

Microbial biosurfactants: prospects of sustainable molecules with promising applications in bioremediation

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Abstract

The dispersal of various chemical pollutants in our environment requires the development of effective approaches that would lead to the bioremediation of contaminated areas. The biological methods such as the application of microbial biosurfactants represent one of the suitable solutions that has gained large attention due to their structural diversity, molecular sizes, and multifunctional characteristics. Thus, they are likely to be applied in the field of bioremediation, where they can lower the surface and interfacial tensions at different interfaces leading to removal of pollutants or improve their biodegradation and bioavailability. The presented review highlights the recent advances and knowledge on the important aspects of microbial biosurfactant applications in bioremediation.

Key words: bioremediation, biosurfactants; chemical pollutants, microbial products.

Abbreviations: CMC, critical micellar concentration; MEOR, microbial enhanced oil recovery; PAHs, polycyclic aromatic hydrocarbons.

Introduction

Human development and greater living standards have been promoted by industrialization. On the other hand, these benefits and human activities have damaged the environment (Gómez-Sagasti et al. 2016). Therefore, pollution causes numerous health disorders and deaths (Doney et al. 2012). In our life, we use many chemicals in consumer products, drugs, industrial solvents, fertilizers, pesticides, food additives, and fuels. However, industrialization is indispensable for faster growth in developing countries, and its concomitant pollutants should not be left in the environment without sustainable management (Das, Dash 2014).

There are several processes to treat these pollutants, such as chemical methods that can only be used under some conditions, physical and biological procedures, but biological processes are more effective, economical, and environmentally friendly than chemical and physical methods. (Paul et al. 2005; Chauhan et al. 2008; Bisht et al. 2015). Therefore, biological processes have attracted much interest in comparison with other physicochemical treatments (Rizi et al. 2012). Furthermore, many studies have shown that the use of pollutant-degrading microbes is increasingly beneficial (Wentzel et al. 2007; Liu et al. 2016). Numerous studies have reported that microorganisms produce diverse metabolic components that can safely remove pollutants by either direct chemical destruction

or conversion of pollutants into a less toxic intermediate (Dash, Das 2012).

Biosurfactant production is among the effective pathways for microorganisms to remove contaminants (Shekhar et al. 2015). These surface-active hydrophobic metabolites are complex biomolecules (glycolipids, exopolysaccharides, proteins, amino acids, fatty acids, etc.) with amphiphilic structures (Ibrahim et al. 2013).

Moreover, biosurfactants can decrease liquid surface/interface tension (Banat et al. 2010), solids, and gases (Satpute et al. 2010). They present many remarkable characteristics in comparison with chemical surfactants, such as biological origin, biodegradability, and a low level of toxicity (Sharma et al. 2016a). These properties can result in inexpensive production of biosurfactants, exploit waste substrates, and simultaneously reduce their polluting effect (Kosaric 1992; Das et al. 2008a). For these reasons, they can also be used in different fields, such as cosmetic, food, textile, pharmaceutical, mining, varnish, and oil recovery industries (Das et al. 2008a; Henkel et al. 2012; Marchant, Banat 2012a).

Significant research on the production and application of biosurfactants has been conducted in recent decades. This review discusses the outcomes of the research related to their diversity and uses. We present general information on biosurfactants production, applications, and their role in the bioremediation and biodegradation of pollutants.

Biosurfactants: chemical structure and classification

Microorganisms produce amphipathic chemicals called biosurfactants, which can modify the surface structure. They are employed in dispersion, foam forming, emulsification, stabilization, wetting, and other commercial activities (Ismail et al. 2013; Pereira et al. 2013). In the natural environment, biosurfactants often occur among degrading bacteria, and by reducing surface and interfacial tension, they can accelerate the mobility, solubility, bioavailability, and biodegradation of pollutants (Aparna et al. 2011; Bezza, Chirwa 2015). Biosurfactants are amphiphilic molecules with a hydrophilic head and a hydrophobic tail. Regarding the biosurfactant structure, mono-, oligo-, or polysaccharides, peptides, or proteins make up the hydrophilic moiety, while saturated, unsaturated, and hydroxylated fatty alcohols or fatty acids make up the hydrophobic group (Rajkumar et al. 2012).

Biosurfactant classification is mostly based on the biosurfactants' chemical composition and origin (Sharma et al. 2016b). They are divided into two categories: (a) surfactants derived from microorganisms, such as glycolipids (rhamnolipids, trehalolipids, and sophorolipids), lipopeptides, surfactins, lichenysin, and phospholipids (Vijayakuma, Saravanan 2015; Gudiña et al. 2016), and (b) surfactants derived from plants such as saponin (Kommalapati et al. 1996; Zhou et al. 2013; Iglesias et al., 2014; Blyth et al. 2015). Biosurfactants are low-molecular-weight surface-active agents that efficiently lower surface and interfacial tension; this group includes glycolipids, lipopeptides, and phospholipids; bioemulsifiers are high-molecular-weight polymers that are more effective as emulsion-stabilizing agents (Xia et al. 2014; Saenz Marta et al. 2015).

Glycolipids

Regarding their high surface activity, glycolipid biosurfactants, produced mainly by *Pseudomonas aeruginosa* as rhamnolipids, have received the most attention. This well-understood, easy-to-cultivate microorganism produces them in relatively high quantities after relatively short incubation periods. In addition to that, they are among the virulence factors related to the pathogenesis of *Pseudomonas aeruginosa* infections (Abdel-Mawgoud et al. 2011). Rhamnolipids are mainly composed of a carbohydrate group linked to fatty acids (Mnif, Ghribi 2016). The carbohydrate portion consists of mono-rhamnolipid or di-rhamnolipid, and the rhamnose moieties are linked together by an α -1,2-glycosidic linkage. The non-glucosidic part consists mostly of one or two (in rare cases three) β -hydroxy fatty acid chains (saturated, mono-, or polyunsaturated with chain length ranging from C_8 to C_{16}) attached via an ester bond between the β -hydroxyl group of the distal (relative to the glycosidic bond) chain and the carboxyl group of the proximal chain

(Abdel-Mawgoud et al. 2010). Rhamnolipid production is necessary for metabolic pathways and gene regulation, and contribute to the diversity of microbial species (Müller et al. 2012).

Sophorolipids are another type of glycolipids mostly synthesized by yeasts such as *Candida* species and consist of a dimeric sugar attached by a glycosidic bond to a hydroxyl fatty acid where the fatty acid configuration and carbon chain length of a given sophorolipid can differ depending on the source of carbon used to produce it (Díaz De Rienzo et al. 2015). The sugar portion represents a sophorose molecule that can be acetylated in two locations, and the carbon chain is 16 to 18 atoms in length, includes one or more double bonds, or is saturated. Depending on the organization of the fatty acid chain, there are two major sophorolipid subtypes: open-chain characterizes the acidic sophorolipids that terminate in a carboxylic acid group, whereas a closed-ring structure characterizes the lactonic sophorolipids where the chain is reattached to the sophorose molecule (Lydon et al. 2017). In addition to the above two mentioned glycolipids, trehalolipids are a class of glycolipids synthesized by *Rhodococcus*, *Corynebacterium*, *Mycobacterium*, and *Nocardia*, containing a non-reducing disaccharide with two glucose molecules linked together via an α , α -1,1-glycosidic link, and long-chain fatty acids of mycolic acid esterified to the C6 position of each glucose (Franzetti et al. 2010; Karlapudi et al. 2018).

Lipopeptides

Lipopeptides are among the best-known biosurfactants and are primarily produced by *Bacillus* species. Their structure consists of a peptide moiety linked to fatty acid (Das et al. 2008b). Surfactin remains the most well-reported lipopeptide, which consists of a heptapeptide conjugated with a β -hydroxy fatty acid of chain length 12 to 16 carbon atoms generating a cyclic lactone ring structure (Seydlová, Svobodová 2008). Furthermore, there are other described families of lipopeptides, including fengycins, iturins, kurstakins, bacillomycins, and mycosubtilin (Bezza, Chirwa 2017).

Fatty acids, phospholipids, and neutral lipids

Fatty acids, phospholipids, and neutral lipids, which are considered biosurfactants, are produced as extracellular metabolites by the microbial oxidation of alkanes, which allows uptake of hydrophobic substrates by microorganisms (Rehm, Reiff 1981; Sharma et al. 2016b). A glycerol unit esterified to two fatty acids and one phosphate group is present in all phospholipids, and the length of the hydrocarbon chain is related to the hydrophilic/lipophilic balance (Cirigliano, Carman 1985; Ahmadi-Ashtiani et al. 2020). The most common fatty acids are corynomycolic acids and other hydroxy fatty acids that have been demonstrated to be far more efficient biosurfactants than simple fatty acids (Ahmadi-Ashtiani et al. 2020). Lipophilic

compounds such as triacylglycerols, diacylglycerols, wax esters, and polyhydroxyalkanoates are part of extracellular neutral lipids produced by degrading marine bacteria (Nakano et al. 2012). The majority of phospholipids are phosphatidylethanolamines (Gautam, Tyagi 2006; Ahmadi-Ashtiani et al. 2020).

Polymeric biosurfactants

The polymeric biosurfactants emulsan, biodispan, alasan, and liposan are widely recognized as emulsifiers (Fenibo et al. 2019). Of these, emulsan is the best reported and is primarily made up of a heteropolysaccharide backbone to which fatty acids are covalently attached via o-ester linkages. The polysaccharide part has different sugar forms including D-galactosamine, D-galactosaminuronic acid, D-glucose, L-rhamnose, D-mannose, and D-glucuronic acid (Das et al. 2008b), whereas liposan consists of a mixture of carbohydrate and protein (Sharma et al. 2016b).

Biosurfactant-producing microorganisms

After microbial growth reaches a high cellular density, biosurfactants are usually produced in the exponential or stationary phase (Suwansukho et al. 2008). They might be found inside cells (intracellular) or secreted outside of cells (extracellular) (Antonioni et al. 2015). Microbial biosurfactants play a key functional role in the uptake of hydrophobic substrates by microorganisms; they are also involved in each phase of the biofilm formation by enhancing motility to avoid cell adherence to the substrates. Additionally, the fluid channels that allow oxygen and

nutrient circulation inside the biofilm and the breakdown product elimination are maintained by biosurfactants. The ability of biosurfactants to reduce the surface tension of the surfaces promotes different types of motility of bacteria such as swarming and twitching (Kumar, Das 2018).

Many biosurfactants have been produced by bacteria from many different genera, including *Pseudomonas*, *Bacillus*, *Mycobacterium*, and *Acinetobacter* (Banat et al. 2010), which can produce biosurfactants naturally or during stress response (Shekhar et al. 2015). Actinomycetes and fungi, in addition to bacteria, produce biosurfactants (Atlas 1995), such as yeast *Candida* production of sophorolipids (Kurtzman et al. 2010; Van Bogaert et al. 2011), and among the best-described biosurfactants produced within the class Actinobacteria are glucose-based glycolipids, the majority of which have a hydrophilic backbone made up of glycosidic-linked glucose units that create a trehalose moiety (Anandan, Dharumadurai 2016).

Applications of biosurfactants in environmental biotechnology

The Biosurfactants are products with vast industrial potential (bioremediation, cosmetics, production of food) and pharmaceutical applications (Magalhães, Nitschke 2013). In different aspects of environmental biotechnology, oil residue recovery biosurfactants can be applied in storage tanks, other oil recovery processes, oil spill cleanup, and bioremediation for soil and water (Sobrinho et al. 2013; Silva et al. 2014). As shown in Table 1, *Pseudomonas* and *Bacillus* species are most commonly

Table 3. Biosurfactant classification, microbial origin, and environmental applications

Microbial origin	Biosurfactant type	Environnemental application	Reference
<i>Rhodotorula</i> sp.YBR	Glycolipoprotein	Hydrocarbon removal from contaminated soils. Enhanced microbial oil recovery from polluted sand	Derguine-Mecheri et al. 2021
<i>Halomonas pacifica</i>	Lipopeptide	Hydrocarbons remobilization and naphthalene degradation. Removal of used motor oil from contaminated soils	Cheffi et al. 2020
<i>Pseudomonas aeruginosa</i>	Rhamnolipid	Microbial-enhanced oil recovery. Enhancing solubilization and biodegradation of slowly desorbing polyaromatic hydrocarbons	Câmara et al. 2019; Posada-Baquero et al. 2019
<i>Pseudomonas aeruginosa</i> S5	Rhamnolipid	<i>In situ</i> remediation of polycyclic aromatic hydrocarbons	Sun et al. 2019
<i>Bacillus licheniformis</i>	Lichenysin	Microbial enhanced oil recovery	Phetcharat et al. 2019
<i>Streptomyces</i> spp.	Unkown	Naphthalene and crude oil degradation. Petroleum degradation ability	Baoune et al. 2018
<i>Paenibacillus dendritiformis</i>	Lipopeptide	Pyrene biodegradation enhancement	Bezza, Chirwa 2017
<i>Bacillus amyloliquefaciens</i> and <i>Bacillus subtilis</i>	Surfactin	Engine oil degradation. Microbial enhanced oil recovery	Sharma et al. 2018
<i>Pseudomonas aeruginosa</i>	Rhamnolipid	Remediation of zinc and cadmium polluted soil	Bendaha et al. 2016
<i>Burkholderia cenocepacia</i>	Glycolipid	Enhanced pesticide solubility	Wattanaphon et al. 2008

reported as biosurfactant producers and can be effectively applied in different bioremediation technologies. The main environmental applications are detailed in the following sections.

Removal of heavy metals

In the chemical galaxy, the two major environmental pollutants are heavy metals and polycyclic aromatic hydrocarbons (PAHs) (Dash et al. 2013; Raj et al. 2014). The problem of heavy metal pollution is highly associated with its toxicity to plants, animals, and people and its lack of biodegradability (Singh, Prasad 2015). Also, heavy metals cause multiple types of biological system malfunctions and can cause deaths (Dotaniya et al. 2018). Arsenic, cadmium, chromium, lead, and mercury are among the priority metals of public health importance due to their high toxicity (Tchounwou et al. 2012). A range of bacterial-mediated processes may improve the mobility of heavy metals in sediments, including interactions of metals with bacterial membrane components (e.g., pigments, polymers, cell-free organic compound complexes) and sulfide production by sulfate-reducing bacteria (Singh et al. 2007).

Many studies have proved the significant role of biosurfactants in heavy metal removal from the environment by facilitating their solubilization, dispersion, and desorption (Venkatesh, Vedaraman 2012; Juwarkar et al. 2007; Bendaha et al. 2016). As demonstrated in Fig. 1, these metabolites can form complexes with heavy metals at the interface between soils, desorb soil matrix metals, incorporate metals into biosurfactant micelles, and thereby increase metal solubility and soil bioavailability (Santos et al. 2016). Interestingly, significant evidence suggests that biosurfactants can increase the mobilization of heavy metals in polluted soils (Juwarkar et al. 2007; Sheng et al. 2008; Venkatesh, Vedaraman 2012; Bendaha et al. 2016). Depending on the biosurfactants' electrical charge, and through ionic bonds, anionic biosurfactants can form non-ionic complexes with metals leading to their detachment

from the soil (Fig. 1). Ion exchange allows the cationic biosurfactants to compete and replace similarly charged metal ions for negatively charged surfaces (Santos et al. 2016). Also, microbial biosurfactants can be involved in other heavy metal removal mechanisms such as plant-microbe-modulated phytoremediation and biofilm-mediated heavy metal bioremediation, which have been proved as essential ways to enhance heavy metal remediation, detoxification, and mediate sustainable plant nutrient dynamics (Rajkumar et al. 2012; Meliani, Bensoltane 2016; Dotaniya et al. 2018).

Removal of polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are widespread soil contaminants and are considered priority pollutants due to their carcinogenicity (Wolf, Gan 2018). Microorganisms play an essential role in the degradation of PAHs in the soil (Bacosa, Inoue 2015). There are two main types of microorganisms participating in the biodegradation of PAHs – aerobic and anaerobic bacteria/degradation metabolism. The aerobic mechanisms rely on the oxidation of the aromatic ring, followed by the systematic breakdown of the compound to PAH metabolites and/or carbon dioxide, whereas anaerobic metabolism of PAHs is thought to occur via the hydrogenation of the aromatic ring (Bamforth, Singleton 2005).

Another way to enhance the bioremediation of PAHs is the production of biosurfactants, which can increase the apparent solubility of PAHs, increasing their mobility and biodegradability (Kobayashi et al. 2009; Tecon, Van Der Meer 2010; Tiwari et al. 2016; Patowary et al. 2018). These biobased molecules are probably more preferable when produced by the degrading microorganisms themselves. In addition to the increased bioavailability of contaminants, biosurfactants act to improve biodegradation (Wolf, Gan 2018) by changing the bacterial cell surface properties or involving the solubilization and emulsification of these hydrophobic hydrocarbons (Fig. 2; Xia et al. 2014), leading

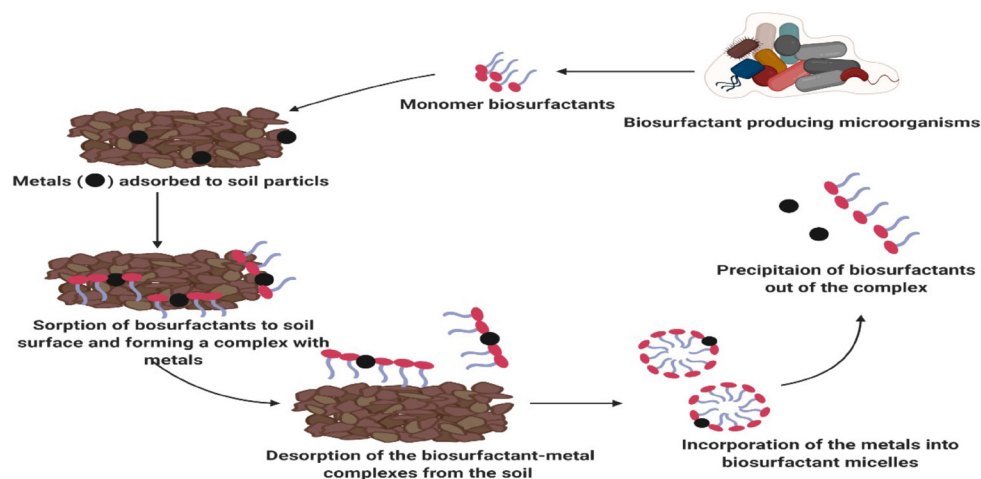


Fig. 1. Removal of heavy metals by microbial biosurfactants.

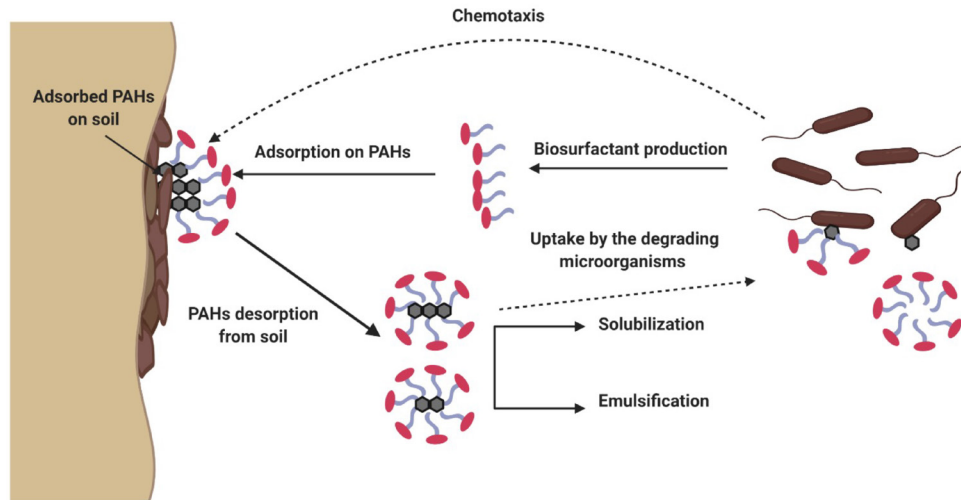


Fig. 2. A simplified process of biosurfactant activity for a better understanding of hydrocarbon bioremediation in soil.

as a result to reduced surface tension around the bacterium as well as interfacial tension between the bacterial cell wall and hydrocarbon (Aparna et al. 2011). It is increasingly evident that interactions between the pollutants and biosurfactants of the contaminant matrix can change the properties of the cell membranes and increase microbial adhesion, which affects the efficiency of biodegradation (Aparna et al. 2011; Ławniczak et al. 2013; Bezza, Chirwa 2017).

Two mechanisms exist to improve the desorption rate of polyaromatic hydrocarbons from soil: micellar solubility and direct modification of the contaminant matrix. Micellar solubilization involves dividing polyaromatic hydrocarbons into surfactant micelles when surfactant concentrations exceed the critical micellar concentration (CMC), leading to an increase in the desorption rate by maximizing the concentration gradient between sorbent and aqueous phases (Adrion et al. 2016). Higher surfactant concentrations are usually necessary for this process because the solubility of the hydrocarbons in the solution depends entirely on the surfactant concentration (Pacwa-Płociniczak et al. 2011). The mobilization process takes place below the biosurfactant CMC. Once the microorganisms are brought into close contact with polluted

soil, and through chemotaxis mechanisms (Kumar, Das 2018), pollutant-degrading microorganisms move toward polyaromatic hydrocarbons and commence to produce monomeric biosurfactants that will adsorb and surround these pollutants, allowing to their desorption from soil by micellar formation (Fig. 2). The PAHs-surrounded micelles facilitate the solubilization, emulsification and the biodegradation of hydrocarbons (Fenibo et al. 2019; Kaczorek et al. 2018).

Microbial-enhanced oil recovery

Microbial-enhanced oil recovery (MEOR) is among the most prominent application field for biosurfactants in the oil industry (Singh et al. 2007; Banat et al. 2010; Marchant, Banat 2012b). This method is an essential tertiary recovery technology, which utilizes microorganisms and their metabolites for residual oil recovery (Banat 1995; Banat et al. 2000; Amani et al. 2010). These metabolites, which can be used as surface agents (amphiphilic molecules), offer to replace chemical surfactants and enhance the life spans of mature reservoir soil (Banat et al. 2010; Alvarez et al. 2015).

Biosurfactants are effective through reduction in surface and interfacial tension, wettability alteration, and oil/water or water/oil emulsion formation (Geetha

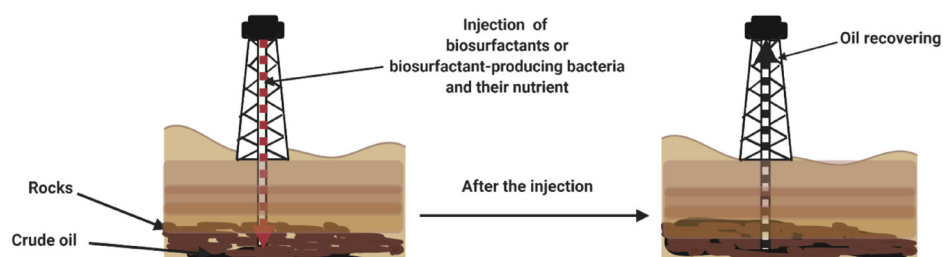


Fig. 3. Principles of microbial enhanced oil recovery.

et al. 2018). These characteristics allow the biosurfactant to be an appropriate microbial-enhanced oil recovery candidate. Emulsification of the oil depends on the surface tension (Cámara et al. 2019). Microbial-enhanced oil recovery (MEOR) is based on the injection of pressing water containing microorganisms, nutrients, and/or biosurfactants that will enhance the emulsification and mobility of oil by reducing surface and interfacial tension; these biosurfactant properties allow to recovery important oil amounts from wells (Fig. 3; Marchant, Banat 2012b; Geetha et al. 2018). Microbial-enhanced oil recovery mediated by biosurfactant can be applied *in situ* or *ex situ* (Geetha et al. 2018). *In situ*, biosurfactant-producing bacteria and nutrients are injected into oil reservoirs, and then the produced biosurfactants increase the recovery of oil. (Youssef et al. 2009; Zhao et al. 2015). In the *ex situ* system, biosurfactants are first produced by aerobic fermentation in a bioreactor and then injected into oil reservoirs (Youssef et al. 2007; Youssef et al. 2013; Zhao et al. 2015); this method suffers from complex bioprocessing techniques and high transport and product purification costs (Albino, Nambi 2010). Thus, *in situ* production of biosurfactants in oil reservoirs is more suitable for microbial-enhanced oil recovery applications due to the cost-effective and straightforward implementation (Youssef et al. 2009). In addition, this is still the preferred application in cases where either the injection of tailors-made selective nutrients to stimulate biosurfactant producing indigenous microorganisms or the injection of oil reservoirs with biosurfactants producing exogenous microorganisms with nutrients is utilized (Geetha et al. 2018).

Bioremediation of pesticides

Pesticides are chemical substances that are used to prevent and control various harmful organisms, including bacteria, viruses, fungi, insects, nematodes, weeds, and unwanted flora and fauna (Al-Jawhari 2018). However, pesticide pollution has become a severe environmental concern. Pesticides can accumulate in plants, soil, and organisms and occur in water and food (Abdel-Shafy, Mansour 2018).

In addition to heavy metals and polycyclic aromatic hydrocarbons, biosurfactants may be a potential bioremediation candidate for a wide range of pesticide residues (Wattanaphon et al. 2008). One of the mechanisms of action of biosurfactants is based on the formation of complex biosurfactant-pesticide micelles that interact with the hydrogen bonds of water molecules leading to achieving better solubilization of pesticides (López-Prieto et al. 2020). There have been several reports on the possible properties of many bacteria such as *Pseudomonas* spp., *Bacillus* spp., and *Acinetobacter* spp. as biosurfactant producers, which remove heavy metals from contaminated soil and even accelerate pesticide biodegradability (Pacwa-Płociniczak et al. 2011). Numerous reports highlight the success of the use of biosurfactants in improving Pb and Cd recovery

and removing the lindane pesticide (Wan et al. 2015). Also, biosurfactants produced by *Pseudomonas aeruginosa* showed a high biodegradation rate of quinalphos as organophosphorus pesticides (Nair et al. 2015). In another recent study, a glycolipid and glycopeptide produced by *Pseudomonas rhodesiae* and *Pseudomonas marginalis*, respectively presented a significant ability to increase the degradation of the insecticide chlorpyrifos (Lamilla et al. 2021). In a patent, it was noted that arachnids, eggs, larva, grasshoppers, and box-elder bugs were all successfully controlled with glycolipid biosurfactants (Awada et al. 2014). Also, microscopic observation of rhamnolipid-treated aphids identified the insecticidal mechanism as cuticle membrane damage (Mnif, Ghribi 2016).

Future prospects and conclusions

This review presents interesting information on the application of biosurfactants in bioremediation applications as promising green molecules for sustainable development. As an enhancer in bioremediation applications, their role has so far turned out to be an superior technique because of their advantages over synthetic surfactants. Efforts have been made in the last decades to minimize the production costs of biosurfactants to promote commercial acceptance, as the main obstacle is their high production costs and complication, which have limited their large-scale development. It is important not only to consider biosurfactants as advanced keys to the challenges of the competitive synthetic surfactant, but also to reduce the environmental pollution crisis and greener production strategies using agro-industrial wastes as a solution to reduce costs on the one hand, and the other hand to optimize production.

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