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Review

Biosurfactants as Multifunctional Remediation Agents of Environmental Pollutants Generated by the Petroleum Industry

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Abstract: Fuel and oil spills during the exploration, refining, and distribution of oil and petrochemicals are primarily responsible for the accumulation of organic pollutants in the environment. The reduction in contamination caused by hydrocarbons, heavy metals, oily effluents, and particulate matter generated by industrial activities and the efficient recovery of oil at great depths in an environmentally friendly way pose a challenge, as recovery and cleaning processes require the direct application of surface-active agents, detergents, degreasers, or solvents, often generating other environmental problems due to the toxicity and accumulation of these substances. Thus, the application of natural surface-active agents is an attractive solution. Due to their amphipathic structures, microbial surfactants solubilize oil through the formation of small aggregates (micelles) that disperse in water, with numerous applications in the petroleum industry. Biosurfactants have proven their usefulness in solubilizing oil trapped in rock, which is a prerequisite for enhanced oil recovery (EOR). Biosurfactants are also important biotechnological agents in anti-corrosion processes, preventing incrustations and the formation of biofilms on metallic surfaces, and are used in formulations of emulsifiers/demulsifiers, facilitate the transport of heavy oil through pipelines, and have other innovative applications in the oil industry. The use of natural surfactants can reduce the generation of pollutants from the use of synthetic detergents or chemical solvents without sacrificing economic gains for the oil industry. Therefore, investments in biotechnological processes are essential. It is predicted that, in the not-too-distant future, natural surfactants will become viable from an economic standpoint and dominate the world market. The application of biosurfactants in these settings would lead to industrial growth and environmental sustainability. The main goal of this paper is to provide an overview of diverse applications of biosurfactants on environmental remediation, petroleum biotechnology, and the oil industry through a scientific literature review.

Keywords: biosurfactants; demulsifiers; heavy oil; environmental contamination; corrosion; soil remediation; oily effluents; biodesulfurization; fuels

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1. Introduction

The extraction of natural resources and the expansion of industries have led to serious environmental problems, requiring greater efforts to solve such problems. The sources of energy most used across industries are oil and petroleum products, the exploration, transport, and use of which are associated with the generation of pollution [1]. The major problem of pollution caused by oil is the fact that hydrocarbons have low biodegradability, placing ecosystems and biodiversity at risk [2].

Among the technologies developed to lower the impact of pollution related to the oil industry, remediation processes involving the use of chemical surfactants are effective; however, these agents are mostly not biodegradable and can produce secondary pollutants [1]. Thus, interest in green surfactants has intensified in recent years. The main reason for this interest is the need to recover oil from great depths, as underground reservoirs are finite and production will eventually reach a peak (known as Hubbert's peak). Another reason is related to the use of synthetic surfactants in dispersants [3].

Natural surface-active agents (green surfactants), such as microbial and plant-derived surfactants, have the potential to replace their synthetic counterparts, offering the advantages of biocompatibility, biodegradability, low toxicity, and stability under extreme environmental conditions [4]. Microbial surfactants—more commonly known as biosurfactants—have been more widely studied due to their versatility, specificity, and efficiency. Biosurfactants of plant origins, on the other hand, include saponins, phospholipids, and proteins or protein hydrolysates, among which saponins have the potential for many biotechnological applications due to their biological and physicochemical properties. Due to their variety of structures and properties, biosurfactants have applications in numerous industrial processes [5] and are expected to become the multi-functional materials of the century [2,6]. These natural biomolecules are particularly useful to the oil and petrochemical industries. The remediation of contaminated soil and water due to the occurrence of oil spills, the removal of oily sludge from storage tanks, the remediation of soil contaminated with heavy metals, and the enhancement of oil recovery processes are among the applications of biosurfactants. These natural compounds can also be used to prevent the corrosion of equipment, oil pipelines, and transportation tanks, to treat oily effluents, and to formulate fuels for many industries [7].

However, the industrial output of synthetic surfactants is much greater than that of green surfactants due to the high production costs of the latter [8]. Thus, methods are needed in the field of biotechnology to lower the production costs of green surfactants, such as the use of agro-industrial waste as culture substrates for surfactant-producing microorganisms and genetic modifications so that microorganisms can produce larger volumes of these natural compounds [9–11]. The oil industry has shown particular interest in biosurfactants due to their versatility [12].

The main goal of this paper is to provide an overview of the diverse applications of biosurfactants on environmental remediation, petroleum biotechnology, and the oil industry through a scientific literature review.

2. Biosurfactants

Biosurfactants are among the most widely-studied biotechnological compounds of the current century [13]. These amphipathic molecules are produced through fermentation processes involving bacteria, yeasts, or filamentous fungi and have a hydrophilic (polar) part and a hydrophobic (non-polar) part, enabling a reduction in the surface tension and interfacial tension of liquids [14,15]. The polar portion of a biosurfactant may be an amino acid, carbohydrate, cationic peptide, or anionic peptide, whereas the non-polar portion is formed by saturated or unsaturated carbon chain broth that may be comprised of proteins, fatty acids, or peptides [1]. Anionic biosurfactants are more effective for cleaning and oil removal processes, whereas cationic biosurfactants are used more for the emulsification of skincare products and non-ionic biosurfactants are more appropriate for low-temperature detergents. Thus, each natural surface-active agent has considerable specificity [10]. The combination of biosurfactants with cost-effective synthetic surfactants at optimized compositions can also provide superior interfacial and surface activity compared to the single surface-active components. In recent years, the use of ionic liquids in green surfactants formulations has gained attention owing to their low toxicity, non-volatility, high thermal stability, and high surface activity [16].

The quality of a surfactant is normally determined based on measures of surface tension and the critical micelle concentration (CMC). Surface tension regards the forces between the molecules of a liquid. Surfactants are generally capable of breaking these forces and reducing tension between phases, enabling two immiscible liquids to interact [17]. A greater concentration of surfactant leads to a greater reduction in tension, which is when the formation of micelles occurs, which are surfactant molecular aggregates (Figure 1) [18]. The CMC is a measure of the efficiency of a biosurfactant and ranges between 1 and 2000 mg/L, whereas surface tension and interfacial (oil/water) tension are around 30–35 and 1–10 mN/m, respectively [19].

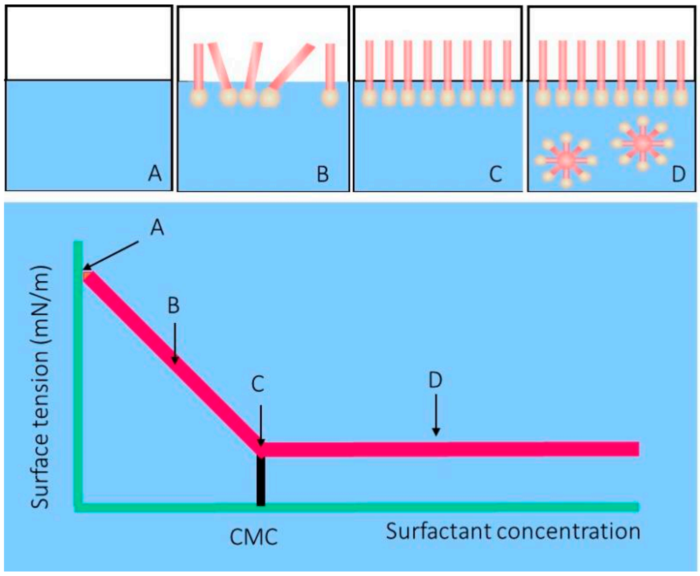

Figure 1. Reduction in surface tension and formation of micelles. A, B, C and D show the sequence of surfactant addition until the critical micelle concentration (CMC) (created by the authors).

Biosurfactants have a variety of chemical structures, such as glycolipids, lipopeptides, phospholipids, fatty acids and neutral lipids, polymeric surfactants, polysaccharides, lipopolysaccharides, proteins, and lipoproteins, which are produced by microorganisms cultivated in soluble (carbohydrates) and insoluble (hydrocarbons, oils, and oily residues) substrates [1,20]. A combination of substrates, such as vegetable oils and waste fry oil, can also be used to enhance the yield of biosurfactants produced by microorganisms [1].

The average molar mass of biosurfactants ranges from 500 to 1500 Da. Low molar mass biosurfactants are more effective at lowering the surface tension at the air–water interface and the interfacial tension at the oil–water interface, whereas biosurfactants with a higher molar mass are used more for the stabilization of oil-in-water emulsions [2]. Proteins, lipoproteins, polysaccharides, and lipopolysaccharides have a high molar mass and are often called bioemulsifiers [21], whereas glycolipids, phospholipids, and lipopeptides have a low molar mass and constitute what is classically known as biosurfactants [12].

The hydrophilic–lipophilic balance (HLB) should also be considered. Biosurfactants that are highly soluble in water are the best stabilizers of oil-in-water (O/W) emulsions and those with considerable stability in oil are the best stabilizers of water-in-oil (W/O) emulsions. Depending on the HLB value, a biosurfactant may serve as a wetter, antifoaming agent, and emulsifier in environmental applications [14].

Glycolipids are the most widely studied class of biosurfactants and are composed of a hydrophilic carbohydrate linked to hydrophobic fatty acid chains of various lengths through an ester group. This class of biosurfactants is characterized based on the carbohydrate portion and the most widely-studied subclasses of glycolipids are rhamnolipids, trehalose lipids, sophorolipids, mannosylerythritol lipids, and sophorolipids [2].

Rhamnolipids have hydroxylated or non-hydroxylated aliphatic acid as their hydrophobic part, which is linked to a carbohydrate with one or two rhamnoses (hydrophilic part). This subclass of biosurfactants is produced by bacteria of the genus *Pseudomonas* and includes powerful removers of petroleum hydrocarbons [22]. Rhamnolipids reduce the surface tension of water from 72 mN/m to around 30 mN/m and reduce water/oil interfacial tension from 43 mN/m to around 1 mN/m. Rhamnolipids in pure form or mixtures have a CMC ranging from 50 to 200 mg/L, depending largely on the chemical composition of the species [23].

The polar part of trehalose lipids comprises trehalose. These glycolipids have low toxicity and versatile properties, and are produced mainly by species of the genera *Nocardia*, *Rhodococcus*, *Corynebacterium*, and *Mycobacterium* [22]. Trehalose lipids lower surface tension to 25–40 mN/m and interfacial tension to 1–5 mN/m [23].

Sophorolipids have antimicrobial properties and are effective in cleaning operations. These biosurfactants are used for the recovery of oil from petroleum reservoirs and during cleaning activities in the textile industry. Sophorolipids may form a closed or open chain. The polar part is comprised of sophorose by lactonization or acetylation and the non-polar part is a fatty acid chain of variable length [1,22]. These surfactants are generally produced by yeasts, such as *Starmerella bombicola* [22], and lower surface tension to around 33 mN/m and interfacial tension to 5 mN/m in n-hexadecane and water. *S. bombicola* is one of the most productive species and is capable of producing an average of 300 g/L of sophorolipids [24].

The bacterium *Bacillus subtilis* produces a cyclic lipopeptide known as surfactin, which has seven hydrophobic long-chain amino acids (13 to 15 carbons) and a set of seven amino acids connected by a lactone bond: L-asparagine (Asn), L-leucine (Leu), glutamic acid (Glu), L-leucine (Leu), L-valine (Val), and two D-leucines [25]. Surfactin is one of the most powerful biosurfactants and is widely used in different applications due to its antibacterial, antifungal, and antiviral properties. Using this biosurfactant, surface tension is lowered from 72 to 27 mN/m with a concentration of less than 5% by volume and the CMC is low [26].

Bacteria and yeasts growing in n-alkanes produce large quantities of phospholipids. *Acinetobacter* spp. and *Thiobacillus trioxidanes* synthesize this subclass of biosurfactants. Emulsan and liposan are polymeric biosurfactants used as emulsifiers and are produced by bacteria as well as yeasts of the genus *Candida* [27].

The properties of biosurfactants are determined based on collapse tests, the solubility of oil droplets, surface tension, the emulsification index, the foaming index, and CMC (Figure 2) [6,28].

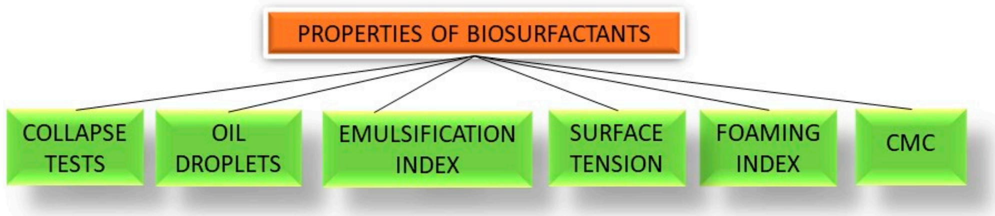

Figure 2. Methods for characterizing the properties of biosurfactants (created by the authors).

Good surfactants reduce the surface tension of water from 72 mN/m to 35 mN/m and the interfacial tension of water and n-hexadecane from 40 mN/m to 1 mN/m [6,29]. For instance, low interfacial tensions between bitumen and sediments achieved by biosurfactants enable oil extraction and subsequent sale of the oil [30].

Surfactants with a low molar mass are used more for pollutant removal, whereas those with a high mass are better for emulsification. Biosurfactants with a low molar mass have a lower CMC, making them usable for industrial applications [1,19].

Biosurfactants remain stable in a broad pH range, at high temperatures of up to 100 °C, and in the presence of saline. This demonstrates the potential of these natural biomolecules in the treatment of oil spills in the marine environment (high salinity) and in enhanced oil recovery, in which high temperatures are employed [3]. Lipopeptides produced by *B. subtilis* and *B. licheniformes* maintain their surface-active properties at 120 °C and in a pH range from 5 to 11, as well as after being kept at a temperature of −18 °C for two months [22]. It is important to state that biosurfactants may degrade over time when they are exposed to a warm environment, sun light, or humidity. In such cases, several additives may be applied in order to extend their shelf life [31].

The emulsification of oily compounds is very important to the dispersion and solubilization of droplets in oil spills, enabling the degradation of hydrophobic compounds by the autochthonous microbiota [4].

The HLB, as mentioned before, determines whether a biosurfactant is more hydrophilic or hydrophobic [20]. A lower HBL indicates a more lipophilic compound. This property enables selecting a biosurfactant for use in more polar or non-polar media [19].

Microbial biosurfactants are innocuous, with low toxicity to animals, larvae, and plants [19]. The amphipathic structure and properties of biosurfactants enable their application as detergents, dispersants, emulsifiers, foaming agents, and solubilizers for use in the oil industry and the environmental field (these applications will be discussed in greater detail in the following sections), as well as in pharmaceuticals, cosmetics, the food industry, agriculture, and the textile industry [19,21,27] (Figure 3). In the food industry, biosurfactants serve as emulsifiers to improve the texture of foods. In cosmetics, green surfactants have low toxicity and, therefore, do not harm human skin cells. In the pharmaceutical field, biosurfactants can be used as an alternative to chemical-based medications, serving mainly as a biofilm anti-adherent agent, impeding the incorporation of pathogens to cell membranes and contributing to the death of cancer cells. Biosurfactants also have a great tendency to interact with biological membranes, exhibiting hemolytic activity [22]. Biosurfactants are also involved in the emulsification and solubilization of dyes and pigments, and adherence to fabrics in the textile industry. These natural compounds can also be used in the fabrication of paints and nanoparticles [22,32].

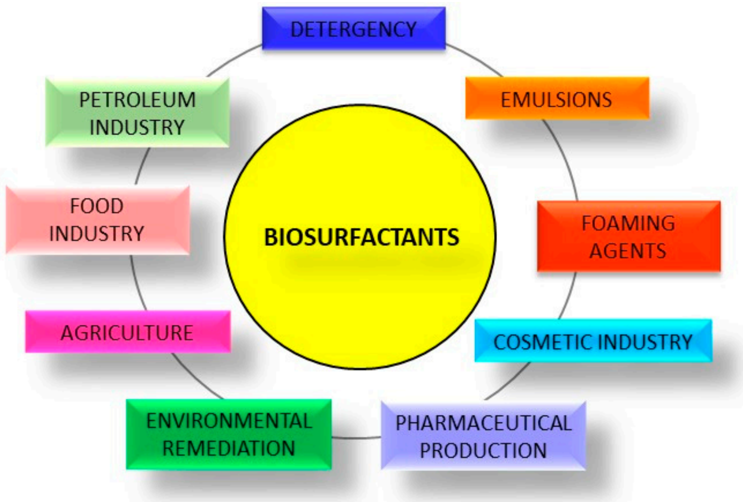

Figure 3. Fields with biosurfactant applications (created by the authors).

3. Application of Biosurfactants in Soil Remediation

Soil can accumulate hydrophobic pollutants from the oil and gas industry. Storage tank leaks, pipeline leaks, and spills related to accidents during the activities of oil exploration, refining, and transportation are some causes of soil contamination. These pollutants are not only toxic, but they are also insoluble and recalcitrant [33].

Physical, mechanical, chemical, and biological soil remediation methods can be employed. The biological method involves the use of renewable resources (plants, microorganisms, and surfactants) and low technology.

Surfactants lower the surface and interfacial tensions of pollutants, enabling their desorption from soil particles and favoring their mineralization and microbial degradation. The contaminant removal process can also occur via the absorption of pollutants by plants (phytoremediation), facilitated by biosurfactants [10].

The adequate use of biosurfactants in the bioremediation of petroleum hydrocarbons, especially in soil systems, requires the consideration of several factors. The administration of low concentrations of biosurfactants in the early stages of biodegradation results in greater efficiency, especially when the hydrocarbon content is high [34,35]. In contrast, high concentrations of biosurfactants lead to the uptake of the biomolecules by the microorganisms rather than the biodegradation of the contaminant [10].

Biosurfactants produced by *Bacillus* sp. and *C. sphaerica* UCP0995 increased the degradation rate of oil by 10 to 20%, indicating the enhancement of hydrocarbon biodegradation in soil [36]. Researchers studied the ability of the biosurfactant alasan in increasing the degradation of polyaromatic hydrocarbons in soils. The mineralization of fluoranthene was higher than 50% and an expressive increase in the mineralization of phenanthrene by *Sphingomonas paucimobilis* EPA505 was found in the presence of the surfactant [37]. The biosurfactant produced by *P. cepacia* CCT6659 cultivated in industrial waste demonstrated potential application in the bioremediation of soils. In experiments involving soil contaminated by hydrophobic organic compounds, an indigenous consortium and the biosurfactant led to the degradation of 95% of the pollutants in 35 to 60 days [38]. The combination of FinasolOSR-5, which is a chemical surfactant, and a trehalose lipid biosurfactant led to the complete degradation of an aromatic hydrocarbon [39].

In agriculture, biosurfactants can be used for the removal of pesticides and the inhibition of plant pathogens [40]. These natural compounds also favor a mutualistic relationship between bacteria that are beneficial to plants, assisting in the formation of biofilms in roots as well as solubilizing metals and dissolving hydrocarbons, which can then serve as nutrients for plants. The biosurfactant itself can serve as a carbon source for plants used in agriculture [10].

Biosurfactants do not harm flora that are beneficial to plants; however, they cause the lysis of pathogen cells and can be used in pest control programs due to the reduction in the life expectancy of mosquitos and nematodes, and, by contributing to the infecundity of these organisms, favor crop growth, especially with the application of sophorolipids [10].

Biosurfactants can also be used as soil cleaning agents, increasing the quantity of nutrients in the soil and serving as biocides, particularly targeting microbes. Biosurfactants increase the bioavailability of pesticides, facilitating the biodegradation of these pollutants of soil and sediments [22].

4. Soil Washing

The washing of soils can be performed with the use of surfactants [34]. Through ex situ washing, the contaminated soil is pretreated and mixed with water containing surfactants, followed by agitation. After washing, the soil dirty particles settle, and the washing solution can be recycled. Ex situ soil cleaning can be used to treat different concentrations of contaminants at a relatively low cost and enable returning the clean portion of the soil to its original site [34]. One study found that a rhamnolipid was the most effective for phenanthrene, removing 63% of the contaminant, followed by surfactin, Triton-100, and Tween 80, exhibiting respective removal rates of 62, 40, and 35% [41]. Another study found a better hydrocarbon removal rate with the use of a single rhamnolipid compared to a mixture of rhamnolipids [42]. The removal of different hydrocarbons requires different biosurfactants; however, rhamnolipids are generally the most efficient at removing this class of pollutants [10].

In situ soil washing is a practical application of surfactants. With this method, the washing solution (i.e., liquid containing surfactants) is injected into the soil and the contaminants are mobilized by solubilization (e.g., formation of micelles with the washing solution) or chemical interactions. After percolating through the contaminated area, the solution containing the contaminants is collected from the injection wells for treatment, disposal, or reinjection into the same site [43]. Soil washing is one of the few forms of treatment that enable the removal of heavy metals and other contaminants. Unlike their synthetic counterparts, biosurfactants can be produced in situ and are more effective, thereby lowering the cost of treatment [34].

Modern bioremediation involving biosurfactants, employing in situ and ex situ methods, constitutes an excellent eco-friendly alternative to synthetic surfactants, as biosurfactants can maintain a high biodegradation rate in contaminated soils [44]. However, some factors must be taken into account for the successful remediation of contaminated soils using surfactants, such as the adsorption behavior of the surfactant, the solubilization/elution capacity of the surfactant in relation to the target contaminant, and the toxicity and biodegradability of the surfactant. Economic factors (cost of surfactants and amount of soil contaminated) must also be considered. Besides the desorption of contaminants, a surfactant should be effective and efficient, with a low CMC to enable a low concentration in washing solutions, reducing the cost of the remediation process [45].

Surfactants alter the biological and physiochemical properties of soil, triggering a set of reactions. Residual surfactants in the soil after cleaning raise concerns of toxicity if the surfactants are not easily biodegraded. The ecosystem can be adversely affected by an excess of surfactants in the water system of the soil. Anionic surfactants can also bind to bioactive macromolecules (e.g., enzymes, peptides, and DNA) and alter the biological function of microorganisms [34].

The flow of a fluid through a porous medium (soil) is a function of the viscosity [46]. Soil washing with surfactants for the removal of hydrophobic organic pollutions occurs through two mechanisms: displacement (mobilization), which occurs below the CMC, and solubilization, which occurs above the CMC [12]. The mobilization mechanism occurs due to monomers of surfactants below the CMC, which accumulate at the soil/contaminant and soil/water interfaces, altering the wettability of the system and increasing the contact angle between the soil and hydrophobic contaminant. Surfactant molecules adsorbed to the surface of the contaminant promote repulsion forces between the main groups of the surfactant molecules and soil particles, causing the separation of the contaminant from the soil [12]. The mobilization mechanism depends on the ionic charge of the biosurfactant. An anionic biosurfactant performs better as a washing agent compared to cationic and non-ionic surfactants, and the anionic residual water is easier to destabilize using charge neutralization mechanisms [47,48]. Surfactants at a concentration above the CMC can increase the solubilization of hydrophobic organic pollutants in the micelles, followed by an increase in the partition of pollutants in the aqueous phase. Contaminants that partition in the micellar phase can be further separated and treated with activated charcoal, electrochemical treatment, and demulsification [45], and the washing solution with the surfactant can be recycled or discarded (Figure 4). However, recycling is preferable and lowers the cost remediation.

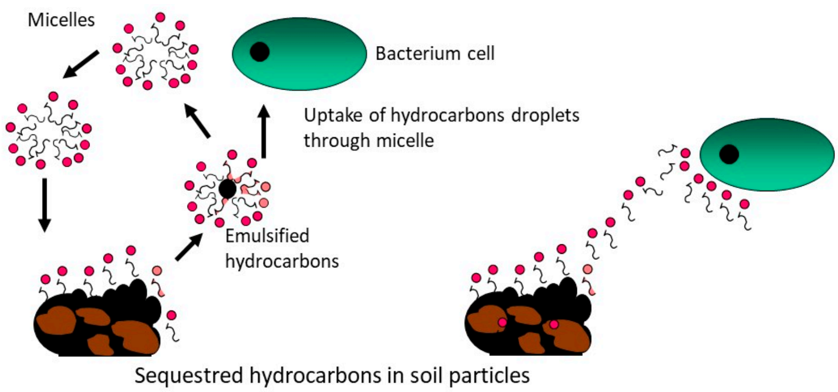

Figure 4. Solubilization (above the CMC) and displacement (below the CMC) mechanisms of biosurfactants during the washing of soils contaminated with hydrocarbons (created by the authors).

The presence of surfactants in the soil enhances the remediation of hydrocarbons by reducing the surface tension of the aqueous phase and promoting greater interaction between the cells and hydrophobic contaminant. This increases the bioavailability of pollutants to microorganisms, consequently enhancing biodegradability [18].

The literature offers numerous studies involving the use of biosurfactants for the remediation of organic pollutants in soil. In a kinetic assay, Rufisan, a biosurfactant from the yeast *C. lipolytica* UCP0988, removed 98% of motor oil and the oil removal rate was not influenced by the concentration of biosurfactant or type of soil [49]. A biosurfactant produced by *P. aeruginosa* increased the oil degradation rate by bacteria by up to 90%, demonstrating potential in oil decontamination processes [50]. The crude biosurfactant produced by *P. cepacia* CCT6659 enabled the recovery of up to 75% of residual oil from sand [51]. Crude and isolated forms of the biosurfactant from *B. cereus* UCP1615 removed 63% and 84% of a petroleum product from sand in static (packed columns) and dynamic (flasks under orbital shaking) tests, respectively [52].

5. Application of Biosurfactants for Removal of Heavy Metals

Heavy metals are inorganic contaminants and do not biodegrade in contaminated effluents or soils, posing considerable risk to the health of humans and other animals. Heavy metal contamination is associated with congenital defects, mental retardation, learning difficulties, liver damage, kidney damage, cancer, etc. [53]. Physicochemical methods (e.g., the use of acids and bases) alter the properties of soil, leading to infertility. The considerable interest in replacing synthetic surfactants with natural compounds has fueled investigations of microbial biosurfactants as components of decontamination processes in different environments [54].

The heavy metals found in soil and sediments are lead (Pb), chrome (Cr), mercury (Hg), zinc (Zn), copper (Cu), nickel (Ni), and cobalt (Co), and must be made to form stable complexes with anionic biosurfactants for precipitation and removal from the soil [22]. Washing with a surfactant solution can be performed for the remediation of soils contaminated by heavy metals. Moreover, the biosurfactant can be reused several times for the cleaning of contaminated soil, also enabling the reuse of the metals in the battery industry [54]. Anionic surfactants form complexes with metals by ionic bonds, which are normally stronger than the bonds between the metal and soil. The interaction between the biosurfactant and the metal is expected to be stronger than the interaction between the metal and the humic acids present in the soil, which act as chelating agents. The reduction in surface tension enables the desorption of the metal–surfactant complex from the soil matrix. The mechanism of removal by ionic surfactants occurs through the sorption of the surfactant to the soil surface, followed by complexation with the metal, detachment of the metal from the soil, and its uptake by micelles of the surfactant, as illustrated in Figure 5. Electrostatic interactions maintain the heavy metal imprisoned in the micelles, enabling subsequent recovery with the use of membrane separation methods [43].

Cationic surfactants can replace similarly-charged ions through competition for some—but not all—negatively-charged surfaces (ion exchange). The removal of metal ions from soil can also be achieved by the micelles of the surfactants [55].

Rocha Júnior et al. [54] showed that, when the concentration is high, a biosurfactant takes the place of metals by reacting with soil particles, as demonstrated with an anionic biosurfactant produced by *C. tropicalis* applied at $\frac{1}{2}$ the CMC, the CMC, and 2 × the CMC for the removal of Zn, Pb, and Cu from sand. A higher removal rate was found with the increase in the concentration of the biosurfactant. While the biosurfactant was effective in the static test, the dynamic test enabled the greater removal of these contaminants. The kinetic test determined that 30 min were sufficient for the biosurfactant to remove the heavy metals. Moreover, recycling tests demonstrated that the first washing removed nearly all the lead and copper from the soil, whereas the second washing removed 9% of these contaminants [54]. Biosurfactant-assisted phytoremediation enabled the following heavy metal removal rates: 41% for Ni, 30% for Cr, 29% for Pb, and 20% for zinc [56]. An anionic biosurfactant produced by *C. sphaerica* UCP0995 was tested in different combinations with NaOH and HCl for removing heavy metals from soil, with Fe, Zn, and Pb removal rates of

95, 90, and 79%, respectively [57]. The anionic crude biosurfactant produced by the yeast *C. guilliemondii* UCP 0992 achieved Fe, Zn, and Pb removal rates of 89.3, 98.8, and 89.1%, respectively [58]. Removal rates of 95 and 52% were found for Cd and Pb, respectively, with the use of a sophorolipid produced by *S. bombicola* [59]. Biosurfactants can also be used to inhibit the corrosion of oil pipelines, as cations such as Na^+ in the environment tend to take the place of the metallic cations of the pipe, whereas biosurfactants tend to adsorb to the surface of the metal, inhibiting corrosion [12].

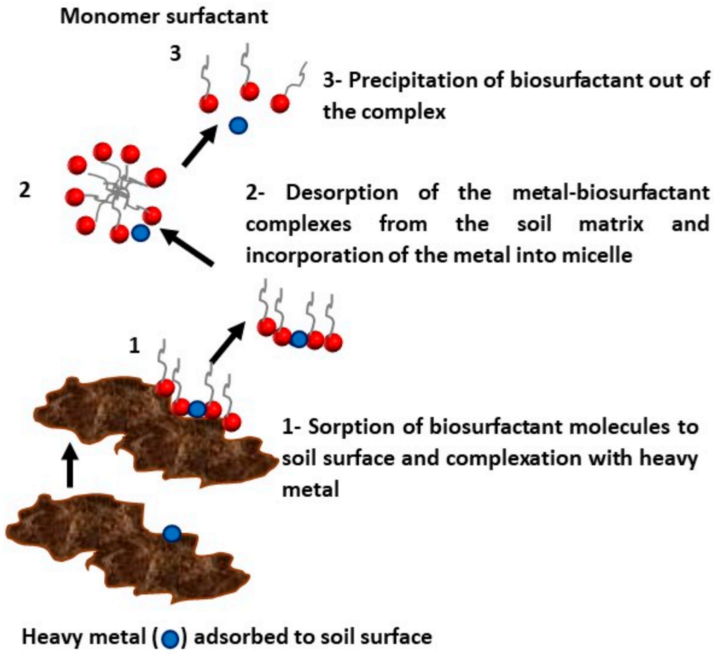

Figure 5. Heavy metal removal mechanism using biosurfactants [23].

6. Application of Biosurfactants for Bioremediation of Marine Environment

Oceans are the home to the largest oil reserves and, hence, the location of the largest oil spills. The transport and use of oil leads to the generation of oily effluents in the marine environment as well as the emission of gaseous pollutants [12]. Conventional remediation is classified as (i) physical or mechanical methods employing floating barriers known as booms and skimmers to contain and suck up the spilled oil and hydrophobic absorbents that repel water and capture the oil; (ii) biological and (iii) chemical, the latter of which includes on-site burning, the use of solidifiers, or gelling agents that transform the oil into a solid compound and dispersants, which are composed of surfactants, organic solvents, stabilizers, and other additives that break up the oil into emulsified droplets (oil-in-water emulsion) and accelerate the natural bioremediation process [60–62].

Although efficient at the remediation of oil spills in the marine environment, chemical dispersants do not biodegrade easily and are toxic to aquatic organisms, underscoring the need to replace these products with effective, biodegradable, ecologically friendly dispersants.

Biosurfactants have considerable stability when exposed to different environmental conditions. Microbial surfactants can maintain their surface-active properties in the presence of 2–10% salinity, a pH range of 3–12, and high temperatures (e.g., 70 °C), making these natural compounds viable for application in extreme environments with high salinity, such as the Dead Sea, where archeobacteria and halophilic bacteria produce polysaccharides and assist in the use of biosurfactants [19].

As in the remediation of oil-contaminated soil, the biosurfactant-mediated biodegradation of hydrocarbons occurs via two mechanisms. One involves the enhancement of the availability of the hydrophobic substrate to microorganisms by reducing the medium surface tension and reducing the interfacial tension between the cell wall and hydrocarbon molecules. The other mechanism involves the interaction between the biosurfactant and the cell surface, leading to alterations in the membrane, facilitating hydrocarbon adherence (increase in hydrophobicity), and reducing lipopolysaccharides of the cell wall without harming the membrane [4]. Biosurfactants block the formation of hydrogen bridges and allow hydrophobic–hydrophilic interactions, promoting molecular rearrangements, reducing the medium surface tension, increasing its surface area, and favoring bioavailability and, consequently, biodegradability [23].

An analysis of dispersion capacity revealed that a biosurfactant with a low CMC was effective; however, the dispersant at a concentration 1.5 times above the CMC was ineffective for biodegradation in marine remediation [63]. Above the optimal concentration, biosurfactants cover the biosurfactant–hydrocarbon complexes, either impeding the access of microorganisms to the hydrocarbons or becoming the substrate used by the microorganisms [64,65].

Several studies report the use of biosurfactants in marine environments. *P. aeruginosa* UCP 0992 grown in corn steep liquor and waste vegetable oil in a bioreactor produced a biosurfactant that reduced the surface tension of the medium to 26.5 mN/m, with a yield of 26 g/L. The biosurfactant was capable of removing 90% and 80% of motor oil adsorbed to sand in kinetic and static tests, respectively. Experiments revealed degradation rates higher than 90% for the oil in the presence of the bacterium and biosurfactant in seawater samples over a period of 75 days, demonstrating the potential of the biosurfactant in the oil decontamination process in a marine environment [50]. Experiments with a marine indicator demonstrated the stability and non-toxicity of the biosurfactant produced by *C. bombicola* URM 3718 cultivated in industrial waste and formulated with the addition of potassium sorbate. Tests involving different pH and temperature values in the presence of salt demonstrated that a commercial biosurfactant has potential applications in the environmental field [66]. A biosurfactant produced by the yeast *C. lipolytica* promoted the degradation of a petroleum by autochthonous microorganisms from seawater [67]. Bacteria isolated from contaminated seawater were studied for the production of biosurfactants and subsequent use in the remediation of marine environments. Genetic sequences revealed promising isolates from the same species (*B. cereus*). The BCS0 strain cultivated in a mineral medium with 2% waste fry oil was the best producer of surfactant, being able to reduce the surface tension to 27 mN/m. The biosurfactant remained stable in a wide range of pH and temperatures and in the presence of salinity, enhancing the degradation of motor oil in seawater up to 96% in 27 days. The biosurfactant also exhibited considerable oil displacement capacity [68]. The compound was characterized as a non-toxic lipopeptide, as survival rates for the fish *Poecilia vivipara* and the bivalve *Anomalocardia brasiliana* were higher than 90% and 55%, respectively. The biosurfactant also promoted the growth of autochthonous microorganisms in seawater [69]. Studying a biosurfactant produced by the same microorganism (*B. cereus*), Ostendorf et al. [70] found that the biomolecule was capable of removing 90% of oil adsorbed to marine rock and dispersed 70% of the contaminant in seawater.

The biosurfactant produced by the bacterium *P. cepacia* CCT6659 cultivated in industrial waste products and formulated with a food conservative had low toxicity to a plant and marine aquatic species and was able to remove about 70% and 84.50% of hydrophobic contaminants adsorbed to sand and marine rock, respectively. The biosurfactant also dispersed 96% of oil in seawater and increased the degradation of the oil by 70% in bioremediation experiments performed in flasks containing seawater [71]. The oil dispersion capacity of a biosurfactant from *C. lipolytica* was tested using a test developed by the US Environmental Protection Agency as a protocol for classifying oil spill dispersants (baffled flask test) [67]. The authors reported a dispersion rate ranging from 50 to 100%, depending on the concentration of the biosurfactant and its form of application (crude or isolated).

Binary systems involving the combination of biosurfactants and chemical surfactants have also been developed for the less-toxic treatment of oil spills. The system formed by ethanediyl-1,3-bis(dodecyl dimethyl ammonium bromide) (cationic surfactant) and surfactin from *B. subtilis* successfully reduced the interfacial tension of crude oil [72]. A binary mixture of the ionic surfactant choline laurate and a sophorolipid produced by *S. bombicola* proved non-toxic to fish and achieved dispersion rates greater than 80% [73].

7. Application of Biosurfactants for Oil Recovery from Reservoirs

Crude oil is a mixture of water, gas, and the organic fraction together with appropriate thermochemical conditions found in sedimentary rock [12]. The process used to extract crude oil involves three steps. The first and second steps are natural pressure and induced pressure (injection of water and gas). These steps extract approximately 40% of the trapped oil. The third step is the recovery of the residual oil. Enhanced Oil Recovery (EOR) involves the use of thermal methods (injection of hot water and carbon dioxide [CO_2]), non-thermal methods (flooding with solvents and chemical surfactants), and biological methods, although removing this oil from rock is difficult [12].

Synthetic surfactants in this process require capital and cause environmental pollution [74]. In contrast, the use of biosurfactants in EOR can provide favorable conditions and solve problems associated with environmental pollution [10]. Microbial Enhanced Oil Recovery (MEOR) involves the use of microorganisms or their metabolic products to retrieve the residual oil [75]. This procedure generally involves the injection of biosurfactant producer microorganisms, followed by the injection of nutrients into the reservoir (in situ biosurfactant production). Ex situ biosurfactant production can also be performed in industrial bioreactors for the subsequent direct injection of these compounds into the reservoir using CO_2. The emulsifiers/surfactants produced by microorganisms lower the surface tension, resulting in the release of the trapped oil. Biosurfactants alter the wettability of the injected CO_2 and the interfacial behavior of CO_2-brine-rock, increasing the flushing efficiency of the injected fluid and CO_2, resulting in the recovery of the oil [12,74,76].

Figure 6 shows the effect of biosurfactants on oil/rock and oil/water interfaces, decreasing the capillary forces that impede the oil from moving through the pores of the rock during the EOR process. Biosurfactants also form an emulsion at the oil/water interface that stabilizes the oil desorbed in water and allows its removal with the injection water [23].

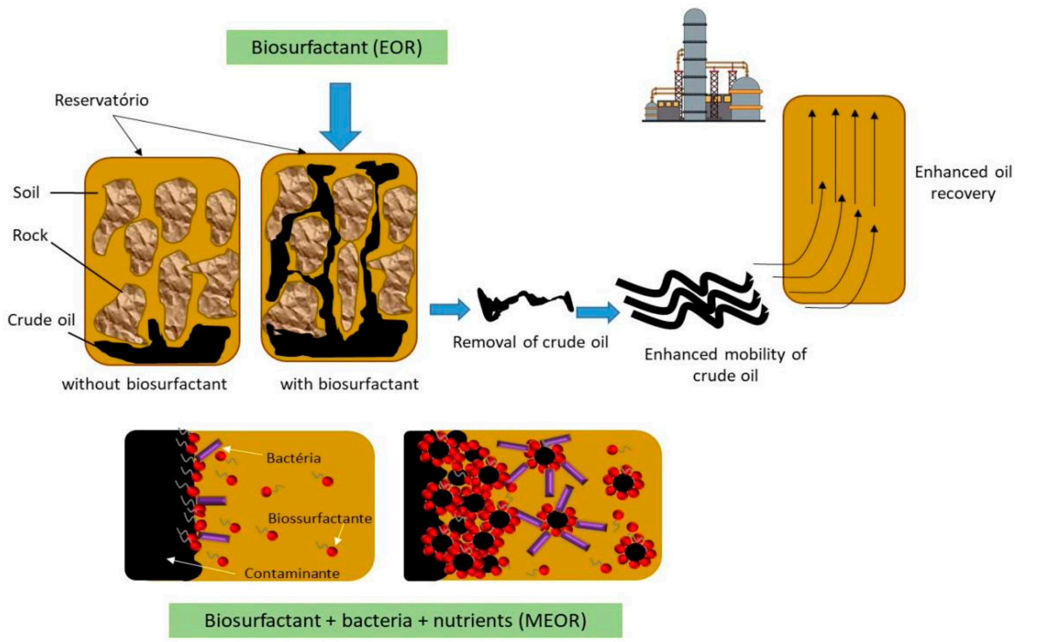

Figure 6. Enhanced Oil Recovery (EOR) and Microbial Enhanced Oil Recovery (MEOR) using biosurfactants (created by the authors).

Another method consists of the injection of only nutrients to stimulate the growth of indigenous biosurfactant-producing microorganisms in reservoirs. With all mechanisms, the biosurfactant lowers the surface tension of the water and the interfacial tension between the oil and rock, enabling the removal of the hydrocarbons [12]. Laboratory experiments involving the use of crude biosurfactants in sand-pack columns reported oil recovery rates of 15% [77], 30% [49], 80% [50], and 65–80% [78], demonstrating the viability of these ecological agents in EOR and MEOR processes.

One study reported the isolation of biosurfactant-producing strains capable of degrading crude oil isolated from the reservoir of an oil field in Assam, India. *B. Tequilensis* exhibited good oil degradation properties and biosurfactant production, which was optimized ex situ. The crude biosurfactant was identified as a surfactin, achieving an interfacial tension of 0.32 mN/m and removing 80% of the oil during washing procedures [79].

MEOR was evaluated by an economic model that simulates oil recovery operations using a bench-scale project [18]. A biosurfactant from *B. methylotrophicus* cultivated in industrial waste was able to remove 63% of oil adsorbed to sand and 25% adsorbed to sandy soil [80]. Contaminant removal rates are affected by the type and concentration of the biosurfactant, the affinity with the contaminant, interactions with acid or alkaline additives, and soil characteristics, as described by Adrion et al. [81].

8. Biosurfactants Applied to Cleaning of Oil Storage Tanks

The cleaning of oil storage tanks poses a challenge due to deposits of heavy oil [82]. Conventional methods are hazardous, time consuming, labor intensive, and expensive. The washing operation may involve the liquefaction of solvents and the spraying of hot water as well as the use of chemical surfactants [10,83]. Thus, the use of microbial surfactants, which are efficient, biodegradable, and environmentally friendly, constitutes an advantageous option for these cleaning processes [12].

The application of biosurfactants leads to oil-in-water emulsions, diminishing the viscosity of the sludge and deposited oil to enable the pumping of these residues. Moreover, the crude oil can be recovered after the emulsion is broken down, which is facilitated by the use of demulsifiers (discussed in Section 11 of this paper).

A rhamnolipid was used in storage tanks applying a vortex, resulting in an oil removal rate greater than 90% [12]. In another study, the sludge at the bottom of a tank was treated with the biosurfactant JE1058BS, achieving good results [84]. Silica nanoparticles, a saponin, and a rhamnolipid were used for oil recovery from refinery sludge in laboratory experiments. The optimal HLB for the extraction process was 10.5 for the mixture containing 62.5% rhamnolipid and 37.5% saponin, and the results demonstrated that the joint use of biosurfactants and nanoparticles led to a significant increase in the efficiency of the process. The maximum residual oil yield was around 4% under optimal conditions (biosurfactants and 3 g/L of silica nanoparticles) [85]. Another study investigated the effect of the metabolic broth of *P. aeruginosa* sp. SH 29 on vessels contaminated with oils, reporting the recovery of the oils from the walls and bottom of the vessels in 15 min, which floated in the supernatant as a distinct phase [86].

9. Application of Biosurfactants in Transport of Crude Oil through Pipelines

The long-distance transport of viscous crude oil from the extraction field to strategic destinations poses a problem due to the high content of asphaltenes and paraffins, which can cause obstructions of the pipeline [87]. This problem could be minimized with the use of larger pipes and stronger pumps to increase the pressure, the heating, or the use of toluene and xylene to dissolve the sludge. Despite the efficiency of these solutions, they are expensive and release toxic substances into the environment [10].

Paraffin wax deposition from crude oils at a low temperature is one of the serious problems in the petroleum transportation industry. As one of the low-temperature resistance properties of crude oil, the wax appearance temperature (WAT) is the temperature at which visible crystallization occurs. When the temperature falls below the WAT, the waxes tend to separate from the oil, crystallizing to form interlocking network structures, thereby entrapping the remaining liquid fuel in the structures [88].

Wax build-up that occurs in conventional middle and light crude oils is also a complicated and very costly problem for the petroleum industry. One viable solution of this problem is the employment of polymeric additives to improve the flow behavior of crude oil at low temperatures. Polymeric additives act as crystal modifiers, flow improvers, or pour-point depressants (PPDs), and are able to build crystals and modify the crystal morphology and growth characteristics into wax, reducing the tendency to interconnect into three-dimensional networks [88].

In this scenario, biosurfactants with emulsifying properties are indicated, as the formation of emulsions is essential to transporting oil. Unlike a water-in-oil emulsion, the stabilization of an oil-in-water emulsion requires the separation of the phases once the crude oil transported through the pipeline reaches its destination (refinery). The reduction in tension is not a concern in such cases and the surfactant should have a high HLB. Thus, biosurfactants with a high HLB (bioemulsifiers) are highly useful to increase the mobility of oil. Such cases involve the use of biosurfactants with a high molar mass, such as alasan, emulsan, and polymeric biosurfactants.

The bacterial surfactant Emulsan has many reactive groups that enable the molecule to secure firmly to oil droplets, avoiding the coalescence of these droplets by forming a barrier [18]. An emulsan produced by *Acinetobacter* sp. was used to reduce the viscosity of oil during transportation. Upon arriving at the destination, treatment can be performed with enzymes or demulsifiers to remove the bioemulsifiers from the emulsion [89]. Working with emulsifiers requires producing large volumes of this type of surfactant for the transportation of oil;however, it could avoid the need for a new pipeline [10]. Two bacterial biosurfactants were successfully added as a mixture or individually to crude oil to reduce the pour point [88]. A biobased surfactant obtained by enzymatic synthesis was tested for wax deposition inhibition. A maximum viscosity reduction of 70% and a drag reduction of 40% for light crude oil flows in pipelines were obtained with the surfactant additive at a concentration of 100 mg/L. Furthermore, a successful field application of the drag-reducing surfactant in a light crude oil pipeline in Daqing Oilfield was demonstrated [90].

10. Biosurfactants as Anti-Corrosion Agents

Corrosion is a process that leads to the deterioration of fundamental properties for the application of different materials, such as metals, plastic, wood, cement, and rubber. Corrosion on metals occurs via chemical and electrochemical mechanisms and is related to the surrounding environment [91]. Corrosion on a metal surface is a natural process that occurs due to the oxidation of metal atoms, which compromises the mineralized structure of the surface. This type of corrosion begins with the adsorption of protons, followed by an electrochemical reaction with the metal atoms. Metallic cations can either dissolve in the aqueous phase or react with anions, exposing the surface to further corrosion [92].

The employment of inhibitors is one of the most practical methods for avoiding corrosion [93]. Carbon steel pipes for the transport of oil and gas are subject to corrosion. Due to their proven effectiveness in industrial processes, biosurfactants could serve as anti-corrosion agents on metal surfaces to slow or even prevent corrosion [94].

Hegazy et al. [32] conducted a test in an acidic medium with 1 M of HCl for the inhibition of corrosion using three synthetic cationic surfactants. The carbon steel structure was assessed in a 1 M HCl medium with and without different concentrations of corrosion inhibitors. Scanning electron microscopy (SEM) revealed the corroded structure without the application of the surfactants and atomic force microscopy (AFM) revealed minimal roughness after the application of the inhibitors. The surfactant molecules were adsorbed physically and chemically to the active sites of the carbon steel structures, which were negatively charged due to the influence of the acidic medium, favoring the binding of the final group of the positively charged alkyl chain and the negatively charged part of nitrogen (N) of the surfactants. Thus, the hydrophobic portion of the alkyl chain favored the electron donor capacity and part of the nitrogen in both surfactants contributed to electron receiving [32].

Some studies report the applicability of plant-derived biosurfactants for the inhibition of corrosion. Extracts from *Citrus sinensis* contributed to the inhibition of corrosion on carbon steel surfaces in a 0.5 M sulfuric acid (H_2SO_4) medium. The metallic structure was composed of Mn, C, P, S, and Fe. Fourier-transform infrared spectroscopy revealed the interaction between the metallic structure and biosurfactant, whereas atomic force microscopy (AFM) revealed that the surfactant formed a smooth protective layer on the carbon steel surface in the H_2SO_4 medium, demonstrated by the assessment of roughness [95]. In another study, Faccioli et al. [33] investigated the use of a non-toxic biosurfactant produced by *P. cepacia* CCT6659 as an inhibitor of metallic corrosion, reporting minimal loss of mass (15.7%) compared to the control condition and demonstrating its potential in industrial applications.

11. Biosurfactants as Demulsifying Agents

Emulsifications are mixtures of water and oil formed with the use of surfactants and manual shaking or centrifugation. Emulsions can remain stable for up to a month in some cases. An emulsion may be oil droplets dispersed in water or water droplets dispersed in oil. Larger droplets form more stable emulsions. Nanodroplets also form stable emulsions, whereas those with microdroplets are unstable. The viscosity of oil-in-water emulsions increases with the reduction in the diameter of the oil droplets [20].

The zeta potential is a measure of the stability of an emulsion, with a higher zeta potential indicating a more stable emulsion due to the greater repulsion of nanodroplets. Greater salinity also leads to a more stable emulsion [20].

The recovery of oil from emulsions requires a different type of biosurfactant—demulsifiers, for which rhamnolipids are quite effective. Biosurfactants with high emulsifying capacity are important for controlling oil spills due to the consequent increase in the biodegradability of the oil by microorganisms. Other types of natural surfactants perform better in demulsification, which is necessary for the removal of the oil and the reuse of water from emulsions [19].

Demulsification occurs through flocculation. A thin layer of biosurfactant is formed on the oil droplets, facilitating coalescence and resulting in instable droplets of a larger diameter, consequently enabling the separation of the oil from the water. The demulsification process is easier when the emulsion has a greater concentration of hydrophobic compounds [20]. Rhamnolipids are the most efficient biosurfactants for this purpose, achieving 100% demulsification. Biosurfactants with a lower molar mass perform better as demulsifiers. Surfactin, as a demulsifier, achieved a greater-than-95% oil removal rate [20].

Demulsification of water and oil emulsions occurs through sedimentation, cremation, flocculation, aggregation, and coalescence. Sedimentation and cremation occur due to the difference in density between the two phases. Flocculation involves the aggregation of water droplets, whereas coalescence involves the collapse of the droplets, resulting in an increase in size [20].

Rocha e Silva et al. investigated the demulsification potential of microbial biosurfactants in aqueous emulsions formed by petroleum products [96]. The biosurfactants were produced by the yeasts *Candida guilliermondii, C. lipolytica*, and *C. sphaerica,* as well as the bacteria *P. aeruginosa, P. cepacia,* and *Bacillus* sp. cultivated in industrial waste products. The bacterial biosurfactants recovered more of the seawater in emulsions with motor oil compared to the yeast biosurfactants (65% vs. 35–40%, respectively). The biosurfactants were also tested with oil-in-water and water-in-oil emulsions with kerosene; no association was found between demulsification capacity and cellular hydrophobicity or interfacial tension of the biomolecules.

12. Application of Biosurfactant in Treatment of Oily Effluents

Over time, the need for the reuse of oily wastewater has increased and the importance of reusing industrial or domestic wastewater has driven the development of novel treatment technologies [13,82]. Technological advances have enabled the use of decantation, centrifugation, filtration, and flotation for the treatment of oily wastewater [97]. However, the use of chemical substances constitutes a problem. For instance, conventional collectors used in flotation processes are toxic and harmful to the environment [21]. Thus, environmentally sustainable alternatives are desirable, such as the use of biosurfactants as collectors in flotation processes [98,99].

Rocha e Silva et al. [100] analyzed the effect of biosurfactants on the separation efficiency of a dissolved air flotation (DAF) system on a pilot scale. While the process was effective with and without the surface-active agents, the biosurfactant produced by *C. sphaerica* improved the separation efficiency from 80 to 95%.

The biosurfactant produced by the bacterial species *B. methilotrophicus* UCP1616 was used as a collector for oil removal in a prototype DAF system involving a centrifuge pump for the generation of microbubbles in the flotation chamber. The results demonstrated that the injection of the biosurfactant increased the oil removal rate from 60 to 92% [101].

Biosurfactants produced by the bacteria *P. cepacia* and *B. cereus* were used as collectors in a DAF system, achieving oil removal rates from wastewater of 94.11% and 80.01%, respectively. Moreover, the study proposed the reuse of the treated wastewater, which is in line with sustainability [89]. An induced pre-saturation tower (set of flotation chambers in continuous sequence from top to bottom) was operated with the biosurfactant produced by *P. cepacia* for the treatment of oily wastewater and a reduction in the diameter of the microbubbles to increase the contact surface with the droplets and enhance the oil removal process, resulting in an oil concentration of 15 mg/L, which is within the limit determined by Brazilian environmental law [102].

13. Biosurfactants in Biodesulfurization

Sulfur and nitrogen constitute one of the problems in the production of fossil fuels. These elements cause corrosion and are associated with the emission of the NO_x and SO_x gases, which cause health problems to humans as well as acid rain, contributing to the degradation of the environment. Thus, environmental legislation limits the sulfur content in oils. This limit is from 10 to 15 ppm in developed countries but can reach around 50 ppm in low-income countries [103].

Sulfur is removed by heating the oil to 200–600 °C at a pressure of 1 to 18 Mpa in the presence of a metal catalyst in conventional technologies. However, this is economically unfeasible and produces pollutants. Hydrodesulfurization (HDS) is employed but has low desulfurization efficiency for thiophene-based aromatic heterocyclic compounds [103]. Thus, an alternative used in the oil industry to remove sulfur from oil is bio-desulfurization (BDS), which involves the application of microorganisms to selectively remove sulfur from organosulfur hydrocarbons to complement HDS without degrading the carbon skeleton. However, it is a great challenge to ensure the availability of these aromatic sulfur compounds for microorganisms. Solubilization and mobilization using chemical or green surfactants could overcome this limitation, for which biosurfactants offer the advantages of specificity, biocompatibility, ease of production, and eco-friendliness. Biosurfactants can make organic sulfur compounds bioavailable for microbe-based removal without affecting the calorific value of the fuel [104].

The degradation mechanism of natural surfactants should also be considered. Enzymes such as dehydrogenase and oxygenase are needed to degrade aromatic polycyclic compounds. Moreover, the final electron acceptor in aerobic environments is oxygen gas, whereas the acceptors are nitrogen and sulfur atoms in anaerobic environments, such as sediments on the sea floor, aquifers, and subsoils [19].

Corrosion can also occur through the biological pathway. Anerobic bacteria use sulfate (SO_4^{2-}) in petroleum products as the final electron acceptor, reducing it to H_2S, which promotes corrosion in oil pipelines. Biosurfactants could be used as biocides of these bacteria and impede the occurrence of corrosion [12]. The removal of sulfur from thiophenes in fuel is achieved by specific microorganisms that use enzymes for this purpose.

Other methods employ genetic engineering to increase the production of microbial enzymes needed to catalyze the hydrolysis of thiophenes in HSO_3^- [10].

14. Application in Formulation of Fuels

Fossil fuels are the main source of energy in the modern world. Diesel is used in transportation, the production of electricity, and industrial activities; however, it is associated with the emission of particulate matter, black carbon, sulfur oxides, nitrogen oxides (NO_x), carbon monoxide, and carbon dioxide (CO_2) [105,106]. Thus, a technology capable of reducing these pollutants without compromising engine performance is needed. Researchers have investigated water-in-diesel emulsions that can reduce emissions of particulate matter and oxides while also improving performance. However, phase separation occurs after a long storage period [12] and surfactants are, therefore, necessary to stabilize the emulsion and ensure that the dispersed water droplets remain suspended within the diesel. Additives can be incorporated to improve performance. Sorbitan monooleate, fatty acid esters, Span 80, Tween 20, and Tween 80 are surfactants that are commonly used for the stabilization of diesel emulsions [107,108] and have HLB values of 9–10. The quantity of these surfactants ranges from 0.5 to 5% by volume, whereas the water content is from 5 to 15% p/p [109]. Water-in-diesel emulsions improve combustion efficiency and reduce the quantity of unburned hydrocarbons, particulate matter, and pollutant emissions [12].

Some studies describe the use of biosurfactants in such formulations as a sustainable alternative to synthetic surfactants. Rhamnolipids produced by *P. aeruginosa* can be used to stabilize water-in-diesel emulsions for use as fuel. This combination is needed to reduce viscosity during the transport of diesel, which is the main source of energy, as well as reduce the emissions of particulate matter and hydrocarbon gases [10]. A test fuel was prepared with lemon peel oil (LPO), 10% water (10W), and 2% of the surfactant sorbitan monolaurate to make the emulsion more stable. The LPO and emulsion with water (LPO10W) performed the best in terms of the heat release rate. Delayed ignition with the LPO10W was solved by the incorporation of cetene. LPO and LPO10W had low opacity and low atmospheric pollutant emissions. The emulsion also had low NO_x emissions. Thus, LPO and LPO10W are feasible options for the replacement of conventional diesel due to their renewability, "green" status (low environmental pollution), and contribution to sustainability [110].

A biosurfactant was used in a microbial fuel cell. A biofilm was grown on the anode, which produced the surface-active agent. The influence of Fe, Ni, and Fe/Ni on the cathode was assessed regarding the production of surfactant and increase in electrical power. Fe nanoparticles reduced internal resistance more than Ni nanoparticles, followed by Fe/Ni nanoparticles, in comparison to the control. The nanoparticles increased biosurfactant production, biofilm growth on the anodes, and the power density of the fuel cell [111]. In another study, an increase in the electrical power of a microbial fuel cell was found after the addition of 5 mg/L of biosurfactant, with a tendency toward a decrease in charge at higher concentrations. This occurred because biosurfactants increase the hydrophobicity of bacterial membranes. Moreover, the hydrophobic part of the biosurfactant reacts easily with the lipid layer of the membrane, causing small perforations at a concentration of 5 mg/L, with an increase in the permittivity of the membrane and a greater ion flow from the cells to the electrode, increasing the electrical potential of the microbial fuel cell [112].

Better results can be obtained when biosurfactants are appropriately incorporated into water-in-diesel mixtures compared to their synthetic counterparts. Biosurfactants, such as rhamnolipids, have a low CMC, which is essential for W/O emulsions. The best option may be heteropolysaccharides and emulsifying proteins [113]. According to Fenibo et al. [10], while a biosurfactant stabilizes an emulsion, water plays an important role in combustion via microexplosion and reduces NOx (due to cooling resulting from the vaporization of the water), reducing the formation of soot, particulate matter, and hydrocarbons due to the slower reaction velocity.

15. Application of Biosurfactants in Degreasing Industry

Industrial activities involve the generation of large volumes of oily wastewater resulting from the lubrication of equipment and the cleaning of floors impregnated with heavy oil [13]. Synthetic detergents, degreasing agents, and organic solvents are currently used to remove grease from equipment; however, these non-biodegradable agents are harmful to the cleaning staff as well as the environment [114]. Conventional chemical detergents form a layer on the surface of aquatic ecosystems, impeding gas exchange and the penetration of sunlight, which compromises photosynthesis and respiration. Such detergents are also toxic to organisms, provoke ecological imbalances, and cause harm to human health through inhalation, absorption, and ingestion. Thus, researchers have been working to produce biosurfactants for the replacement of synthetic surfactants in these degreasing formulations [13].

Biodetergents or biodegreasing agents have biodegradable components and can have microbial or plant-based biosurfactants or a chemically-synthesized green surfactant. An ecological biodegreasing agent is non-toxic and environmentally compatible when present in soil, rivers, or marine environments. Such sustainability meets the demands of consumers for "greener" cleaning products [6,115].

Detergents and degreasing agents are composed of a volatile organic solvent to reduce viscosity and dissolve the components, stabilizers to provide color to the system, adjust the pH, reduce the occurrence of corrosion and make the heavy water content more stable, and a non-ionic or anionic surfactant comprising 1 to 50% of the product. In a biodetergent, the synthetic surfactant is replaced with a natural correlate [11].

Farias et al. [116] investigated biosurfactants produced by the bacterial species *P. cepacia* CCT 6659, *P. aeruginosa* UCP 0992, *P. aeruginosa* ATCC 9027, and *P. aeruginosa* ATCC 10,145 for use in a biodetergent. Emulsion and dispersion tests revealed that the biosurfactant produced by *P. aeruginosa* ATCC 10,145 was more effective at removing heavy oil. The biodetergent was also more efficient at removing oil compared to detergents that are commonly employed. Toxicity tests with the microcrustacean *Artemia salina* and cabbage (*Brassica oleracea*) revealed that the biodetergent was non-toxic. The product was also biodegradable [116].

However, the yield of biosurfactants was low and the costs of upstream and downstream processes still limit the production of natural surfactants on the industrial scale [117]. On the other hand, studies reveal that compounds containing more than one surfactant can reduce the interfacial tension of water and oil systems in a more satisfactory way [73]. This combination and the search for particular substrates and specific biosurfactant production conditions could make biodetergents and biodegreasing agents economically feasible for the removal of heavy oil [23].

The biosurfactant from *S. bombicola* ATCC 22,214, which reduced the surface tension of water to 32.30 mN/m, was used in a commercial formulation that had low toxicity, and heavy oil dispersion capacity and emulsification capacity, demonstrating potential as an ingredient of industrial degreasing agents [78]. Rocha e Silva et al. [114] described a sustainable plant-based biodetergent composed of cotton seed oil (natural solvent), saponin (plant-derived surfactant), and natural stabilizers (carboxymethylcellulose and glycerin). The biodetergent was non-toxic, stable, and highly efficient (capable of removing 100% of heavy oil from glass and metal surfaces). This product can be applied in industries that require the cleaning of equipment and floors encrusted with oil and grease.

Sophorolipids are used in detergents sold by SyntheZyme (USA), Soliance (France), and Ecover (Belgium). Moreover, alcohols increase the length of the hydrophobic chain when reacting with biosurfactants, enhancing the oil removal efficiency. Other options include the genetic recombination of biosurfactant-producing microorganisms and the optimization of fermentation processes with the use of agroindustrial waste products as the source of carbon and nitrogen, which eliminates the use of expensive yeast extract. These are the main ways to make biodetergents and biodegreasing agents viable for the market [118].

16. Patents with Biosurfactants Applied in Environmental Field and Oil Industry

Various biosurfactants have been patented for use in the environmental field and are sold by different countries. Different MEOR processes have also been patented (Table 1).

Table 1. Patents involving biosurfactants in the environmental field and oil industry (created by the authors).

Title of Patent	Biosurfactants/Microorganisms	Patent Number	Reference
Enhanced oil recovery process using microorganisms	Mixture of biosurfactant-producing microorganisms	US 4450908	[119]
Recovery of oil from oil reservoirs	Indigenous biosurfactant-producing microorganisms	US 5083610	[120]
Nutrient injection method for subterranean microbial processes	Injection of nutrients to stimulate microbial biosurfactant production	US 5083611	[121]
Biosurfactant and enhanced oil recovery	Lipopeptide	US 4522261	[122]
Methods for improved hydrocarbon and water compatibility	Diverse biosurfactant-producing microbial population	US 7992639	[123]
Process for stimulating microbial activity in a hydrocarbon- bearing, subterranean formation	Microbial consortia	US 6543535	[124]
Complex biological oil displacement agent and application thereof	Combination of biosurfactant (rhamnolipid) and chemical surfactant (fatty acid amide sulpho monoester potassium maleate)	CN 102492409	[125]
Composition for increase of oil recovery	KshAS-M biosurfactant	RU 2143553	[126]
A process for preparation of biosurfactant useful as microbial emulsifier for the recovery of oil	Microbial bioemulsifier	IN 189459	[127]
A process for enhanced recovery of crude oil from oil wells using novel microbial consortium	Three hyperthermophilic, barophilic, acidogenic, anaerobic bacterial strains producing variety of metabolic products, especially CO_2, methane, biosurfactant, volatile fatty acids and alcohols	WO 2005005773	[128]

Table 1. *Cont.*

Title of Patent	Biosurfactants/Microorganisms	Patent Number	Reference
Microbial enhanced oil recovery and compositions therefor	Microbial consortia	US 4905761	[129]
Microbial enhanced oil recovery methods	Exogenous biosurfactant-producing microbes that degrade hydrocarbons	US 8316933	[130]
Biotechnological process for hydrocarbon recovery in low permeability porous media	Biostimulation and bioaugmentation of biosurfactant-producing bacterial consortia	US 8631865	[131]
Steady state anaerobic denitrifying consortium for application in in-situ bioremediation of hydrocarbon-contaminated sites and enhanced oil recovery	Anaerobic denitrifying biosurfactant-producing consortium	US 8753865	[132]
Heavy oil recovery process using extremophile anaerobic indigenous microorganisms	Extremophile anaerobic indigenous biosurfactant-producing microorganisms	US 8895479	[133]
Alkaline microbial enhanced oil recovery	Haloalkaliphilic biosurfactant-producing microbes	US 9290688	[134]
Method and installation for flooding petroleum wells and oil-sands	Glycolipids	CA 1119794	[135]

Most of these patents are from the United States and describe the application of biosurfactant-producing microorganisms or biosurfactant-producing consortia for oil recovery, followed by the use of biosurfactants. It is clear that other applications described in this review must be explored to generate patents and commercial products for the oil industry. The addition of nutrients is also mentioned. The future promises greater use of biosurfactants in oil industries [12].

17. Conclusions

The goal of this review was to show biosurfactant contributions to the petroleum industry, as well as to discuss interesting and less-explored research areas for these biomolecules in the oil sector. In terms of practical implications, the current findings provide insights for industrial entities that value the green economy, as biosurfactants can be used to achieve organizational or national-level sustainability goals.

Biosurfactants have a wide range of applications in industries and the environmental field. These natural compounds can be used in soil and marine remediation processes to mitigate impacts caused by the oil industry. Biosurfactants can also be used for the removal of heavy metals, oil recovery enhancement in mature oil fields, the inhibition of metal corrosion, the formulation of fuels, and the fabrication of "green" detergents, reducing the occurrence of toxic substances in the environment. They are also suitable as additives for improving the flow properties of crude oil.

Biosurfactants have high specificity due to their considerable molecular variability and structural diversity, enabling the selection of the best biosurfactant for a particular application based on the results of previous studies.

The biosurfactant industry has demonstrated remarkable growth in recent decades, although the large-scale production of these biomolecules remains a challenge from the economic standpoint. This is mainly due to the enormous difference between the financial investment required and viable industrial production. Advances in research in this field have indicated the importance of yields and productivity improvements achieved through bioengineering strategies, changes in fermentation modes, and the statistical design of experiments. Biosurfactants are expected to become viable for industrial scale production with the use of low-cost substrates. Other solutions include the use of biosurfactants in crude form, the employment of nanoparticles, foam fractioning methods, directed and solid-state fermentation, and novel extraction systems. Identifying efficient and significant surfactants is also of paramount importance for industrial scale-up because the final yield compound is influenced by nutrients, microorganisms, micronutrients, and environmental factors. The high cost of biosurfactant production could be circumvented by the elimination of purification processes, which account for 60% of production costs. Regarding the oil industry, the use of crude fermentation broths can be a viable solution, especially if the application is in an environmental context, as biosurfactants in such cases do not need to be pure and can be synthesized using a blend of inexpensive carbon sources, which would allow the creation of an economically and environmentally viable technology for bioremediation processes. The development of recombinant DNA techniques to increase the production of biosurfactants by microorganisms could also reduce the cost of these natural compounds. Thus, the future of studies on biosurfactants depends on the understanding of the genetics and physiology of producing microorganisms and the reduction of downstream processes to make biosurfactant production viable from the industrial standpoint.

Despite the high cost of biosurfactant production, companies are already selling these compounds. However, only a few small industries are already producing microbial biosurfactants for commercial use in the global sector. Large industries should take meaningful steps to incorporate microbial biosurfactants in their commercial products to enhance the use of biosurfactants in the global market.

Moreover, one can see limitations in oil production, the technical and economic impacts of which on industries will need to be addressed while respecting environmental laws. The extensive atmospheric pollution caused by noxious gases, the pollution of aquatic ecosystems, and the contamination of soils by oil and heavy metals leave few natural environments that offer quality of life. Thus, biosurfactants are promising products in the current scenario. Despite the challenges, solutions are being found, such as the use of agro-industrial byproducts as low-cost substrates and the genetic recombination of biosurfactant-producing microorganisms. Another option is the use of crude biosurfactants and the optimization of these products. Due to their stability, biodegradability, and low toxicity, natural surface-active agents offer advantages over chemical correlates, promoting greater quality of life and social wellbeing. The careful and controlled use of these interesting microbial surface-active molecules will surely help in the enhanced cleanup of toxic environments and provide us with a clean environment.

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