatable as a scientific and technological endeavor. Gentry et al. [10] detailed several new approaches that may increase the persistence and activity of exogenous microorganisms and/or genes following introduction into the environment. They also reviewed the generation of genetically engineered microorganisms for use in bioaugmentation along with methods for the control of the engineered microorganisms in the environment, and the potential effects of the release on indigenous organisms.

Biostimulation is the introduction of additional nutrients or products in the form of organic and/or inorganic fertilizers into a contaminated system, which increases the population of the indigenous microorganisms [11]. Microbial degradation of organic contaminants in groundwater can occur naturally, supported by available electron donors, electron acceptors, and nutrients, or through human intervention using enhanced or engineered bioremediation technologies. The indigenous microorganisms may or may not primarily target the hydrocarbons as a food source. Smets and Pritchard [12] reviewed recent approaches involving the addition of electron acceptors, electron donors, or nutrients to stimulate naturally occurring microbial populations (biostimulation).

CONNECTION BETWEEN BIOREMEDIATION AND BIOSURFACTANTS

Bioremediation of xenobiotics in the environment is motivated by their ubiquitous distribution, their low bioavailability and high persistence in soil, and their potentially deleterious effect on human health. Due to high hydrophobicity and solid–water distribution ratios, these xenobiotics tend to interact with nonaqueous phases and soil organic matter and, as a consequence, become potentially unavailable for microbial degradation since bacteria are known to degrade chemicals only when they are dissolved in water. Conversion of the chemicals during bioremediation by the microbial cells is governed by the rate of uptake and metabolism (the intrinsic activity of the cell) and the rate of transfer to the cell (mass transfer). These factors regulate the so-called bioavailability of a chemical. In a nutshell, the research efforts in bioremediation have been spent on the optimization of the microbes' activity by the addition of nutrients or bioaugmentation, but the lack of success of these measures to enhance the intrinsic microbial activities during bioremediation is often attributed to the reduced bioavailability of the chemicals of concern.

The bioavailability of a chemical in general is controlled by a number of physical–chemical processes such as sorption and desorption, diffusion, and dissolution. For example, the hydrocarbons are hydrophobic compounds with low water solubility, thus, microorganisms have developed several mechanisms to increase the bioavailability of these compounds in order to utilize them as potential carbon and energy source. Therefore, one of the major factors limiting the degradation of hydrocarbons such as *n*-alkanes is their low availability to the microbial cells. Microorganisms employ several strategies to enhance availability of those hydrophobic pollutants, such as biofilm formation and biosurfactant production [13]. In this sense, growth of microorganisms on oil hydrocarbons has often been related to their capacity of producing polymers with surfactant activity.

SURFACTANTS AND BIOSURFACTANTS

Surfactants are amphipathic molecules with both hydrophilic and -phobic moieties that partition preferentially at the interface between fluid phases that have different degrees of polarity and hydrogen bonding, such as oil and water, or air and water interfaces. Usually, the hydrophobic domain is a hydrocarbon, whereas the hydrophilic domain can be nonionic, positively or negatively charged, or amphoteric [14,15]. This dual nature causes surfactants to adsorb at interfaces, thus reducing the interfacial energies [16–18]. Depending on the surfactant head group, they are classified as anionic, cationic, nonionic, or zwitterionic (cationic and anionic groups). At low concentrations, surfactants exist solely as monomers. These monomers will accumulate at interfaces present in the system (e.g., air–water, oil–water, soil–water). As the interfacial areas are satisfied and the aqueous surfactant concentration increases, the monomers aggregate to form micelles. The concentration at which micelles first begin to form is known as the critical micelle concentration (CMC). At concentrations above the CMC, surfactants have the ability to solubilize more of a hydrophobic compound than they would in water alone.

The most common nonionic surfactants are ethoxylates, ethylene and propylene oxide copolymers, and sorbitan esters. Examples of commercially available ionic surfactants include fatty acids, ester sulfonates or sulfates (anionic), and quartenary ammonium salts (cationic). These properties make surfactants suitable for an extremely wide variety of industrial applications involving emulsification, foaming, detergency, wetting and phase dispersion, or solubilization. Table 1 shows the various areas of their applications. Characteristic properties of the surfactants such as their abilities to lower surface tensions, increase solubility, detergency power, wetting ability, and foaming capacity enhance their ability to be an important industrial product for numerous applications. Surfactants have been applied as adhesives, flocculating, wetting and foaming agents, de-emulsifiers, and penetrants [19–21]. Although the petroleum industry has traditionally been the major users, as in enhanced oil removal applications, recent reports and reviews extend their applications to the food, cosmetics, and pharmaceutical industries [22–25].

Table 1 Types of modern surfactants used in industries.*

Surfactant type	Examples	% of total production	Major uses
Anionic	Carboxylates, sulfonates, sulfuric acid esters	66	Washing powders
Cationic	Amine oxides, monoamines quaternary ammonium salts	9	Fabric softners, shampoos
Nonionic	Carboxylic acids and carbohydrate esters, glycerides, and their ethoxylated derivatives	24	Laundry cosurfactants, washing up liquids, personal care products, and foods
Amphoteric	Alkyl betaines, alkyl dimethylamines, imidazonilinum derivatives	~1	Specialty uses

*Adapted from Banat et al. [103] and Desai and Banat [15].

From the current perspective of green chemistry and the environment, the toxicity factor of the chemical surfactants and the cost of their production are major obstacles for their applications in solv-

ing major environmental problems. Many biological molecules are amphiphilic and partition preferentially at interphases [20]. Microbial compounds, which exhibit particularly high surface activity and emulsifying activity, are classified as biosurfactants [20]. The biosurfactants, a term derived from the biological surface-active agents, are a molecule of the future because of the numerous advantages over their chemical counterparts because they are biodegradable and less toxic, are effective at extreme temperatures or pH values, and can be produced from several inexpensive waste substrates, thereby decreasing their production cost [20,22,24,25]. Biosurfactants have a wider availability and higher diversity in terms of several and different genres of microbes producing them [16,18,26]. Different groups of biosurfactants have different natural roles in the growth of the organisms in which they are produced (see Tables 2 and 3). These include increasing the surface area and bioavailability of hydrophobic water-insoluble substrates, heavy metal binding, bacterial pathogenesis, quorum sensing, and biofilm formation [27–30]. Although the literature is full of reports with many types of biosurfactants (Fig. 1), no single surfactant is suitable for all the potential applications. Thus, the development of even more multifunctional biosurfactants is important in order to broaden the spectrum of properties available. The losing economics of biosurfactant production makes it more worthy for better and efficient bioprocesses to make them competitive because they are unable to compete economically with the chemically synthesized compounds in the market, due to high production costs. This is due to inefficient bioprocessing methodology available, poor strain productivity, and the need to use expensive substrates.

Table 2 Major types of glycolipids produced by microorganisms.

Biosurfactant type	Producing microbial species	Application
Sophorolipids	*Candida bombicola* ATCC 22214	Emulsifier, MEOR
	Candida bombicola	Alkane dissimilation
Trehalose lipids	*Rhodococcus sps.*	Bioremeidation
	Tsukamurella sp	Antimicrobial properties
	Arthrobacter sp. EK 1	
	Rhodococcus ruber	Oxidize the gaseous alkanes
Rhamnolipids	*Pseudomonas aeruginosa* 57SJ	Bioremediation
	Renibacterium salmoninarum 27BN	Bioremediation
	P. putida Z1 BN	Bioremediation
	P. aeruginosa PA1	Bioremediation
	P. chlororaphis	Biocontrol agent
	P. aeruginosa GL1	Hydrocarbon assimilation
	P. aeruginosa GL1	Surface-active agent
	Pseudozyma fusiformata VKM Y-2821	Antifungal activity
	Bacillus subtilis 22BN	
Rubiwettins R1 and RG1	*Serratia rubidaea*	Swarming and spreading
Liposan	*Candida lipolytica*	Emulsifier
Schizonellins A and B	*Schizonella melanogramma*	Antimicrobial and antitumor agent
Mannosylerythritol lipids	*Candida antarctica*	Neuroreceptor antagonist, antimicrobial agent
	Kurtzmanomyces sp. I-11	Biomedical applications
Ustilipids	*Ustilago maydis and Geotrichum candidum*	Dopamine D3 receptors antagonist

(*continues on next page*)

Table 2 (*Continued*).

Biosurfactant type	Producing microbial species	Application
Cellobiose lipid (microcin)	*Cryptococcus humicola*	Antifungal agent
Flocculosin	*P. flocculosa*	Antifungal, biocontrol agent
Anionic glucose lipid	*Alcanivorax borkumensis*	Biomarkers

Table 3 Lipopeptides produced by various microorganisms.

Name	Producer organism	Properties and activities
Amphomycin	*Streptomyces canus*	Antibiotic, inhibitor of cell wall synthesis
Chlamydocin	*Diheterospora chlamydosporia*	Cytostatic and antitumor agent
Cyclosporin A	*Tolypocladium inflatum* (*Trichoderma polysporum*)	Antifungal agent, immunomodulator
Enduracidin A	*Streptomyces fungicidicus*	Antibiotic
Globomycin	*Streptomyces globocacience*	Antibiotic, inhibitor of cell wall synthesis
HC-toxin	*Helminthosporium carbonum*	Phytotoxin
Polymyxin E1 (ColistinA)	*B. polymyxa*	Antibiotic
Surfactin	*B. subtilis*	Antifungal, antibacterial, and antiviral agent
Bacillomycin L	*B. subtilis*	Antifungal, antibacterial, and antiviral agent
Iturin A	*B. subtilis*	Antifungal and antiviral agent
Mycosubtilin	*B. subtilis*	Antimicrobial agent
Putisolvin I and II,	*P. putida*	Biofilm formation inhibitor
BL1193, plipastatin and surfactin.	*B. licheniformis* F2.2	Antimicrobial agent
Bacilliomycin/Plipastatin/Surfcatin	*B. subtilis BBK1*	Inhibitor of phospholipase A(2)
Plipastins	*B. cereus BMG 302*	Antmicrobial agent
Surfactant Bl-86	*B. lichiniformis*	Antimicrobial agent
Halobacillin	*Bacillus*	Acyl-CoA and cholesterol acyltransferase inhibitor
Lichenysin G	*B. licheniformis IM 1307*	Hemolytic and chelating agent
Arthrofactin	*Arthrobacter*	Oil displacement agent, antimicrobial agent

(*continues on next page*)

Table 3 (*Continued*).

Name	Producer organism	Properties and activities
Fengycin	*B. thuringiensis* CMB26,	Biocontrol agent, fungicidal, bactericidal, and insecticidal activity
	B. subtilis F-29-3	Antifungal lipopeptide
Mycobacillin	*B. subtilis*	Antifungal

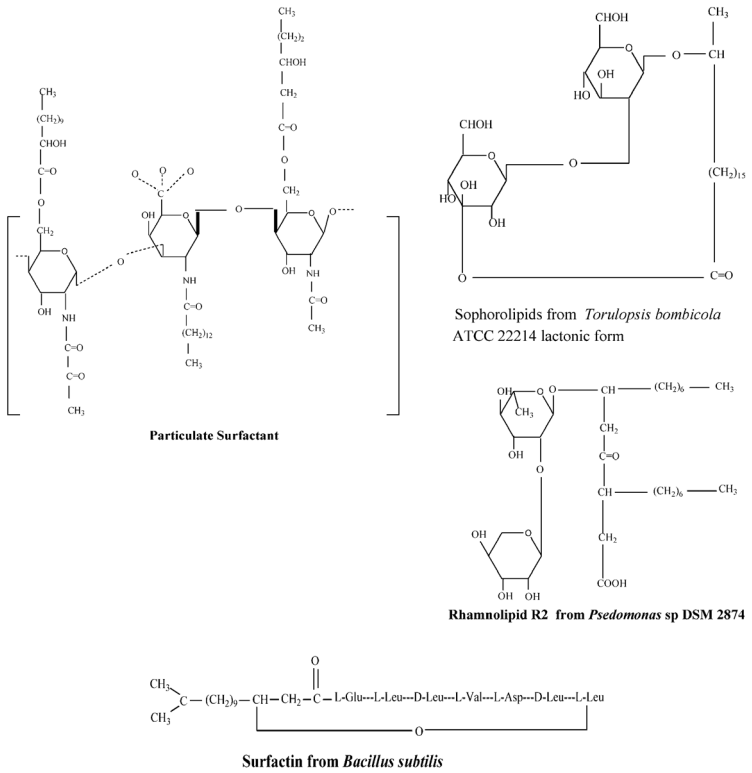

Fig. 1 Structures of most common biosurfactants produced by microbes.

APPLICATIONS OF BIOSURFACTANTS IN BIOREMEDIATION OF HYDROPHOBIC POLLUTANTS

In the last few decades, the research focus has been on surfactant-mediated bioremediation [27–29,31]. The increasing interest is attributable to the fact that surfactants can enhance the solubilization of pollutants from contaminated soil and increase their solubility, which in turn improves their bioavailability [32,33]. Many surfactants of different kinds have been so far investigated for their possible applications in facilitating the biodegradation of organic contaminants such as PAHs [19]. There are two mechanisms of surfactant-enhanced soil washing depending on the concentration of surfactants.

A process that occurs below the CMC, when surfactant monomers increase the contact angle between the soil and hydrophobic contaminant, promotes the separation of contaminant from soil particles and finally displaces the oil from the soil. The second process, solubilization, occurs above the CMC, when contaminants are partitioned from the soil into the hydrophobic core of surfactant micelles

[28,34–36]. Solubilization using surfactant at concentrations above their CMC values is widely explored in in situ soil washing [28,36,37]. Solubilization depends on the type and dose of the surfactant, the hydrophobicity, the surfactant–soil interactions, and the time that the contaminant has been in contact with the soil [19]. Nonionic surfactants with high hydrophobicity such as Triton's, Tween's, and Brij's, are considered to be suitable for enhancing solubilization of hydrophobic organics in soil. However, in some cases, the inhibition of contaminant biodegradation in soil systems at surfactant concentrations above CMC has also been reported [19,29,38]. A variety of factors and mechanisms have been proposed to explain the inhibition process, including cellular toxicity from interaction of surfactant molecules with cell membranes or cell membrane bound proteins, inhibition of enzymes of the catabolic pathway either by association with the enzyme or with the substrate, decreased bioavailability due to sequestration of the substrate compound into surfactant micelles, or the accumulation of toxic intermediates due to incomplete metabolism incurred from substrate–surfactant interactions.

There are many studies involving laboratory and field experiments of above-ground and in situ soil washing [39]. A few experiments have indicated the potential for enhanced biodegradation of subsurface hydrophobic contaminants in the presence of surfactants. Overall, surfactants are viewed as being potentially useful for aiding the bioremediation of the contaminated sites if issues such as variable efficacy can be resolved. An improved strategy to surfactant-enhanced remediation (SER) is to reduce the concentrations of surfactants and cost level while maintaining the efficiency of remediation. In practical applications, mixtures of surfactants, rather than individual surfactants, are often used. In most cases, when different types of surfactants are purposely mixed, synergism is observed, i.e., the condition when properties of the mixture are better than those attainable with individual components by themselves. Various kinds of surfactants usually coexist in water and soils. The sorbed surfactants may significantly affect the properties of soils/sediments and suspended particles, and thus the transport and transformation of organic pollutants. Thus, a further understanding of the properties of mixed surfactant systems is desirable. [40]. Furthermore, mixtures of anionic and nonionic surfactants exhibit synergistic solubilization for hydrophobic contaminants, which would enhance the efficiency for contaminated soil washing and flushing and facilitate the bioavailability of pollutants by microbial population [41,42].

Studies on biosurfactant production-aided polynuclear aromatic hydrocarbon (PAH) bioremediation

PAHs are a class of compounds found throughout the environment in the air, soil, and water with known harmful effects on humans and wildlife [43]. These compounds are widely distributed and can be produced by natural or anthropogenic sources [44]. Natural sources of PAHs include events such as volcanic releases and forest fires. Anthropogenic sources include incomplete fossil fuel combustion, industries, internal combustion and diesel engine exhausts, aviation exhaust, and cigarette smoke [45]. Although cigarette smoke accounts for the highest exposure threat to humans, vehicle exhaust is the main source of PAHs in the environment. Exposure to PAHs has long been identified as an environmental concern. Over 120 PAHs have been detected in urban pollution. Of these 120, there are 16 PAH compounds that the U.S. Environmental Protection Agency (EPA) has classified as priority pollutants. Three PAHs are not commonly associated with adverse health affects in humans. However, the U.S. Department of Health and Human Services has determined that some PAHs, particularly 4- and 6-ringed PAHs, can potentially cause cancer.

PAHs have been identified in soil, surface water, groundwater, sediment, and air; they are very hydrophobic organic compounds and are relatively insoluble in water. They have a high affinity for organic matter and, when present in soil or sediments, tend to remain bound to solid particles and dissolve slowly in water. Their release in the environment in conjunction with transport phenomena can cause soil contamination [46,47], and effective and viable treatments of organic wastes and soils contaminated with PAHs have become a major focus of recent research. Poor solubility of PAHs is the major prob-

lem in biodegradation processes leading to the low bioavailability of PAHs as a substrate. A variety of physical, chemical, and biological remediation techniques have been proposed and have been demonstrated to have varying degrees of success [48–50]. Surfactants are able to increase hydrophobic substrate solubility and provide a less aggressive environment for bacterial cells [51,52]. Naturally, many microbes are able to degrade PAHs and can produce amphiphatic compounds similar to synthetic surfactants that can form micellar systems [53,54]. Organisms with excellent pollutant-degrading capability are not capable of producing biosurfactants; application of synthetic surfactants/biosurfactants can further improve PAH bioconversion processes by increasing PAH bioavailability and mass transfer rates to cells. This has been seen in biodegradation improvement in whole-cell bioconversion of naphthalene, phenanthrene, and anthracene by *E. coli* JM109 recombinant strains carrying naphthalene dioxygenase and regulatory genes cloned from *Pseudomonas fluorescens* N3 in the presence of surfactants such as Tween 60 and Triton X100. [51,52,55]. We have listed some of the important studies involving biosurfactants and PAHs in the last decade.

Dean et al. [56] investigated bioavailability of phenanthrene to two phenanthrene-degrading bacteria (*Pseudomonas* strain R and isolate P5-2) in the presence of rhamnolipid biosurfactant and/or a biosurfactant-producing bacterium, *Pseudomonas aeruginosa* ATCC 9027. They observed different rates and extent of phenanthrene mineralization by two strains in aqueous studies. Rhamnolipid addition increased phenanthrene mineralization by *Pseudomonas* strain R in Fallsington sandy loam with high phenanthrene-sorption capacity. However, the phenanthrene mineralization in soils inoculated with P5-2 was minimal and no enhancement in phenanthrene degradation was observed when biosurfactant was added. Even co-inoculation of Fallsington sandy loam with the biosurfactant producer did not affect phenanthrene mineralization by isolate P5-2, but significantly enhanced phenanthrene mineralization by *Pseudomonas* strain R. Authors suggested that the addition of rhamnolipid at concentrations above the CMC resulted in enhanced phenanthrene release from test soils, which was accessed by the phenanthrene-degrading strains. The study indicated the presence of some degree of commensalisms between the bacteria with the catabolic potential to degrade sorbed hydrophobic contaminants and surfactant-producing strains by an unknown mechanism to hasten the biodegradation of aromatic hydrocarbons. Further studies to understand the interactions among microbes may provide opportunities to further enhance biodegradation of soil-bound organic contaminants.

Lan Chun et al. [57] investigated the effect of the presence of multiple PAHs on micellar partitioning of single compounds by three different anionic surfactants. The three surfactants were sodium dodecylbenzene sulfonate (SDDBS), monoalkylated disulfonated diphenyl oxide (MADS-C12), and dialkylated disulfonated diphenyl oxide (DADS-C12). The results showed markedly different tendencies with coexistent PAHs. In the presence of less hydrophobic solutes, the solubility of more hydrophobic solute was increased. Phenanthrene was greatly influenced by cosolutes other than naphthalene, and the solubility of phenanthrene was greatly enhanced in the presence of less hydrophobic naphthalene but reduced in the presence of more hydrophobic pyrene. A plausible explanation for these results could be that less hydrophobic compounds can be solubilized at the interfacial region of a hydrophobic core, which reduces the interfacial tension between the core and water, and then the reduced interfacial tension can support a larger core volume for the same interfacial energy.

The study focused on the comparison of the enhanced solubility of PAHs among three surfactants with different structure attributes, the difference in solubilization power among three anionic surfactants to the structure of surfactants, the shape and size of micelles, and their CMC values. The findings were very important as using the results obtained for individual compounds might be unreasonable for the remediation of real contaminated sites where the contaminants exist as mixtures, because the solubilization power and CMC value of surfactants were remarkably changed with the coexisting PAHs. Therefore, the components of the contaminants should be investigated first in the field, and the effect of coexistence of multiple contaminants should be considered in order to effectively remove all of them.

Garcia-Junco et al. [58] conducted the study to see the effect of two different biological factors, microbial surfactants, and biodegradation, on the kinetics of partitioning of PAHs from nonaqueous-

phase liquids (NAPLs). The effect of rhamnolipid biosurfactants on partitioning into the aqueous phase of PAHs was determined in multiple-solute experiments. They found the enhanced partitioning rate of fluorene, phenanthrene, and pyrene, but were ineffective with naphthalene when biosurfactants at a concentration above the CMC was used. Similar enhancement of partitioning was also observed in the presence of suspended humic acid-clay complexes. Biosurfactants sorbed to the complexes modified PAH partitioning between the NAPL and these solids, increasing the fraction of solid-phase PAH. The study suggests that in NAPL-polluted sites, partitioning of PAH may be efficiently enhanced by in situ treatments involving the use of biosurfactants and biodegradation.

Chang et al. [59] studied the effects of trehalose lipid biosurfactants produced by *Rhodococcus erythropolis* on the solubilization and biodegradation of phenanthrene experiments in soil water slurry and showed that the biosurfactants increased the rate, but not the extent, of phenanthrene mineralization. Their experimental set-up with different culture conditions suggested that the biosurfactants increased the rate, but not the extent, of phenanthrene mineralization. The results obtained in the present study indicate that the trehalose lipid biosurfactants produced by *R. erythropolis* have good solubilization capacity for hydrophobic organic compounds and great potential for applications in bioremediation of sites contamination with PAHs. Shin and Kim [60] examined the potential of biosurfactants and their operating conditions in soil remediation. They investigated the effects of flow rate, biosurfactant concentration, and surfactant type in biosurfactant-enhanced soil flushing process. They could achieve as much as 70 % of the phenanthrene and 60 % of the diesel in the sand under optimal conditions, indicating that the use of biosurfactants in the flushing process is favorable, not only with respect to the environment, but also on removal efficiencies.

Cheng et al. [61] found that the combination of surfactant and biosurfactant can enhance bioremediation of soils contaminated with PAHs by reducing sorption of PAHs or increasing desorption rates. They tested the effectiveness of nonionic surfactant (Tween 80) and biosurfactants to enhance the solubilization and desorption of PAHs in soil-aqueous systems under thermophilic conditions using batch studies. A linear relationship between the concentrations of surfactants at concentrations above their respective CMC and solubilization of PAHs was observed. When the surfactants' concentrations in aqueous phase were above their respective CMCs, substantial amounts of PAHs were desorbed from soil into the aqueous phase. Enhanced solubilization and desorption of PAHs, by use of surfactants mixture, imply that it might have the potential to be further applied in the bioremediation of PAH contaminated soils. Wong et al. [62] investigated the effect of surfactants on the bioavailability and biodegradation of PAHs under thermophilic conditions. They used Tween 80, Triton X100, and biosurfactants produced from *P. aeruginosa* strain P-CG3 and *P. aeruginosa* ATCC 9027. They observed varied results for different microbes used. Surfactants effectively enhanced the solubility of phenanthrene at 50 °C, and the biosurfactant from P-CG3 was most effective with a 28-fold increase in apparent solubility of phenanthrene at a concentration of 10 times CMC compared with the controls. But experiments with addition of synthetic surfactants or biosurfactants inhibited the biodegradation of phenanthrene in mineral salts medium by an isolate *Bacillus* sp. B-UM. Degradation of phenanthrene diminished with increasing surfactant concentrations, and phenanthrene degradation was completely inhibited for all the surfactants tested when the concentrations were greater than their respective CMC.

It was found that the four surfactants enhanced the phenanthrene solubility; phenanthrene biodegradation did not correspondingly increase. Authors discussed the likely causes for this inhibition as use of surfactants as preferential growth substrates, toxicity effect, and/or lower uptake of phenanthrene by microorganisms [63]. The growth test suggested that Tween 80 and biosurfactants were degradable, but preferential utilization of these surfactants as substrates was not the mechanism for explaining the inhibition of phenanthrene biodegradation. Therefore, uptake reduction of phenanthrene by microorganisms in the presence of surfactants was probably the major cause for inhibition.

As described by Makkar and Rockne [19] on the mechanisms for bacteria to take up solid hydrocarbons, that is, either by dissolution of the target molecules in the aqueous phase, or by adhesion of bacteria directly to the solid–water interface. For very low-solubility hydrocarbons, microbial cells may

secrete surface-active substances to increase the mass transfer and result in increasing uptake. The strain B-UM did not show any ability to produce surfactants for increasing dissolution of phenanthrene in MSM. Therefore, the direct adhesion of cells to PHE surface would be the major pathway for B-UM to take up phenanthrene. The hydrophobic property of B-UM supports this hypothesis. Earlier studies have also shown that surfactants can inhibit biodegradation of hydrocarbons by de-adhesion of cells from the liquid/solid–water interface (Neu, 1996 #5221; Volkering, 1998 #10775). The inhibitory effect of Triton X100 on the growth of an *Arthrobacter* sp. on *n*-hexadecane has been attributed to diminishing bacterial adhesion to the liquid–liquid interface [64].

Stelmack et al. observed similar inhibitions on anthracene degradation by two bacterial strains, *Mycobacterium* sp. and *Pseudomonas* sp., by Triton X100 and Dowfax 8390 [63]. The plausible explanation of these inhibitions is that free energy of adhesion was expected to increase by surfactants, which diminished bacterial adhesion to solid surfaces. Adsorption of surfactants onto nonpolar, hydrophobic surfaces is primarily by dispersion force interaction and results in aggregation of surfactant molecules at solid–liquid interfaces in aqueous solution. When the concentrations of surfactant were near or above the CMCs, the surfactant molecules at the solid–water interface reached saturation, and micelle-accommodated phenanthrene began to form at this point. Hence, surfactants could entirely prevent the cells from contacting the surface of phenanthrene and the phenanthrene in micelle; thus, the degradation did not occur any more.

Considerable work on biosurfactant production by microorganisms when grown on hydrocarbon has been done by Tuleva et al. In their first report in 2002 [65] they observed the both tensio-active and emulsifying activities of the rhamnolipid biosurfactant produced by newly isolated and promising strain *Pseudomonas putida* 21BN. In 2004 [66], they reported the production of two rhamnolipids RLL and RRLL from a new strain *Renibacterium salmoninarum* 27BN when grown on *n*-hexadecane as sole carbon source. Growth on *n*-hexadecane by *R. salmoninarum* 27BN was directly related to the biosurfactant production that was increasing the bioavailability of the substrate for the cells. Further in their studies, they found capability of biosurfactant (rhamnolipids) production by a new *Bacillus subtilis* 22BN in relation to hydrocarbon degradation [67]. This report is important since it is the first report of *B. subtilis* strain that degrades hydrophobic compounds and at the same time produces rhamnolipid biosurfactant. In 2005, the same group reported the biosurfactant activity and naphthalene degradation by a new strain identified as *Bacillus cereus* 28BN. The strain grew well and produced effective biosurfactants in the presence of broad substrate range. The significance of this study comes from the fact that it was the first report for a *B. cereus* rhamnolipid-producing strain that utilized naphthalene under aerobic conditions and has a vast potential for application in environmental technologies. In their recent study [68], they isolated a glycolipid-producing bacterial strain *Rhodococcus wratislaviensis* BN38, when grown on 2 % *n*-hexadecane. The synthesis of surface-active glycolipids make the *R. wratislaviensis* strain BN38 an excellent potential candidate for use in bioremediation applications or in biosurfactant exploration.

Kuyukina et al. [69] studied the enhanced crude oil desorption and mobilization with the biosurfactants from *Rhodococcus ruber* in the model soil column systems. The biosurfactants were about 2.5 times more effective in comparison to the similar synthetic surfactant (Tween 20). Biosurfactant-enhanced oil mobilization was temperature-related, and could remove 65–82 % of crude oil from the soil columns. The extracted oil from the columns suggests it to be the most nonbiodegradable compound, compared to initial oil composition which could be easily biodegraded by soil bacteria. *Rhodococcus* biosurfactants can be used for in situ remediation of oil-contaminated soils.

Kumar et al. [70] characterized a newly isolated bacterium, designated as IR1, with respect to its ability to degrade PAHs and to produce biosurfactants. 16 S rRNA analysis showed that isolated IR1 was as *P. putida* and capable of utilizing two-, three- and four-ring PAHs but not hexadecane and octadecane as a sole carbon and energy source. The presence of both tensio-active and emulsifying activities indicated that biosurfactants were produced by IR1 during growth on both water miscible and immiscible substrates, which lowered the surface tension of medium and formed a stable and compact

emulsion with an emulsifying activity. These findings indicate that this isolate may be useful for bioremediation of sites contaminated with aromatic hydrocarbons.

Shin et al. [71] used biosurfactant to remediate phenanthrene-contaminated soil by the combined solubilization–biodegradation process. The removal efficiency of the process was compared at various pH values since the pH of biosurfactants solution could be an important factor in this system. This study provides the information on the impact of biosurfactant-enhanced flushing operations on followed microbial processes and the possibility of combined remediation process. Ruberto et al. [72] studied the biodegradation of PAHs in Antarctic soils in microcosm systems (1-l glass flasks containing Antarctic soil supplemented with phenanthrene). In the study, they determined the effect of biostimulation with a complex organic source of nutrients (fish meal) + surfactant (Brij 700), the effect of bioaugmentation with a psychrotolerant PAH-degrading bacterial consortium, and the combination of both strategies. The results obtained show that the combination of biostimulation and bioaugmentation caused a significant removal of phenanthrene after 56 days under Antarctic environmental conditions. However, the individual strategy did not show any significant reduction in phenanthrene concentration. This was an important study as it proved that "in situ" bioremediation process of phenanthrene-contaminated soils is possible in Antarctic stations and a bioaugmentation strategy involving inoculation with a psychrotolerant PAH-degrading bacterial consortium and a mix of fish meal (Nutrient) and a high-molecular-weight should be the selected strategy when the number of hydrocarbons degrading bacteria in the target soil is low.

Cui et al. [73] applied the biosurfactants produced by *P. aeruginosa* W3 to enhance the anthracene-degrading ability of the two strains, *Sphingomonas* sp. 12A and *Pseudomonas* sp. 12B. Anthracene is a PAH that is not readily degraded, plus its degradation mechanism is still not clear. It was found that the rhamnolipids dramatically increased the solubility of anthracene by both strains. They did observe the metabolism of biosurfactant by *Sphingomonas* sp. 12 A and thus attributes to important fact to be considered during application of biosurfactant for bioremediation. Das et al. [74] undertook the study to assess the capability of a marine bacterium to increase bioavailability and consequent biodegradation of anthracene, a model PAH. An anthracene-supplemented glycerol medium appeared to be better for growth and biosurfactant production. Various analytical parameters showed a high degree of emulsification and solubilization of various hydrocarbons by the biosurfactants. Thus, organic pollutant anthracene was metabolized and converted to biosurfactants facilitating its own bioremediation. This study is unique and has wider implications for bioremediation at organic matter rich contaminated sites, where initial growth and production of biosurfactant by the organism is because of organic matter metabolism and sustained growth and production of biosurfactant is because of metabolism of the pollutant, in this case, anthracene.

Zhu and Zhou [75] investigated the distribution of PAHs in solid–water–surfactant systems with five nonionic surfactants and a natural bentonite. The study covered the partition behavior of PAHs to various solid-sorbed nonionic surfactants on surfactant mole basis. The corresponding mechanism modeled the partition of PAHs to solid-sorbed surfactant and the distribution of PAHs in solid–water–surfactant systems. The results of the study increase our insight for understanding and predicting the partition of hydrophobic organic compounds to solid-sorbed surfactant and the distribution of hydrophobic organic compounds in soil–water–surfactant systems. It also gave valuable information in predicting the efficiency of surfactant-enhanced desorption or washing for contaminated soils and designing SER technologies. Kolomytseva et al. [76] investigated and optimized the biodegradation of fluorene by *Rhodococcus rhodochrous* VKM B-2469 by nonionic surfactants laden liquid media. Poor solubility of fluorene is the major problem in biodegradation process leading to the low bioavailability of fluorene as a substrate. It has been recently demonstrated that dilute direct micellar systems formed utilizing nonionic surfactants are able to increase hydrophobic substrate solubility and provide a less aggressive environment for bacterial cells [52,55]. The authors observed that Tween 60 was useful to enhance the fluorene biodegradation rates by *R. rhodochrous* VKM B-2469 by being an additional carbon source and by decreasing the fluorene toxicity to the bacterial cells. The data reported here show consistent im-

provements in PAH bioremediation and the possible use of these strategies for increasing yields of PAH intermediate possibly useful as high added-value fine chemicals.

In this section, we compared some of the important papers related to biosurfactants and PAH biodegradation. In regard to the surfactants, sorption and sequestration of PAHs within the sediment are very critical and define contaminant mobility, toxicity, and their long-term persistence. All the studies involved with surfactants have been focused on an increase in apparent solubility and their interaction within degrading bacteria or with surfactants and PAHs. With the use of biosurfactants, toxicological disability of surfactants is overcome. All studies related to biosurfactants discussed here suggest an increase in PAH biodegradation rates, however, their effects on PAH bioremediation in the field are still not predictable. This gives us a better understanding in relation to actual application of biosurfactants as remedial technology with respect to PAH bioavailability and their toxicology and ecology.

Studies on biosurfactant production-aided hydrocarbon bioremediation

Petroleum is a very important resource to our environment and one of most widely used chemicals in society today. It is a primary fossil fuel and a major source of not only crude oil but also raw materials for almost everything used in our daily life. Petroleum is the world's largest energy source; it is 60 % of all energy consumed. 30 % of petroleum is in light and medium crude oil, 14 % is in heavy crude oil and is natural gas endowment. Although an indispensable part of our lives, petroleum hydrocarbons (crude oils) are is also one of the most serious and threatening environmental pollutants because of their persistence and high toxicity to all biologic systems. Petroleum contamination results from leaking above- and underground storage tanks, spillage during transport of petroleum products, abandoned manufactured gasoline sites, other unplanned releases, and current industrial processes. Prince et al. [77] reported that 40 % of the petroleum contamination is due to non-tanker operational discharges and urban run-off. Consequently, risks and concern over petroleum hydrocarbon contamination of soil and water with its attendant ecotoxicological effects is significant [31].

Subsurface contamination by these hydrocarbons is a complex process, and their remediation, mainly initiated by microbes, is limited by low water solubility (Banat, 2000 #696; Desai, 1997 #718), adsorption of contaminants onto soil matrix, and limited rate of mass transfer for the biodegradation. The organic contaminant based on physical state can be of two types—a solid or a liquid. In soil, hydrocarbons remain partitioned in a separate NAPL, which may be present as droplets or films on soil particles, thus, hardly available to microbial cells [78]. Two general types of microbial cell interaction with liquid alkanes have been postulated: specific adhesion of cells to large oil drops and pseudosolubilization involving the cellular assimilation of emulsified small oil droplets [19]. Biosurfactants serve to decrease tension at the hydrocarbon–water interface and can result in hydrocarbon pseudosolubilization via micelle or vesicle formation, leading to increased mobility, bioavailability, and subsequent biodegradation [16,20]. The literature is loaded with research, and reports on the biodegradation of hydrocarbons in soils have been thoroughly reviewed [19,22,79], which justify the use of biosurfactants and biosurfactants producing organisms for bioremediation of hydrocarbons. Historically, biosurfactants were produced using hydrocarbons as sole carbon sources [78]. Biosurfactants have more adaptable physicochemical properties; thus, they are more suitable for applications in the oil industry, which explains why the large majority of the biosurfactants produced (estimated to be of the order of 400–500 tons yr^{-1}, including captive use for tertiary oil recovery or tank cleaning) are used in petroleum-related applications [13]. They have been used for applications in tank oil recovery; oil spill management; microbial enhanced oil recovery; and as heavy oil dispersants and demulsifiers [20,24,25]. Recently, much wider applications have been sought for these compounds. As in previous sections, we have compiled some of the important findings in the literature, which associate the biosurfactants with the hydrocarbon degradation.

Nazina et al. [80] isolated 20 pure cultures from formation waters of the Daqing oil field, which were characterized for their capacity to produce surface-active compounds in media with individual

hydrocarbons, lower alcohols, and fatty acids. They found that aerobic saprotrophic bacteria belonging to the genera *Bacillus*, *Brevibacillus*, *Rhodococcus*, *Dietzia*, *Kocuria*, *Gordonia*, *Cellulomonas*, *Clavibacter*, *Pseudomonas*, and *Acinetobacter* were capable of decreasing the surface tension of cultivation media appreciably. Biosurfactants were mostly produced by strains of *B. cereus*, *R. ruber*, and *Bacillus licheniformis*, while bacteria of the genera *Rhodococcus*, *Dietzia*, *Kocuria*, and *Gordonia* produced exopolysaccharides in media with hydrocarbons. This study suggests the significant presence of indigenous biosurfactant producing strains in the oil fields and their capability as oil-releasing factors. A similar study by Bodour et al. [26] to see how common surfactant-producing bacteria are distributed in undisturbed and contaminated sites found that the distribution was dependent on soil conditions, with Gram-positive biosurfactant-producing isolates tending to be from heavy metal- or uncontaminated soils and Gram-negative isolates tending to be from hydrocarbon- or co-contaminated soils.

Lu et al. [81] investigated the indigenous bacteria from the oil-contaminated site of Dawu water source area in Zibo city of China, for production of biosurfactants and their role in the biodegradation of oil hydrocarbons. Two high-effective species of bacteria (Z1 and Z2) were isolated on their growth on paraffin capable of biosurfactant production. The CMC of Z1 ferment liquid was 0.4 (volume content). When reaching the CMC, Z1 ferment liquid demonstrated the effect of dissolution enhancement, that is, it could enhance the dissolved concentration of phenanthrene to above 1 mg/l, concentration higher than the saturated solubility of phenanthrene under standard condition.

In last decade, the Russian group of Vasileva–Tonkova have done considerable work on isolation and characterization of microbes associated with hydrocarbon degradation in a broad range of habitat. They [82] isolated 17 pure aerobic microbial isolates from soil samples of three regions of Antarctica: Casey Station, Dewart Island, and Terra Nova Bay. The isolated mostly Gram-positive coryneform were tested for their ability to grow on mineral salt agar plates supplemented with one of the following model *n*-alkanes or aromatic hydrocarbons: hexane, heptane, paraffin, benzene, toluene, naphthalene, and kerosene. The study provided important information about the broader abilities of degraders capable of growing on both types of hydrocarbons, good production of glycolipids, and emulsifying activity. These mixed cultures of strains will be suitable for application for bioremediation at temperate temperature of soil environments polluted with different hydrocarbons.

In continuation with this work, they isolated an actinomycete identified as *Nocardioides* sp. A-8 grown on *n*-paraffin as a carbon source-produced biosurfactant. The strain produced rhamnolipids, which lowered the surface tension of the medium below 35 mN/m and efficiently emulsified aromatic hydrocarbons and *n*-paraffin. The results showed with effective surface and emulsifying properties, the Antarctic *Nocardioides* sp. A-8 represent a promising potential for application in bioremediation of soil environments polluted with hydrocarbons [83]. In addition, they [84] isolated a facultative anaerobe *Pantoea* sp. strain A-13, ornithogenic soil of Dewart Island (Frazier Islands) Antarctica, which produced rhamnolipids when grown on *n*-paraffin's or kerosene as the sole source of carbon and energy. Surface activity analysis suggested it to be rhamnolipids with excellent surface tension lowering capacity and a low CMC value of 40 mg/l. According to the results, the Antarctic biosurfactant-producing strain *Pantoea* sp. A-13 appears to be valuable source for application in accelerated environmental bioremediation.

In other separate studies, they isolated the *P. fluorescens* strain HW-6 from industrial waste waters capable of producing glycolipid biosurfactants at high concentrations (1.4–2.0 g/l) when grown on hexadecane as a sole carbon source [85]. The biosurfactants produced were able to decrease the surface tension of the air–water interface by 35 mN/m and possessed a low CMC value of 20 mg/l, which indicated high surface activity. Biosurfactants were able efficiently emulsify the aromatic hydrocarbons, kerosene, *n*-paraffins, and mineral oil sand contributed to a significant increase in cell hydrophobicity correlated with an increased growth of the strain on hexadecane. The results reflect possible application of the newly isolated strain of *P. fluorescens* for bioremediation of hydrocarbon-polluted sites.

Recently, they isolated various bacteria from the industrial water based on probable modes of hexadecane uptake. Their results based on analysis of cell surface hydrophobicity, emulsifying activity,

glycoside content, and surface tension of cell-free culture medium suggest that both modes of biosurfactant-enhanced hexadecane uptake by bacterial strains take place, direct uptake and alkane transfer. The biosurfactant-enhanced interfacial uptake of the alkanes was predominant for three isolates, *Staphylococcus* sp. HW-2, *Streptococcus* sp. HW-9, and *Bacillus* sp. HW-4. While secreted biosurfactants enhanced alkanes emulsification was in most hydrophobic isolates *Arthrobacter* sp. HW-8, and *Streptococcus* sp. HW-5 (micellar transfer). Other strains (67 %) followed both mechanisms of biosurfactant-enhanced hexadecane uptake and interfacial uptake and alkane emulsification. These results clearly indicate diverse natural relationships between the members of ecosystem studied and reveal potential producers of surface-active compounds [86].

Prabhu and Phale [53] have found that growth-associated extracellular biosurfactant production and modulation of cell surface hydrophobicity plays an important role in hydrocarbon assimilation/uptake in *Pseudomonas* sp. strain PP2. When grown on hydrocarbon or dextrose, the culture showed good extracellular biosurfactant production, growth-dependent changes in the cell surface hydrophobicity, and emulsification activity. Urum et al. have done considerable work, which has helped our understanding of biosurfactant application in washing of contaminated soil. They [87] evaluated the ability of aqueous biosurfactant solutions (aescin, lecithin, rhamnolipid, saponin, and tannin) for possible applications in washing crude oil contaminated soil. A comparison of the biosurfactant behavior in soil–water, water–oil, and oil–soil systems (such as foaming, solubilization, sorption to soil, emulsification, surface and interfacial tension) with a well-known chemical surfactant (sodium dodecyl sulfate, SDS) at varying concentrations were done. The results indicated that the biosurfactants were able to remove significant amount of crude oil from the contaminated soil at different solution concentrations. Most of the oil removed was due to mobilization, caused by the reduction of surface and interfacial tensions rather than the solubilization and emulsification effects. The study put emphasis on the fact that the knowledge of surfactants' behavior across different systems is paramount before their use in the practical application of oil removal.

In a continuing study, they investigated the removal of crude oil from soil using air-sparging-assisted stirred-tank reactors [88]. They applied two surfactants (rhamnolipid and SDS) in conjunction with the effects of different parameters. Their results showed that more than 80 % crude oil was removed from non-weathered soil samples by the SDS, while rhamnolipid showed similar oil removal at the third and fourth levels of the parameters tested. This approach of soil washing was noted to be effective in reducing the amount of oil in soil. And a further field scale test was required to assess the efficiency of these surfactants. To better understand the process of washing crude oil-contaminated soil with biosurfactants in terms of its chemistry, Urum et al. [89] investigated the removal of crude oil from soil with two biosurfactants (rhamnolipid and saponin) and a synthetic surfactant, SDS. Results showed that SDS removed the most crude oil from soil, followed by rhamnolipid and then saponin. However, a preferential removal of crude oil components from the contaminated soil was observed with the different surfactants. Chemical surfactants were more effective for the aliphatics than aromatic hydrocarbons, whereas biosurfactants had more preference for the aromatic hydrocarbons than for the aliphatic hydrocarbons. This study will help design the surfactant treatment strategy in regard to degree of aromaticity in the crude oil-contaminated soil.

Menezes et al. [90] characterized biosurfactants producing microbial populations from a Long Beach soil, California (USA) and a Hong Kong soil (China), contaminated with diesel oil. Based on surface tension and the E24 index results and 16S rRNA gene sequencing, the strains were identified as *B. cereus*, *B. sphaericus*, *B. fusiformis*, *Acinetobacter junii*, a noncultured bacterium, *Pseudomonas* sp., and *B. pumilus*, respectively. These bacterial isolates displaying substantial potential for production of biosurfactants can be applied in the bioremediation of soils contaminated with petroleum hydrocarbons. Lu et al. [91] investigated the effects of culture conditions in vitro on bacterial strains capable of degrading gasoline from contaminated soils near gas stations. They isolated three strains, *Pseudomonas* sp., *Flavobacterium* sp., and *Rhodococcus* sp., respectively, as efficiently degrading strains. Production of biosurfactant compounds as indicated by the value of surface tension was evident. Some of the

hydrocarbons degraded by three isolates were gasoline, diesel oil, BTEX. The consortium was more effective than the individual cultures in degrading added gasoline, diesel oil, and BTEX. This paper emphasized use strains in consortium has great potential for in situ remediation of soils contaminated by gas station leaking.

Kumar et al. [70] studied the biodegradation of oil by hydrocarbon degrading *P. putida* in the presence of a biosurfactant-producing bacterium. Improved degradation was exhibited by organisms in co-culture, in comparison to the individual bacterium culture in both aqueous and soil matrix. The detailed experiments suggested that the in situ biosurfactant production not only resulted in increased emulsification of the oil but also changed the adhesion of the hydrocarbon to cell surface of other bacterium. This is good work in understanding of interactions between microbes and may provide opportunities for further enhancement of contaminant biodegradation by making a suitable blend for bioaugmentation. Plaza et al. [92] isolated 16 bacterial strains from petroleum hydrocarbon-contaminated soils and screened for biosurfactants/bioemulsifiers production in liquid culture containing crude oil under thermophilic conditions. All isolates reduced surface tension at varying degrees with strains: T/1 resulting in the highest reduction (35 mN/m). This study is more significant for the hydrocarbon remediation as discussed in previous sections because of the thermophilic adaptability of the strains and the biosurfactants produced by them. This paper is an addition to the few reports that are available on biosurfactant-producing strains in thermophilic conditions. Some of the earlier reports by Banat [93], Busscher et al. [94,95], and Zeinali et al. [96] have suggested the existence of the biosurfactant production under extreme conditions. The authors also have done extensive work on biosurfactant production under extreme condition [21,97,98]. This is the one area where more investigations are required for successful application of surfactant-aided bioremediation.

Martienssen and Schirmer [99] have demonstrated the efficiency of surfactant (BioVersal FW) for the in situ remediation of a highly contaminated site at Halle/S. (Germany). They could achieve 50 g hydrocarbons per kg soil elimination during the field-scale investigation over a period of 15 months. Coimbra et al. [100] considered the adhesion ability of cells to porous media as one of the key factors influencing the efficiency of treatment and studied the probable modes of hydrocarbon uptake in cells of *Candida* based on cell hydrophobicity, emulsifying activity, surface tension, and interfacial tension of the cell-free culture medium. They found the potential of yeasts for application in the removal of hydrophobic compounds. Depending on the strain and substrate used, the adhesion ability of yeast cells and the production of surfactants and emulsifiers can take place simultaneously, thus increasing the efficiency of bioremediation treatment of petroleum pollution. This study enhances our understanding of the mechanisms of uptake of insoluble substrates by *Candida* species related to biosurfactant production, aiming to gain knowledge of the potential of such yeasts for future application in the oil industry and in bioremediation. Lai et al. [101] developed a screening method to evaluate the oil removal capability of biosurfactants for oil-contaminated soils collected from a heavy oil-polluted site. They identified the capability of two biosurfactants to remove total petroleum hydrocarbon from soil and compared with that of synthetic surfactants. Their results indicate that biosurfactants exhibited much higher total petroleum hydrocarbon removal efficiency compared to the synthetic ones. The total petroleum hydrocarbon removal efficiency was concentration-dependent in range from 0 to 0.2 mass %, but it did not vary significantly for the contact time of 1 and 7 days.

In the preceding section, we have summarized the findings by various researchers in last decade. The summation of whole section is that microorganisms degrading various types of hydrocarbons are ubiquitous in nature. Many of them usually produce potent surfactants, and these surfactants help them to degrade insoluble substrates. Biosurfactant-aided bioremediation or utilization of hydrocarbons for biosurfactant production is of great interest from the viewpoint of in situ remediation because the biosurfactants excreted at the remediation site will facilitate hydrocarbon degradation. However, understanding the interactions between oil-degrading microorganisms is required, not only when predicting the fate of hydrocarbons in the environment but also for the development of successful bioremediation

process. Authors in this section were able to highlight some of the studies associated with hydrocarbon uptake facilitated by biosurfactants.

CONCLUSIONS AND FUTURE CHALLENGES

Microbial processes responsible for the biodegradation of organic contaminants in environment are the driving forces behind natural attenuation and can be harnessed in enhanced bioremediation technologies such as biostimulation and bioaugmentation. Increasing demands by regulators to provide evidence for bioremediation opens up new applications for the rapidly emerging field of molecular microbial ecology. Recently, more consorted efforts have been made toward the use of bioremediation as an environmentally friendly clean-up approach for the enormous quantities of industrial pollutants that have been released into the environment over the last century. Use of microbial metabolism is one of the environmentally friendly and economic means for eliminating environmental pollutants. The sections above clearly demonstrate that the microorganisms are capable of mineralizing a variety of toxic compounds under laboratory and field conditions. However, the accumulation in the environment of highly toxic and persistent compounds stresses the fact that the natural metabolic diversity of the microbes is insufficient to withstand this challenge. Although bioremediation holds great promise for dealing with intractable environmental problems, it is important to recognize that much of this promise has yet to be realized.

A better knowledge of the metabolic potential of microbes and their products is required. Just relying on the biochemical or microbial ways for the degradation is not sufficient, and deeper investigations are required into the factors, such as bioavailability of the substrates, diffusion and transport of substrates into the cell, the degradation potential, and the site of degradation. This all can be achieved by the combined approach of microbiology, biochemistry, and metabolic and genetic engineering, thus elucidating the microbial metabolic diversity required to understand the metabolic and organismic network necessary for activity under environmental conditions. Modern technologies like microarrays and quantitative polymerase chain reaction target the environmentally important functional genes or specific biodegrading organisms. Integration of these new technologies with existing processes will permit a deeper exploration of the microbial ecology of organisms that make chew on the contaminants, as suggested recently by Wood [103] for the use of metabolic engineering or synthetic biology approaches for construction of designer microbes for their effective clean-up of pollutants. Transcriptome and proteome profiling of the microbes will further improve the effectiveness of microbes in the practical use of bioremediation. The whole concept of using designer microbes is very attractive, but their application is constrained by the interaction with the local microbial population and the ability to retain activity.

Overall, the authors were able to emphasize the use of biosurfactants as a practical solution of the bioremediation of hydrophobic contaminants. However, more efforts are required to understand SBR technologies for organic contaminated soil and water. Recently, Paria [31] stressed that application of surfactants dramatically expedites the remediation process. SBR technologies for organic contaminated sites, is one of the most innovative technologies. Better understanding of the mechanisms involved in the surfactant-aided remediation is required and studies should be focused on adsorption patterns of surfactant to soil, micellar solubilization of organic hydrocarbons, and degradation and partitioning behavior of surfactants onto soil and liquid organic phase. In the end, the overall success of the technology points to economics of the process. Compared to the last decade, biosurfactants are competitive enough now costwise, as they can be produced using renewable substrates. However, more work is still needed for process optimization at the biological and engineering levels.

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