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Biosurfactants as Useful Tools in Bioremediation

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Additional information is available at the end of the chapter

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Abstract

Environmental pollution by organic contaminants is a major problem today because it has affected many environments. Hydrophobic contaminants are of special concern since their molecules can be bound to the soil particles, but because of its low solubility in water and high interfacial tension, those contaminants cannot be easily removed. To help with desorption of contaminants, surfactants can be used in soil and water remediation technologies. Amphiphiles that can form micelles are termed as surface active agents or *surfactants* and are among the most commonly used chemicals in everyday life. Chemically produced surfactants have increasingly been replaced by biotechnology-based products, obtained either by enzymatic or microbial synthesis, because they can be produced using natural resources. The group of surface active biomolecules produced by living organism is called *biosurfactants*. Originally, biosurfactants attracted attention as hydrocarbon-dissolving agents in the late 1960s and as potential replacements for synthetic surfactants (carboxylates, sulfonates and sulfate acid esters) in the food, pharmaceutical, and oil industries. Synthetic surfactants currently used are usually toxic and hardly degraded and as such are also a contaminant in the environment. To replace synthetic surfactants, biosurfactant production needs to be cost-effective; therefore, it is important to develop culture conditions with low-cost materials using efficient biosurfactant-producing microbial strains. Although bacteria have been extensively studied for biosurfactant production, yeasts are also potential biosurfactant-producing microorganisms. Because of their unique structures, biosurfactants may have a greater range of properties that can be

exploited commercially. This review article will describe microorganisms related to biosurfactant production, including yeasts, as well as their role in bioremediation.

Keywords: Biosurfactant, soil yeast, microbial communities in soil, bioremediation

1. Introduction

Recently, there are many reports of soil and surface water locations that are contaminated with organic pollutants, with a great impact on soil and groundwater. Because of its low solubility in water and high interfacial tension, those contaminants cannot be easily removed. Bioremediation has become one of the methods used in the remediation of contaminated sites; bioremediation strategies are based on the use of different microorganisms: bacteria, yeasts, or fungi isolated from soil or from a place where there is a presence of contaminants such as hydrocarbons, which facilitate the cleaning of the contaminated sites. Bioremediation studies begin with the isolation and identification of microorganisms from soil and water that are able to degrade these contaminants. Some hydrocarbon-degrading microorganisms are also able to produce biosurfactants. Biosurfactants produced by the microorganisms in the environment help them to take the hydrocarbons as carbon source, either by making available the hydrocarbon by releasing biosurfactant into the environment or by changing its cell surface so that the contaminant can be absorbed.

Originally, biosurfactants attracted attention as hydrocarbon dissolving agents in the late 1960s as potential replacements for synthetic surfactants (carboxylates, sulfonates, and sulfate acid esters), especially in the food, pharmaceutical, and oil industries. Synthetic surfactants currently used are usually toxic and hardly degraded by microorganism, causing damage to the environment. Most of the biosurfactants are high molecular weight lipid complexes, which are normally produced under aerobic conditions. The classification of biosurfactants is based on their chemical composition, their mode of action, and the microorganisms that produce it. Biosurfactants can be of high or low molecular weight, and based on their composition, they can be glycolipids, phospholipids, lipopeptides, or a mixture of amphiphilic polysaccharides, proteins, lipoproteins, or lipopolysaccharides. Microorganisms also produce surfactants that are in some cases a combination of many chemical types referred to as the *polymeric microbial surfactants*. Because of their unique structures, biosurfactants may have a large range of properties that can be exploited commercially.

Regarding their mechanism of action, some compounds are better at decreasing the surface tension (biosurfactants), and others are able to produce stable emulsions (bioemulsifiers). Best known biosurfactants are produced by bacteria, and there are many studies on them, especially on *Pseudomonas* spp., strains that produce rhamnolipids. However, it is necessary to find new types of biosurfactants and bioemulsifiers, and the studies of other organisms are increasing recently. Yeast and fungi have demonstrated to produce biosurfactant and bioemulsifiers with very good results. The aim of this chapter is to describe microbial biosurfactant, especially those produced by yeasts and to propose their use in bioremediation.

2. General characteristics of biosurfactants

Recently, there are many reports of soil and surface water locations that are contaminated with organic pollutants, with a great impact on soil and groundwater. During this process, molecules of the pollutant have bound to the soil particles as it has moved toward the groundwater and are therefore difficult to remove it from soil because many of these pollutants have low solubility and high interfacial tensions with water [1].

To help on the solution of this problem, surfactants can be used to clean the contaminated soil and water. Surfactants are one of the most commonly used chemicals in everyday life. Since the beginning of the 20th century, the production of a wide spectrum of synthetic surfactants from petroleum resources has increased intensively. The amphiphiles that can improve surface–surface interactions by forming micelles are termed as surface active agents or *surfactants* [2]. Surfactants are amphiphilic molecules consisting of a hydrophobic and a hydrophilic portion [3]. Usually, the hydrophobic portion is a nonpolar long chain of fatty acids, whereas the hydrophilic domain can be nonionic, positively or negatively charged, or amphoteric, frequently a carbohydrate, an amino acid, or a phosphate [4–6].

Increasing concentrations of surfactant into an oil/water or water/air systems causes a reduction in surface tension up to a critical point where the surfactant can form structures like micelles, bilayers, or vesicles. This concentration defines the critical micelle concentration (CMC). To determine this value, the solution containing the surfactant is diluted severalfold; surface tension is measured for each dilution, and the CMC is calculated from this value. Surface tension can be easily measured with a tensiometer. There are surfactant molecules that are able to reduce the surface tension of water from 72 to around 27 mN m⁻¹ [7]. When water, oil, and a surfactant are mixed, the surfactant rests at the water–oil interface; these systems are called *emulsions* or *microemulsions* depending on their stability [8, 9]. These characteristics confer excellent detergency and emulsifying, foaming, and dispersing capacities, which make surfactants one of the most versatile chemicals in industrial processes [10].

Current, worldwide surfactant market is around \$9.4 billion annually, while their production has been reported to be approximately 10 million tons, and their use is divided almost equally between household detergents and several industrial applications [10, 11]. Synthetic surfactants have increasingly been replaced by biotechnology-based compounds, derived either from enzymatic or microbial synthesis, because they can be produced using natural sources [12]. The group of surface active biomolecules produced by living organism is called *biosurfactants*.

Synthetic surfactants currently used are toxic and hardly degraded, causing damage to the environment. Initially, biosurfactants were considered to have applications in the food, pharmaceutical, and oil industries [13–15]. Biosurfactants have several advantages over chemical surfactants, including lower toxicity, higher biodegradability, effectiveness at extreme temperatures or pH values, biocompatibility, and digestibility. Also, biosurfactants can be produced using agroindustrial waste material; they can be economically produced and show better environmental compatibility. The microorganisms that produce the biosurfactant

can be modified by genetic engineering or biological and biochemical techniques. Because the possibility of practical applications for biosurfactants depends on whether they can be produced economically, there have been many efforts to optimize its biological production [16–18]. To replace synthetic surfactants, biosurfactant production needs to be of low-cost, and up to now, there are few studies on the use of low-cost materials on the pilot plant or industrial scale [4].

Most of the biosurfactants are lipid-containing molecules, which are normally produced under aerobic conditions [16]. The classification of biosurfactants is based on their chemical composition, their mode of action, and the microorganisms that produce it. Biosurfactants can be of high or low molecular weight, and based on their composition, they can be glycolipids, phospholipids, lipopeptides, or a mixture of amphipathic polysaccharides, proteins, lipoproteins, or lipopolysaccharides. Biosurfactants with low molecular mass are efficient in lowering surface and interfacial tensions, whereas biosurfactants with high molecular mass are more effective at stabilizing oil-in-water emulsion (Figure 1) [19]. Microorganisms also produce surfactants that are in some cases a combination of many chemical types referred to as the *polymeric microbial surfactants* [8].

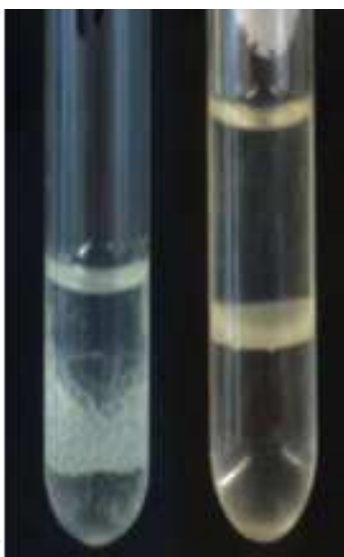


Figure 1. Stable emulsions (EI_{24}) produced by mixing a cell-free supernatant from a biosurfactant-producing yeast and hexadecane. The tube on the left shows a clear emulsion characteristic of polymeric biosurfactants. The tube on the right shows a compact stable emulsion that is characteristic of low molecular weight biosurfactants.

Low molecular weight biosurfactants are usually glycolipids or lipopeptides; the later are usually produced by bacteria from the *Bacillus* genus and is composed of a cyclic peptide and a fatty acid residue. Among the glycolipids, the most studied is rhamnolipid, which is produced by *Pseudomonas aeruginosa* strains; it is composed of a backbone of two rhamnose moieties and two fatty acid residues. Other glycolipid biosurfactants include trehalolipids, produced by *Rhodococcus erythropolis* and other bacterial genera, and sophorolipids, produced by several yeast strains (Figure 2a and 2b). The physicochemical properties of low molecular weight biosurfactants are influenced by the fatty acid residues that contain, and those in fact

depend on the bacterial strain used and on the growth conditions and nutrients present. High molecular weight biosurfactants are usually a complex mixture of macromolecules containing proteins, polysaccharides, and lipid residues. The most studied polymeric biosurfactant is emulsan, produced by *Acinetobacter calcoaceticus* (Figure 2c) [1, 10]. Because of their unique structures, biosurfactants may have a greater range of properties that can be exploited commercially [20].

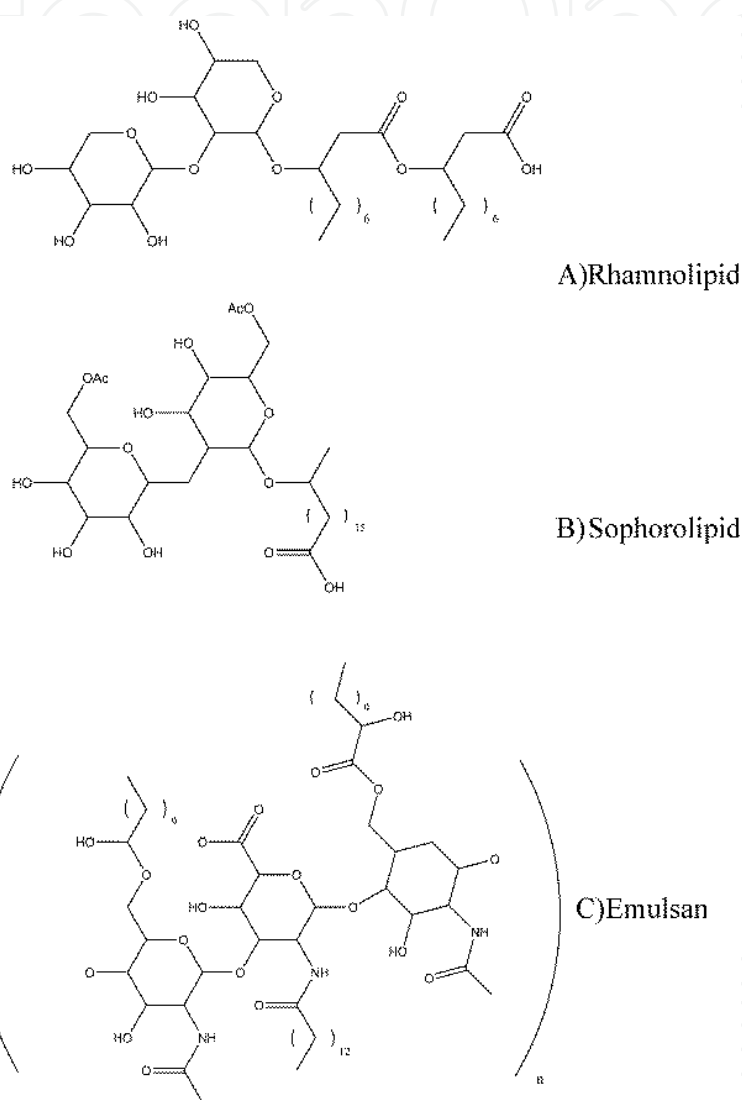


Figure 2. Chemical structures of some of the most common biosurfactants. Low molecular weight glycolipids: (a) rhamnolipid and (b) sophorolipid; high molecular weight glycolipids: (c) emulsan.

3. Biosurfactant-producing microorganisms

The ability of microorganisms to degrade hydrocarbons was first described in 1895 by Misyoshi, who reported the microbial degradation of paraffin. Many different microbial

species of bacteria, yeast, and mold are capable of degrading hydrocarbons, and bacteria are the best described biosurfactant producer [21]. The exact reason why some microorganism can also produce biosurfactants is still not clear [22].

Bushnell and Hass (1941) were the first to demonstrate the bacterial production of biosurfactants, using a strain of *Corynebacterium simplex* and a strain of *Pseudomonas* grown in a mineral media containing kerosene, mineral oil, or paraffin. Since then, numerous studies on the structure and mechanisms involved in the production and action of biosurfactants have been reported [22]. It can be stated that biosurfactants are produced by a variety of microorganisms, and there is also a wide variety on the chemical composition and nature of the biosurfactant produced, as well as on the location (membrane-bound, extracellular) of the produced molecule [23]. The most reported genera of biosurfactant-producing bacteria include *Pseudomonas* sp., *Acinetobacter* sp., *Bacillus* sp., and *Rhodococcus* sp., among others. Table 1 shows some of the most studied bacteria and the type of biosurfactant produced.

Microorganism	Biosurfactant	Reference
<i>Pseudomonas aeruginosa</i>	Rhamnolipids	[24]
<i>Pseudomonas fluorescens</i>	Ornithine lipids	[25]
<i>Pseudomonas stutzeri</i>		[25]
<i>Pseudomonas cepacia</i>		[25]
<i>Acinetobacter calcoaceticus</i>	Lipopolysaccharides (biodispersant)	[10]
<i>Acinetobacter radioresistens</i>	Heteropolysaccharide protein (alasan)	[10]
<i>Bacillus subtilis</i>	Lipopeptides and lipoproteins (surfactin)	[16]
<i>Bacillus licheniformis</i>	Lipopeptides (lichenysin)	[5]
<i>Rhodococcus erythropolis</i>	Trehalolipids	[26]
<i>Mycobacterium</i> sp.		[2]
<i>Nocardia</i> sp.		[5]
<i>Tsukamurella</i> sp.	Di- and oligosaccharide lipids	[27]

Table 1. Biosurfactant-producing bacteria

Microorganisms that produce biosurfactants are isolated mainly from sites that are or were contaminated with petroleum hydrocarbons: contaminated soils, effluents, and wastewater sites. Thus, these have an ability to grow on substrates considered potentially noxious for other nonbiosurfactant-producing microorganisms. Biosurfactants play a physiologic role in increasing bioavailability of hydrophobic molecules, which are involved in cellular signaling and differentiation processes, which facilitate the consumption of carbon sources present in soil [23, 28].

The physiological role of biosurfactants is not clear yet, but it might be related to an increase in the nutrient uptake of hydrophobic substrates, in enhancing the growth on hydrophobic

surface, and in cellular motility and biofilm formation by reducing the surface tension at the phase boundary [10, 15]. The mechanism of uptake of liquid hydrocarbon substrates by microbial cells involves interfacial phenomena. The significant influence on the biodegradation process is observed after the addition of surface active compounds [21]. Another physiological role of biosurfactants can be their observed antimicrobial activity [3].

Biosurfactants are produced predominantly when hydrophobic substrates provided as carbon source is used for microbial growth; they can be either secreted extracellularly or attached to the microbial cell wall. On the contrary, some microorganisms may produce biosurfactants in the presence of different types of substrates, including carbohydrates and other water soluble compounds. It has been reported that the carbon source used for biosurfactant production influences the structure of the compound produced by the microorganism. It is also affected by nitrogen sources as well as by the presence of minerals such as iron, magnesium, manganese, phosphorous, and sulfur [3, 23]. This capacity of modification of the biosurfactant molecule by the composition of the culture media can be used to produce compounds with specific applications. Industrial production of microbial metabolites is a very complex process, and for industrial production, many variables are needed to be considered; in the case of biosurfactants, media composition is a key element to control yield and specific productivity [29]. The success of biosurfactant production depends on the development of cheaper processes and the use of low-cost raw materials, which account for 10% to 30% of the overall production cost. The literature shows that a wide range of carbon sources, including agricultural renewable resources, like sugars and oils, are suitable carbon sources for production of ecologically safe biosurfactants with good properties [30]. The use of agroindustrial waste products such as bagasses, molasses, and plant material residues can be good candidates for use in biosurfactant production.

Interest in microbially produced biosurfactants has led to a need for the further development of rapid and efficient qualitative and quantitative methods for screening and analyzing biosurfactant-producing microorganisms [20]. The development of rapid and reliable methods for screening and selection of microbes from thousands of potentially active organisms and the subsequent evaluation of surface activity holds the key for the discovery of new biosurfactants. Among the most important characteristics needed for rapid screening methods is the ability to identify microorganisms capable of biosurfactant production in large culture collections, as well as the use of reliable methods to quantify the compounds produced [31].

4. Biosurfactant-producing yeast

Yeasts are unicellular cells of dimorphic fungi that are usually classified in the subdivision Ascomycotina and Basidiomycotina. They are ubiquitous in most environments, although they are more related to sites with high organic matter content and/or high water availability. They have been isolated from leaves, flowers and fruits, trees exudates, insects, soils, and other natural environments. Nowadays, approximately 100 genera and 700 species of yeast have been classified based on their morphological, physiological, and biochemical characteristics.

The most frequently isolated yeast genera from soils are *Candida*, *Cryptococcus*, *Debaryomyces*, *Hansenula*, *Lipomyces*, *Pichia*, *Rhodotorula*, *Schizoblastosporion*, *Sporobolomyces*, *Torula*, and *Torulopsis* [32, 33]. Yeasts are involved in the production of a wide variety of foods, including fermented foods, alcoholic beverages, and bread. Yeasts are also involved in industrial fermentations for the production of antibiotics and vitamins among other commodities [34].

There are only few studies on biosurfactants synthesized by yeasts because most reports are related to bacteria and marine microorganisms, but the number of reports has increased, especially for *Candida* sp., *Pseudozyma* sp., and *Yarrowia* sp. [35]. Table 2 shows yeast strains and the type of biosurfactant produced.

Microorganism	Biosurfactant	Reference
<i>Candida bombicola</i> (most studied system)	Sophorolipid	[36]
<i>Candida apicola</i>		[37]
<i>Candida rugosa</i>		[15]
<i>Candida mucilaginos</i>		[15]
<i>Rhodotorula bogoriensis</i>		[38]
<i>Pichia anomala</i>		[39]
<i>Candida lipolytica</i>	Carbohydrate–protein (Liposan)	[40]
<i>Yarrowia lipolytica</i>	Carbohydrate–protein–lipid complex (Yansan)	[41]
<i>Saccharomyces cerevisiae</i> 2031	Mannoprotein	[42]
<i>Pseudozyma</i> (<i>Candida antarctica</i>)	Mannosylerythritol lipids	[43]
<i>Pseudozyma rugulosa</i> NBRC 10877		[44]
<i>Pseudozyma churashimaensis</i>		[45]
<i>Schizonella malanogramma</i>	Erythritol and mannose lipid	[10]
<i>Ustilago maydis</i>		[10]

Table 2. Biosurfactant-producing yeast

Yeasts can be preferred to bacteria as sources for biosurfactants because of their GRAS (generally regarded as safe) status, that is, they do not present risk of inducing toxicity or pathogenic reactions. Yeasts are also known for producing biosurfactants in higher concentrations than bacteria, which is an advantage for the development of production schemes [28, 46]. On the other hand, when comparing bacteria and filamentous fungi to yeast, the latter has many advantages, including faster growth rate than filamentous fungi; still, they can resist unfavorable environments such as filamentous fungi, being useful in biological treatment of effluents [47].

Yarrowia lipolytica was the first yeast used experimentally for the degradation of aliphatic hydrocarbon; this yeast also produces a highly efficient emulsifier [48]. Most of the biosurfactants produced by yeasts are better emulsifiers than biosurfactants, mainly because of the chemical structure of the molecules [49]. The widespread occurrence of yeasts with hydrocarbon-degrading activities has been extensively investigated. *Candida* species, especially *Candida lipolytica*, has been isolated from diesel oil storage tanks and fuel systems. *Candida tropicalis* and *Candida maltosa* are also noted for their use of saturated hydrocarbons. *Debaryomyces hansenii* and *Candida guilliermondii* can grow on hydrocarbons and have been isolated from hydrocarbon contaminated sites. There are many reports on the metabolism of hydrocarbons from yeasts, but very few information on the metabolites is produced [50].

The influence of the carbon source in biosurfactant production has been extensively studied in some microorganisms. For the study of yeast, different types of carbon sources have been used, depending on the yeast strain. For example, Silva et al. [51] found that biosurfactants produced using vegetable and mineral oils have different stability properties when incorporated into aqueous solutions, with better stabilization properties when vegetable oil was used. Daverey et al. [52] reported that a *C. bombicola* strain can produce sophorolipids when growth on a mixture of hydrophobic and hydrophilic substrates. Amaral et al. [53] reported that for the production of Yansan by *Y. lipolytica*, it is important to use glucose as carbon source.

Changes in yeast cell hydrophobicity have been related to the ability of the microbial strain to degrade hydrocarbons [54]. Amaral et al. [41] observed that the interaction of *Y. lipolytica* cells with hydrophobic surfaces is mediated by proteins or glycoproteins present in the cell wall. Furthermore, they suggested that van der Waals forces were involved in the interactions between the yeast cell surface and the nonpolar solvent and biosurfactant production improved these interactions. Regarding biosurfactant chemical properties, yeast biosurfactants maintained their functionality at different pH values as well as over a wide range of temperatures [55]. *Pichia anomala* and other yeasts are thermophilic, and so their biosurfactants could have a wide range of industrial applications [39].

5. Role of biosurfactants in bioremediation

Waste or used lubricating oils have caused a serious environmental problem because once in the environment, it can bind to organic matter, mineral particles, and organisms, with the consequent persistence and toxicity of oil components in the environment. Research on the interaction between hydrocarbon and microorganisms has supported the hypothesis that petroleum and its derivatives are subjected to microbial degradation. In the environment, with the presence of emulsifying agents, hydrocarbons are more bioavailable for degradation; it has been observed that the greater the oil–water interface of hydrocarbons, the faster the rate of decomposition by the microbial community present [56].

Bioremediation can be done in two different ways: *in situ* and *ex situ*. The *ex situ* process can be carried out in a prepared bed or in a slurry reactor system. *In situ* processes are usually accomplished by the addition of microbial nutrients to the soil, which allows considerable

growth of soil microbial indigenous population [16]. Biodegradation efficiency depends on the ratio of hydrocarbon-degrading microorganisms in soil, the composition and physical state of hydrocarbon mixture and oxygen availability, and the condition of water, temperature, pH, and inorganic nutrients. The physical state of the hydrocarbon can also affect biodegradation. In addition, the biodegradation of hydrocarbon in bioremediation might be enhanced by the addition of surfactant. For use in bioremediation procedures, biosurfactants are more promising than synthetic surfactant because they are produced by microorganisms in soil and are commonly considered as low- or nontoxic compounds [57, 49].

Bioremediation involves the acceleration of natural biodegradative processes in contaminated environments by improving the availability of materials (e.g., nutrients and oxygen), conditions (e.g., pH and moisture content), and prevailing microorganisms [58]. Biosurfactants can improve bioremediation effectiveness by the following two mechanisms. The first mechanism includes the increase of substrate bioavailability for microorganisms; for bacteria growing on hydrocarbons, the growth rate can be limited by the interfacial surface area between water and oil. When the surface area of microorganisms with hydrophilic solvents like water is limiting, biomass increases arithmetically rather than exponentially. The second mechanism involves interaction with the cell surface, which increases the hydrophobicity of the microbial cell wall, allowing hydrophobic substrates to associate more easily with bacteria [1, 19]. Microbial cell hydrophobicity can be described as the affinity to adhere to hydrophobic substrates, such as hydrocarbons. This capacity can give the microbial cells the ability to better degrade hydrocarbons, and it can be a factor to understand microbial biodegradation rate differences [54]. The increase of microbial adhesion to hydrocarbons is directly related to the ability of such microorganisms to grow in the medium where hydrocarbons or other hydrophobic substrates are present [56]. If the biosurfactant compound is bound to the microbial cell wall, the cell surface will be more hydrophobic. Microorganisms can use their biosurfactants to regulate their cell-surface properties to attach or detach from surfaces accordingly to their needs [1].

There are many research reports dealing with the degradation of hydrocarbons and production of biosurfactants by microorganisms, as stated in Section 3, and there are some *in field* reports on the use of biosurfactants for bioremediation. For example, Thavasir et al. [59] demonstrated the enhanced degradation of hydrocarbons by the addition of biosurfactants to the culture media, as well as the enhancement of degradation by the addition of mineral nutrients (fertilizers). There are also reports on the identification and characterization of biosurfactant-producing microorganisms, including some genera not usually related to bioremediation, such as *Staphylococcus*. Studies include the determination of functional characteristics of the biosurfactants produced and their potential use in bioremediation [60]. Also, there are reports on the production of biosurfactants by microorganisms isolated from particular environments, such as marine sediment, that could be helpful in the bioremediation of those particular sites [61]. Furthermore, the efficiency of different surfactant solutions in removing crude oil from contaminated soil has been tested. Urum et al. [62] demonstrated the efficiency of surfactant solutions used in a soil-washing process. The synthetic surfactant SDS (sodium dodecyl sulfate) was as efficient as a biosurfactant derived from bacteria (rhamnolipid), and both were more efficient than saponins.

There are some recent literature reviews on the production and use of biosurfactants for bioremediation [63–66], but in those revisions, there are only few cases described where biosurfactants have been used on bioremediation processes at pilot-scale or field-scale studies [64]. Calvo et al. [63] focused on the need for the optimization of biosurfactant production and the tools from molecular biology that can be used to obtain hyperproducing microbial strains. This approach leads to the strategy of producing the biosurfactant and then using it to amend contaminated sites [62]. The question remains if it is possible to inoculate biosurfactant-producing microorganisms in contaminated sites and then promote the production of tensioactive agents on site so that it can be a continuous source of biosurfactant.

Sachdev and Cameotra [66] proposed that biosurfactant-producing microorganisms might have different roles in soil, which can help on agricultural production. They described the use of biosurfactants for the recovery of organic pollutant contaminated soil, with the consequent improvement in the plant-microbiota beneficial interactions, but they also suggested that biosurfactants can be used to disperse fertilizers. Considering the antimicrobial effect of some tensioactive molecules, the authors also suggest that biosurfactants can help on the control of phytopathogens.

A recent review [65] has a more critical point of view on the efficiency of biosurfactants on bioremediation. The authors did a critical analysis of reports on the use of biosurfactants and described that there are many cases on the amendment of contaminated soil with biologically synthesized surfactants showing no differences with control experiments or even showing negative results. A question that needs to be addressed is the variability of experiments reported, as well as the actual role of biosurfactants in noncontaminated environments. As with many other biological processes where the microorganisms are taken from their natural habitats and places in restricted and controlled environments, the contribution of a particular metabolite can be misled. This has always been a major concern in environmental microbiology because there are still few methods that can help us on the understanding of the actual interactions of microbiota in their environments. Therefore, questions about the role of a particular metabolite in the microhabitat and the concentration of such compound in nature are still unanswered.

6. Conclusion

This chapter presents a description of biosurfactants and their uses in bioremediation. Biosurfactants are molecules produced by microorganisms that help them on the absorption and degradation of hydrophobic compounds. Bacterial strains have been extensively studied for biosurfactant production, but recent studies have also reported production of biosurfactants by yeasts. Yeasts are considered as GRAS microorganisms and are often used in the food and pharmaceutical industries and also in bioremediation.

The addition of chemical surfactants to enhance biodegradation efficiency in bioremediation processes is not acceptable because of its toxicity and persistence in the environment; hence, it is better to use biosurfactants. However, to use biosurfactants in bioremediation, the

optimization of large-scale production is needed, as well as studies on the use of alternative carbon sources derived from agroindustrial wastes. Also, it is important to evaluate the possible on-site production of biosurfactants in contaminated sites to expedite contaminated soil restoration.

It is then necessary to continue the isolation of biosurfactant-producing microorganisms on the characterization of their metabolites, on the identification of factors and conditions for production optimization, and on their use in field studies. As with any important subject in science, there are more questions than answers; research in biosurfactants for use in bioremediation of contaminated sites is still an innovative subject that needs more reliable scientific data.

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