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Recent advances in environmental biotechnology: role of biosurfactants in remediation of heavy metals

Recentes avanços na biotecnologia ambiental: papel dos biossurfactantes na remediação de metais pesados

Avances recientes en biotecnologia ambiental: papel de los biosurfactantes en la remediación de metales pesados

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Abstract

Soil contamination by heavy metals is a result of different activities, such as minings, metal smelting, and car battery production. Heavy metals can cause environmental hazards such as contamination of biological systems and the subsoil through the leaching process, and various disturbances and diseases in humans, if they enter the ecosystem, due to their high environmental toxicity and difficult degradability. Non-toxic and biodegradable biological surfactant compounds, also known as biosurfactants, are being used to remediate soils contaminated by heavy metals. The objective of this review is to address the potential application of biosurfactants in the removal of heavy metals and in processes involved in bioremediation. The present study is a narrative review, consisting of a broad analysis of the literature. The searches for the articles used to compose this study were carried out in digital scientific databases, using terms and keywords. This review provides information on biosurfactant application as a promising alternative in heavy metal removal and oil spill bioremediation.

Keywords: Biosurfactants; Heavy metals; Remediation.

Resumo

A contaminação do solo por metais pesados é resultado de diferentes atividades, como mineração, fundição de metais, produção de baterias de automóveis. Os metais pesados podem causar riscos ambientais como contaminação dos sistemas biológicos e do subsolo através do processo de lixiviação, vários distúrbios e doenças em humanos se entrarem no ecossistema devido a sua alta toxicidade ambiental e difícil degradabilidade. Compostos tensoativos biológicos não tóxicos e biodegradáveis também conhecidos como biossurfactantes estão sendo utilizados para remediar solos contaminados por metais pesados. O objetivo da presente revisão aborda o potencial de aplicação dos biossurfactantes, na remoção de metais pesados e em processos envolvidos na biorremediação. O presente estudo é uma revisão narrativa, que consiste em uma ampla análise da literatura. As buscas dos artigos utilizados para compor este estudo foram realizadas em bases de dados científicas digitais, utilizando termos e palavras-chave. Esta revisão forneceu informações sobre a aplicação de biossurfactantes como uma alternativa promissora na remoção de metais pesados e biorremediação de derramamento de óleo.

Palavras-chave: Biossurfactantes; Metais pesados; Remediação.

Resumen
La contaminación del suelo por metales pesados es resultado de diferentes actividades como la minería, la fundición de metales, la producción de baterías para automóviles. Los metales pesados pueden causar peligros ambientales como la contaminación de los sistemas biológicos y el subsuelo a través del proceso de lixiviación, diversas perturbaciones y enfermedades en los humanos si ingresan al ecosistema debido a su alta toxicidad ambiental y difícil degradabilidad. Los compuestos tensioactivos biológicos no tóxicos y biodegradables, también conocidos como biotensioactivos, se están utilizando para remediar suelos contaminados por metales pesados. El objetivo de la presente revisión aborda la aplicación potencial de los biosurfactantes en la remoción de metales pesados y en los procesos involucrados en la biorremediación. El presente estudio es una revisión narrativa, que consiste en una extensa revisión bibliográfica. Las búsquedas de los artículos utilizados para componer este estudio se realizaron en bases de datos científicas digitales, utilizando términos y palabras clave. Esta revisión proporcionó información sobre la aplicación de biosurfactantes como una alternativa prometedora en la eliminación de metales pesados y la biorremediación de derrames de petróleo.
Palabras clave: Biosurfactantes; Metales pesados; Remediación.

1. Introduction

Heavy metals can arise both naturally or from anthropogenic processes, and end up in different environmental compartments, such as soils, water, air and their various interfaces. The emission of heavy metals through anthropogenic sources occurs from industrial activities, mine tailings, disposal of residues with high metal content, gasoline and lead paints, application of fertilizers to the soil, residues from coal combustion, spills of petrochemicals and several other means (Masindi & Muedi, 2018).

Heavy metals, even at very low concentrations, are generally toxic and, as they are not biodegradable, resist conventional disposal treatments. In addition to serious environmental problems, they are harmful to fauna and flora, and exposure to heavy metals can cause several serious human diseases such as respiratory problems, kidney pathology, neurological disorders and cancer (Sall et al., 2020).

Soil pollution by heavy metals has become a global environmental issue due to growing concern about the safety of agricultural products. Globally, there are 5 million places with polluted soils covering 500 million hectares of land. These soils are contaminated by different heavy metals or metalloids. Heavy metal pollution in soil has an estimated global economic impact of more than $10 billion a year (Li et al., 2019).

On 5th November 2015, the Fundão Ore Tailings Dam, operated by mining company Samarco in the Mariana district in the state of Minas Gerais, Brazil failed, releasing more than 50 million m^3 of mud contaminated with toxic heavy metals, causing a major environmental disaster. The soil and water of the Rio Doce were analyzed and high levels of iron, arsenic, mercury and manganese were recorded (Paulelli et al., 2022).

The District of Lavras do Sul (RS) has been known since the 19th century for its great lithological diversity. The region is characterized by the occurrence of mineralization of base metals associated with sulfides, gold, copper, lead, zinc and silver (Gomes et al., 2020). In recent years, the significant increase of metal extraction in Brazil has caused serious environmental problems. Lavras do Sul suffers the effects of metal mining in its surroundings. Effluents containing chemical compounds from mining are released into water courses without any adequate prior treatment (Gomes et al., 2019).

In recent years, the Ingá Mercantile and industrial company, a zinc industry, located 85 km from Rio de Janeiro, deactivated for almost 15 years, has become the largest area of toxic waste contamination in Brazil. Heavy metals such as zinc, cadmium, mercury and lead continue to pollute the soil, water and reach the mangroves, affecting the lives of the population (Avila-Campos, 2022).

Therefore, considering the serious environmental problems, a potential solution for the remediation of soils contaminated by metals and oils exists in the use of biosurfactants, molecules of an amphipathic nature, which can be added in solutions, facilitating the solubilization, dispersion and desorption of soil contaminants, and also allowing their reuse (Sarubbo et al., 2018). The ionic nature of these agents, as well as their biodegradability, low toxicity and excellent surface properties make them potential candidates for the removal of heavy metals contained in soils and sediments.

This review addresses the potential applications of biosurfactants such as heavy metal removal and bioremediation, the main features of microbial biosynthesis of biosurfactants, their physicochemical properties, and the use of industrial waste as promising alternatives for biosurfactants production.

2. Methodology

The present study is a narrative review, consisting of a broad analysis of the literature. According to Vosgerau and Romanowski (2014), in this type of study, bibliographic productions in certain areas are analyzed, providing the state of the art on specific topics, where it is possible to highlight new ideas and methodologies.

The searches for the articles used to compose this study were carried out in digital scientific databases of academic google, Sciense direct and Scielo, where research articles and review articles were selected and analyzed.

The terms and keywords used in the research were: biosurfactants, microbial surfactants, natural surfactants, biosurfactant property, biosurfactant classification, biosurfactant production, remediation, bioremediation, heavy metals and contaminated soils. The research took place from December 2020 to February 2022, 97 works were selected, covering articles that were published from 2010 to 2022.

3. Results and Discussion

3.1 Biosurfactants of Microbial Origins

Biosurfactants offer advantages over synthetics, such as biodegradability, compatibility with the environment and low toxicity, which allows for their use in the cosmetic, pharmaceutical and food industries. Other advantages may include stability under extreme conditions of temperature, pH and salinity, as well as high selectivity, due to the presence of specific functional groups, which allows specificity in the removal of pollutants. These characteristics contribute to the biosurfactant applicability in different industries (Rocha e Silva et al., 2019).

3.2 Properties of Microbial Biosurfactants

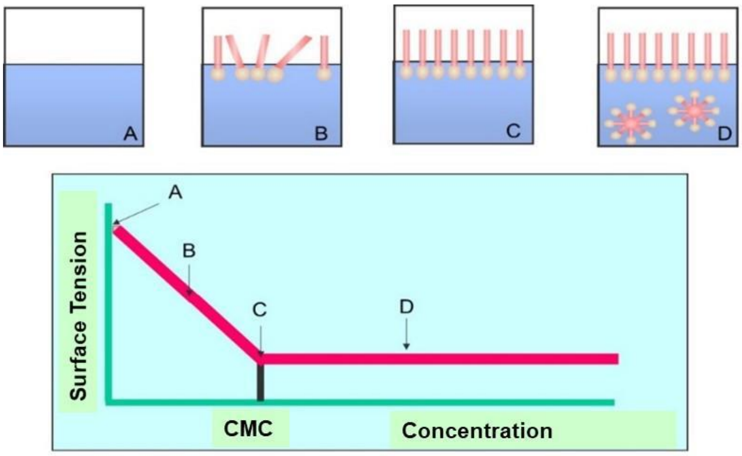
The most important property for surfactant agents is surface tension, which is the force of attraction between liquid molecules (Williams & Trindade, 2017; Santos et al., 2016).

A surface is defined as the boundary between a liquid and air, and an interface as the boundary between two liquids. Thus, the tensions between the air/water and oil/water phases are known as surfaces tension and interfacial tension, respectively (Banat et al., 2014; Bezerra et al., 2018).

Surface tension is easily quantitatively measured by a tensiometer. This measurement is the basis for most initial assessments to identify the presence of a surfactant in the medium. The air/water surface tension for distilled water is approximately 72 mN/m and the interfacial tension for distilled water versus n-hexadecane is approximately 40 mN/m. Typically, surfactants can decrease these values to around 30-40 mN/m and 1 mN/m, respectively (Marchant & Banat, 2012).

The surface tension decreases when the concentration of surfactant in the aqueous medium increases, resulting in the formation of micelles, which are amphipathic molecules aggregated with the hydrophilic portions positioned outside of the molecule and the hydrophobic portions to the inside. The concentration of these micelles forms the Critical Micellar Concentration (CMC) (Figure 1). This concentration corresponds to the minimum surfactant concentration necessary for the surface tension to be reduce to a maximum, beyond which an increase of surfactant has a non-significant effect. When the CMC is reached, several micelles are formed (Ribeiro et al., 2020).

Figure 1 – Micelle formation at Critical Micellar Concentration (CMC).



Source: Santos et al. (2016).

An emulsion is formed when one liquid phase is dispersed as microscopic droplets in another continuous liquid phase. Two types of emulsions can be formed: water-in- oil (w/o) (more oil-soluble surfactant) and oil-in- water (o/w) (more water-soluble surfactant)

The stability of an emulsion depends on many factors, including the droplets' dispersed size, which is favored by reducing the interfacial tension. The presence of emulsifiers and desemulsifiers stabilize or destabilize the emulsions, respectively (Dell'Anno et al., 2018).

Emulsifying ability is assessed by the ability of the surfactant to generate turbidity due to the suspension of hydrocarbons, such as n- hexadecane, in the aqueous system under analysis, whereas demulsifying ability is generally evaluated by the effect of the de-emulsifying agent on normal emulsions prepared with synthetic surfactants (Haq et al., 2017).

The physical and chemical properties of biosurfactants, as described above, such as surface tension reduction, foaming capacity, emulsifying and stabilizing capacity, low critical micellar concentrations, solubility and detergent power, are very important in the evaluation of their performance and potential production of these agents (Held, 2014; Rocha e Silva et al., 2018).

3.3 Classification

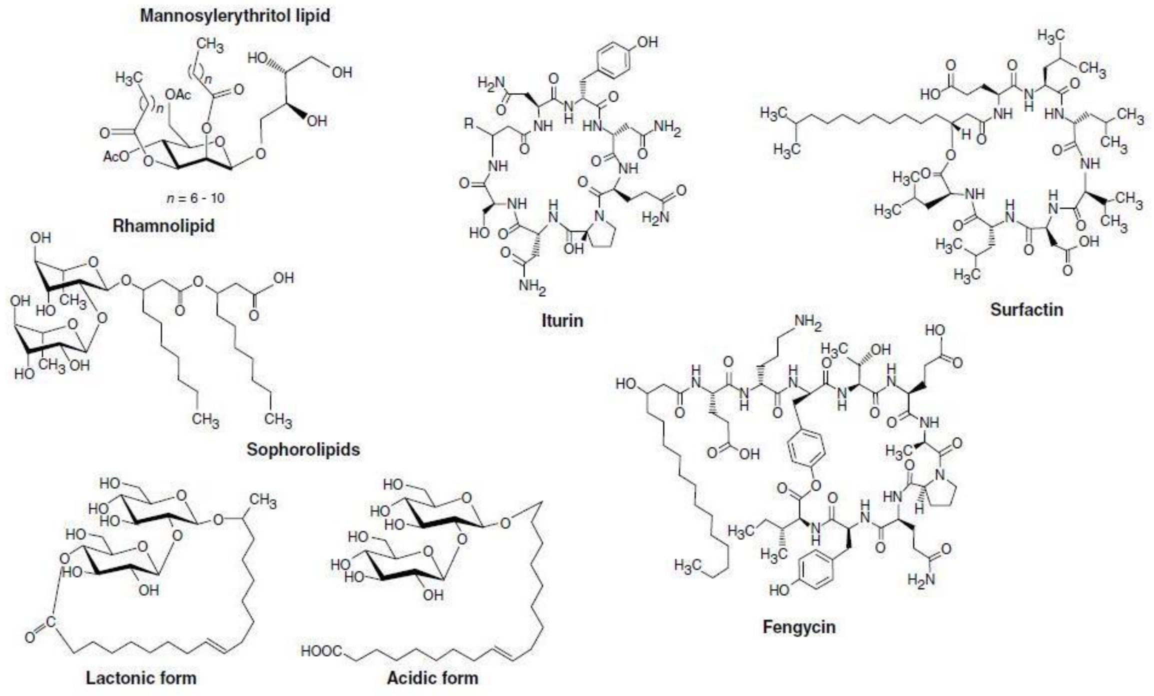
Biosurfactants are classified according to their microbial origin. They are naturally produced from bacteria, mainly from the genera *Pseudomonas*, *Bacillus* and *Acinetobacter;* fungi of the genera *Aspergillus* and Fusarium; yeasts of the genera *Candida* and Pseudozyma and some living organisms such as plants (saponins). Their composition and variety of chemical structures give them to multifunctional properties such as solubilization, detergency, lubrication and they have the ability to stabilize and foam. The polar hydrophilic part is composed of sugars, amino acids, peptides, proteins or carboxylic acid. Their hydrophobic part may contain saturated, unsaturated, or hydroxylated fatty acids or fatty alcohols. These amphiphilic molecules are divided into two classes based on their molecular weight: low molecular weight and high molecular weight (Jahan, 2020; Fenibo et al., 2019).

Low molecular weight biosurfactants are more effective in terms of reducing surface tension at the air/water interface and oil/water interfacial tension, whereas those with higher molecular weight, known as bioemulsifiers, are most effectively used in stabilizing oil-in-water emulsions (Drakontis & Amin, 2020).

In general, there are different approaches to classifying microbial biosurfactants. In addition to the possibility of differentiating molecules by size, leading to a classification of low molecular weight biosurfactants such as glycolipids, and high molecular weight biosurfactants, such as lipopolysaccharides, another common classification, mainly related to low molecular weight biosurfactants, is by liquid charge, which makes it possible to differentiate between ionic, anionic, cationic and amphoteric biosurfactants. Due to the vast structural diversity of biosurfactants, another classification method can be performed by categorizing according to structural composition, such as glycolipids, lipopeptides and proteins (Henkel & Hausmann, 2019).

The main classes of biosurfactants include glycolipids, lipopeptides /lipoproteins, phospholipids/fatty acids, polymeric and particulate surfactants (Figure 2). The glycolipid and lipopeptide groups are the best known. In the glycolipids group, rhamnolipids (RMLs), sophorolipids (SPLs) and mannosylerythritol lipids (MELs) are the most common, while in the group of lipopeptides, surfactin is the most popular (Marcelino, 2020).

Figure 2 - Structure of the main classes of biosurfactants.

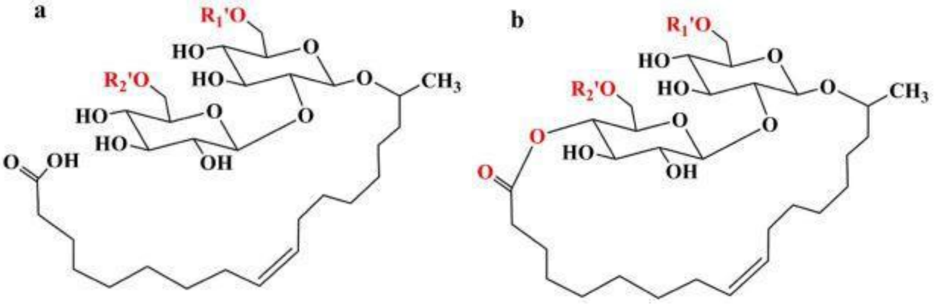


Source: Marcelino (2020).

Biosurfactants produced by microorganisms (bacteria, yeasts and filamentous fungi) are generally classified according to their chemical composition. For the production of biosurfactants, carbon and nitrogen sources are used in the culture medium, where the main sources of carbon are carbohydrates and lipids (Marcelino, 2020; Alwaely et al., et al., 2019).

Biosurfactants have general chemical surfactant properties such as solubility, ability to emulsify, saponify, disperse and reduce surface tension. Sophorolipid biosurfactants (SLs) have been used for soil remediation, in petroleum, food, cosmetic, detergent and pharmaceutical sectors. SLs are composed of a hydrophilic sophorose and a hydrophobic fatty acid, and have a wide structural diversity (Figure 3). The *Candida bombicola* is a promising SL producing strain (Li et al., 2020; Lin et al., 2019).

Figure 3 - Structures of the sophorolipids produced by *Candida bombicola*.



***SLs produzidos por *Candida bombicola*. Acid form SLs (A); lactonic form SLs (B). R1, R2 = –OH, unacetylated; R1 = –OH, R2 = –OCH3, mono-acetylated; R1, R2 = –OCH3, diacetylated. Source: Li et al. (2020). Lin et al. (2019).

3.4 Biosurfactant producing microorganisms

Biosurfactants are a natural class of surface active molecules with a variety of structures that are produced by different microrganisms (Souza et al., 2017). Bacteria, filamentous fungi and yeasts are the microrganisms commonly used for the production of biosurfactants. Microrganisms produce biosurfactants to improve cell mobility, provide access to nutrients or facilitate growth in the environment (Ahmadi-Ashtiani, 2020).

The microrganisms that are able to produce biosurfactants are abundant. They can be found in ecological places such as soil, in polluted soils, sediments and muds; in sources of water such as in fresh water, underground and in the sea; as well as in some extreme environments, such as oil reservoirs, where microrganisms are able to grow in a wide range of temperatures, pH values and salinity. Yeasts have proven their proficiency in the biosurfactant production, even though they are isolated from different sources, they have the capacity to produce biosurfactant in greater amounts than bacteria. Yeasts of the Candida genera are well known for being biosurfactant producers, as in the case of the species *C. albicans*, *C. antarctica*, *C. bombicola*, *C. glabrata*, *C. guilliermondii*, *C. lipolytica*, *C. sphaerica*, *C. tropicalis*, *C. utilis* (Lira et al., 2020; Ribeiro et al., 2020; Nwaguma et al., 2019).

According to Fenibo; Douglas & Stankey (2019), some microrganisms stand out for the production of biosurfactants. Among bacteria genera, *Pseudomonas*, *Bacillus* and *Acinetobacter* dominate the literature space. Among yeast genera, *Torulipsis*, *Pseudozyma*, *Saccharomyces*, *Rhodotorula* and *Kluyveromyces* stand out, and in the genera of filamentous fungi, *Aspergillus*, *Ustilago*, *Fusarium*, *Trichoderma* and *Penicillium* genera are widely reported in research.

One of the great advantages of using yeasts for biosurfactant production are their GRAS status (Generally Considered As Safe), that is, some of the species do not have pathogenicity and risk of toxicity, as is the case of *Yarrowia lipolytica*, *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. Several factors are able to affect the biosurfactant production by microrganisms, such as inoculum size, pH, temperature, nutrient source, carbon-nitrogen ratio, metals ions, stirring speed, and other factors. Among all the factors mentioned, the sources of carbon and nitrogen are the most critical. The influence of the carbon source on biosurfactant production by different strains of microrganisms plays an important role in the survival of the producing microrganisms, due to the increase in solubility of the insoluble compounds in water and thus facilitating the transport to the cell. They also participate in adhesion processes and cell aggregation, biofilm formation and defense against other microrganisms. The nitrogen source is an indispensable factor that acts in the regulation of yield and the efficiency of biosurfactant production (Bravo et al., 2020; Ezebuiro et al., 2019).

Table 1 - Biosurfactants production by *Candida* reported in the literature.

Class/Type of Biosurfactant	Microrganism	References
Sophorolipids	*Candida bombicola*	Roelants et al. (2013); Luna et al. (2016)
Sophorolipids	*Candida glabrata*	Luna et al. (2009); Gusmão et al. (2010)
Liposan	*Candida lipolytica*	Santos et al. (2016); Rufino et al. (2014)
Glycolipids	*Candida sphaerica*	Sobrinho et al. (2013); Luna et al. (2015); Chaprão et al. (2015)
Glycolipids	*Candida guilliermondii*	Sitohy et al. (2010)
Fatty acids	*Candida utilis*	Campos et al. (2013)
Mannosylerythritol-lipids	*Candida antarctica*	Kim et al. (2002); Hua et al. (2003)
Mannose-lipid-protein	*Candida tropicalis*	Batista et al. (2010); Priji et al. (2013)

Source: Autors.

Research around the world has been focused on these microbial agents since they were discovered. As well, the potential of filamentous fungi on biosurfactants production has also been reported by some investigations. However, most studies have used the ability of bacteria and yeast to synthesize biosurfactants using low-cost substrates (Pele et al., 2019).

3.5 Biosurfactant production from industrial waste

Over the last decade, a wide variety of low-cost waste materials have been explored as substrates for biosurfactants production, this is an effective cost-cutting and waste-management strategy. A series of renewable and low-cost industrial wastes can be used on biosurfactant production, among them, food and agro-industrial wastes (Table 2). The transformation processes are mediated, in part, by yeasts, bacteria and filamentous fungi whose metabolic characteristics make them suitable for the synthesis of high value-added molecules from agro-industrial residues (Durán; Reyes & Durán, 2020).

Table 2 - Differents low-cost residual substrates explored for biosurfactant production.

Residues	References
Palm, soy, coconut, cottonseed, peanut, olive oils	Zambry et al. (2021); Durval et al. (2019); Suryawanshi et al. (2021); Silva et al. (2021); Santos et al. (2021); Souza et al. (2018).
Slaughterhouse waste, fish processing, animal fat	Ramani et al. (2012); Patil et al.(2016); Sellami et al. (2021)
Vegetable fat	Gusmão et al. (2010); Almeida et al. (2015).
Whey	Alkan et al. (2019); Vera et al. (2018).
Dregs	Santos et al. (2013); Santos et al. (2014).
Orange, pineapple, banana, carrot, apple and rice peels, potato and sugarcane bagasse	Vieira et al. (2021); Rajasimman et al. (2021); Das & Kumar, (2019); Suryawanshi et al. (2021); Mohanty et al. (2021).
Molasses, Corn steep liquor	Sarubbo et al. (2018); Lira et al. (2020); Ostendorf et al. (2019); Kanna, (2018); Almeida et al. (2018).
Cassava flour	Rajasimman et al. (2021).
Petroleum distillery waste	Luna et al. (2011); Luna et al. (2013).
Glycerol	Moshtagh; Hawboldt & Zhang, (2021)

Source: Autors.

When agroindustrial waste is disposed incorrectly, it has a great impact on the environment and the well-being of humanity. So, an interesting strategy to reduce such impacts is the use of these agroindustrial residues in biotechnological processes, such culture media production. This is possible due to their rich amount of organic matter, which contains macronutrients (proteins and carbohydrates), and micronutrients (minerals) that are essential for the growth of microrganisms (Zanotto, 2019). The sources of lipids and carbon that are present in agroindustrial residues are one of the main requirements for the growth of microrganisms and biosurfactant production (Kumar et al., 2020).

Different agricultural products such as sugarcane straw, molasses, wheat, rice straw, bran, beet molasses, rice, sugarcane bagasse, soy speel, corn, cassava flour and their waste water are agroindustrial residues and good substrates for biosurfactant production, where carbohydrates and lipids necessary for microbial growth are obtained from these sources. Microrganisms use a wide variety of hydrocarbons, organic compounds, hydrophobic mixtures and chemical compounds as a source of carbon and energy (Lin, 2019; Patowary, 2017).

Among the accessible, renewable and low-cost sources for the synthesis of biosurfactants are the residues produced in the petroleum processing industries. These materials are sources of carbon and energy for surfactant biomolecule synthesis (Jimoh & Lin, 2019).

To choose a good agroindustrial residue for biosurfactant production, it is necessary to consider many basic conditions, such as availability of the material and costs related to transport; and the minimization or avoidance of pre-treatment steps so that there is no extra addition of refined raw material to medium production. The use of renewable agroindustrial and low-cost substrates can lead to a significant reduction in operating costs involved in biosurfactant production processes (Rivera et al., 2019). The availability and type of raw material can significantly contribute to the cost of production. Its estimated that the raw materials account for 10 - 30 % of the total cost of a biotechnology product and the purification accounts for 60 % of the total produtcion cost, however, the cost of purification can be avoided if the biosurfactant is applied in crude form (Silva et al., 2019).

3.6 Environmental Applications

Due to their structural diversity and functional properties, biosurfactants play important roles in environmental applications. Remediation technologies using biosurfactants and producer microrganisms help to remove hydrocarbons and heavy metals from polluted areas (Jahan et al., 2020).

3.6.1 Bioremediation

Many remediation strategies involving biological, chemical and physical activities have been developed over the years. Due to advances related to sustainable technologies, the exploration of natural methods has increased in relation to hydrocarbon contamination of water and soil. Bioremediation is a process that involves the action of microrganisms and their respective enzymes to degrade contaminants from the contaminated area, whereby bacteria, filamentous fungi and yeasts are isolated from the polluted site and used to clean contaminated soil and water. Many of these microorganisms isolated from the contaminated area produce biosurfactants, and these trap heavy metals and hydrocarbons and degrade them into products that can be used by the environment (Singh et al., 2020). Their final products are environmentally friendly substances such as carbon dioxide, fatty acids, water or cellular biomass.

Bioremediation is an emerging strategy to clean oil contaminated environments. Fossil oil is made up of a sophisticated mixture of hydrocarbons of varied molecular weights and numerous other compounds. Fossil oil hydrocarbons are mainly alkanes, cycloalkanes and numerous aromatic carbides, while the other compounds are various metals such as iron, nickel, copper and others. The molecular composition of hydrocarbons can vary. Chemically, fossil oil contains paraffin (15 - 60 %), hydrocarbons (30 - 60 %), aromatics (3 - 30 %), and minerals (6 %). The microrganisms widely used for oil, diesel and polycyclic aromatic hydrocarbon spill remediation belong to the genera *Pseudomonas*, *Bacillus*, *Rhodococcus*, *Candida*, *Lactobacillus*, *Arthobacter* and *Acinetobacter* (Trudgeon, 2020; Borah, 2018).

Biosurfactant-enhanced oil spill bioremediation is an attractive spill mitigation approach. However, it is worth noting that biosurfactants and microbial cultures need to be synergistic for maximum oil biodegradability. The physicochemical characteristics of oils vary from oil to oil, which decides their rate of degradation. Oils can be a complex mixture of aliphatic and aromatic compounds in varied proportions. Alkanes can be hexanes, decane, tetradecane, hexadecane, and others, while polycyclic aromatic hydrocarbons can be benzene, naphthalene, phenanthrene, pyrene, and benzopyrene. Microrganisms have the ability to degrade alkanes and aromatics into volatile fatty acids under anaerobic conditions (Patel, 2019). Biosurfactants have also achieved potential interest for other environmental applications in the remediation of organic and inorganic contaminants, particularly in the removal of heavy metals from soil and water (Akbari et al., 2018).

3.6.2 Application in oil reservoir cleaning

The petroleum industries generate approximately 28,220 tons per year of oil sludge at the bottom of the tanks. This oily sludge is one of the main wastes generated during oil exploration, transport and refining. The composition of the oily sludge contains aged crude oil, including saturated hydrocarbons, aromatic hydrocarbons, asphaltene, colloid, heavy metals such as Ni, Cr, Zn, Mn, Cd and Cu, as well as other harmful substances. Oily sludge is considered a hazardous waste due to the threat it poses to the environment, and for human health if handled incorrectly (Ren et al., 2020; Suganthi et al., 2018).

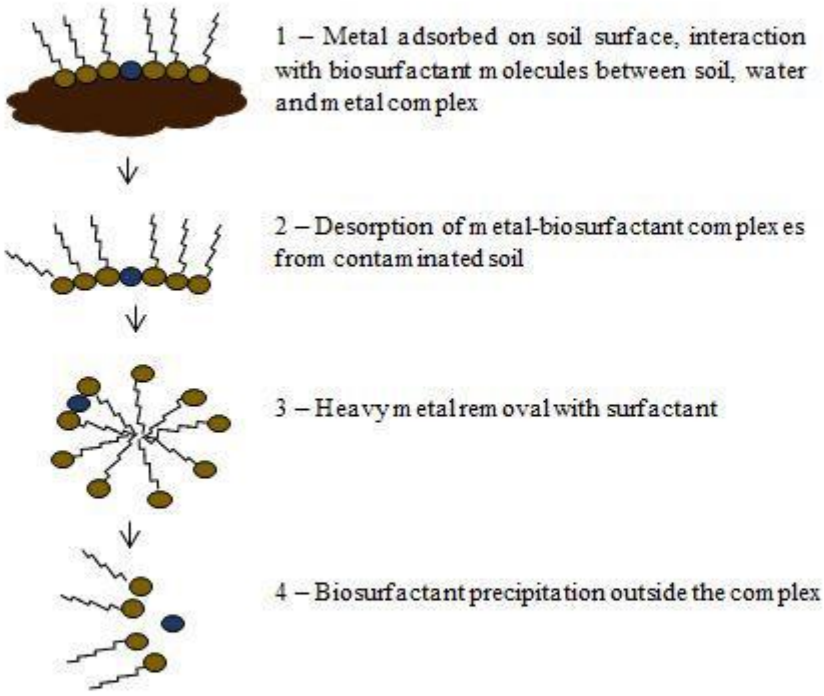
Biosurfactants are used to clean oil tanks, oil contaminated tanks, barges, storage tanks, wagons tank and trucks, pipelines and containers used to transport or store crude oil or petroleum fractions. The oil floats and remains as a distinct phase, then, for cleaning, biosurfactant monomers can accumulate at the solid-oil interface and reduce the capillary force holding the oil and solid together due to the reduction in surface tension. Then, the oil undergoes a displacement if the surface tension between oil-solid is greatly reduced to overcome the capillary force (Al-Tamimi, 2019).

3.6.3 Biosurfactants applications in heavy metal removal

Over the years, modernization and industrialization have led to contamination of soils and water, with heavy metals and hydrocarbons being the most common environmental pollutants (Makombe & Gwisai, 2018). The increasing load of heavy metal contamination has caused severe deterioration to the environment. Among the various contaminations by heavy metals, the presence of arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), mercury (Hg), lead (Pb), zinc (Zn) and nickel (Ni) in the environment is of great concern, because they are non-threshold toxins and are present in air, aquatic and terrestrial systems in concentrations many times above those established by international agencies. Further, they can cause many health problems categorized as inorganic chemical hazards to humans, animals, and plants (Rahman & Singh, 2020; Akbari et al., 2018).

Compared with chemical surfactants, biosurfactants derived from microrganisms better perform, and are considered suitable in removing heavy metals from contaminated soil (Tang et al., 2017; Luna; Rufino & Sarubbo, 2016). There are three main steps involved in removing heavy metals from soil by washing with a biosurfactant solution. The heavy metals adsorbed on the surface of soil particles are separated by the sorption of biosurfactant molecules at the interfaces between the wet soil and the metal in aqueous solution. Subsequently, the metal is adsorbed by biosurfactants and trapped within the micelle through electrostatic interactions. Finally, the biosurfactant can be recovered through the membrane separation process according to Figure 4 (Guan et al., 2017; Ibrahim et al., 2016).

Figure 4 - Mechanism for removal heavy metals from contaminated soil using biosurfactants.



Source: Adapted from Ahmad et al. (2018).

The effective removal of heavy metals from soil is a major challenge. Biosurfactant leaching mainly aims to remove metals from soils through chelation with their respective function groups and transformation of metal speciation fractions to increase their mobility (Yang et al., 2018).

The use of biosurfactants (rhaminolipids, saponin and mannosylerythritol lipids (MEL) to remove heavy metals from soil from a construction site in canada and a lake sediment from Japan was evaluated (Mulligan et al., 2007).

The potential of biosurfactants in the mobilization and decontamination of soils contaminated with heavy metals was confirmed by Juwarkar et al. (2008), who used the di-raminolipid biosurfactant produced by *Pseudomonas aeruginosa* BS2 for the mobilization of metals from a soil contaminated by various heavy metals.

Gnanamani et al. (2010) studied the bioremediation of chromium (VI) by an isolate of *Bacillus sp*. MTCC 5514 biosurfactant producer.

Slizovskiy et al. (2011) studied the remediation of soils contaminated by heavy metals reinforced by a cationic surfactant (DPC), non-ionic A (mmonyxKP surfactant) and by an ionic biosurfactant (JBR-425). It was concluded that JBR-425 had the best elution effect for Zn (39%), Cu (56%), Pb (68%), and Cd (43%).

Biosurfactants produced by *Candida* species have also been successfully applied in the flotation of heavy metals, being able to remove more than 90% of cations in columns and in Dissolved Air Flotation processes (Albuquerque et al., 2012).

The literature reports that the bioremediation technique using biosurfactants is the best method to eliminate heavy metals from the soil, showing 100% efficiency. According to Guan et al., (2017), the efficiency of biosurfactants to removing heavy metals from sludge and soils reached removal rates of 90 - 100% for copper (Cu), zinc (Zn), chromium (Cr) and cadmium (Cd). Natural surfactants are considered effective in treating soils contaminated with crude oils and diesel (Akbari et al., 2018).

Biosurfactants produced by *Pseudomonas sp*. and *Alcaligenes sp*. were used for flotation and separation of calcite and scheelite mineral formations, reaching recovery percentages around 95% for $CaWO_4$ (calcium tungstate) and 30% for $CaCO_3$, (Calcium carbonate) respectively, emphasizing that conventional chemical reagents are unable to separate these two minerals (Sarubbo et al., 2015).

Biodispersan, an anionic polysaccharide produced by *A. calcoaceticus*, was evaluated in the prevention of flocculation and dispersion of limestone and water mixtures. *C. bombicola* biosurfactants, on the other hand, demonstrated efficiency in coal solubilization (Sarubbo et al., 2015).

Luna et al. (2016) obtained in their results removal rates of 95, 90 and 79% for Fe, Zn and Pb, respectively, with the biosurfactant produced by *Candida sphaerica* to remove organic pollutants. Others microrganisms such as *Bacillus* and *Pseudomonas sp*. have been widely used for the removal of heavy metals from effluents and soil. Due to their high metal-binding affinities, these microrganisms can absorb a substantial amount of heavy metals ions, resulting in the transfer of metals to a contaminated biomass matrix (Jacob et al., 2018).

Tang et al. (2018) used a combination of rhamnolipids and saponin in a process of multiple washes of sludge contaminated with metals. The results indicated that after several washes the efficiencies of removal of Cu, Zn, Cr, Pb, Ni and Mn were 62%, 74%, 60%, 15%, 68% and 64%, respectively. Subsequent to the washes to remove the metals, the sludge was suitable for use in agriculture.

Rocha Junior et al. (2019) performed tests involving crude and isolated biosurfactant to remove heavy metals from contaminated sand under dynamic and static conditions. Removal rates ranged from 30 to 80% and 45 to 65% for Zn and Cu removal in dynamic and static tests, respectively. The biosurfactant was also able to remove Pb under dynamic conditions, with a removal rate of 15%.

4. Conclusion

This review provides information on the application of biosurfactants as a promising alternative in heavy metal removal and oil spill bioremediation. Biosurfactants are candidates to replace synthetic surfactants. Thus, Novel discoveries, improvement of production conditions, development of more cost-effective recovery and downstream processes and development of new microbial strains from screening programmes may allow the use of biosurfactants in large scale. The versatility and efficiency demonstrated in biosurfactant application make these compounds promising biomolecules.

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