Chapter 2

Biosurfactants in Improving Bioremediation Effectiveness in Environmental Contamination by Hydrocarbons

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Abstract Recent biotechnological advances currently evidence new surfactant production technologies. Biocompounds produced by fermentative processes appeared as an economic and sustainable alternative to many synthetic molecules. Thereby, biosurfactants have become a promising substitute due to their synthesis potential by a wide variety of microorganisms. Biosurfactants are a highly diverse group of structures, such as glycolipids, lipopeptides, polysaccharide-protein complexes, phospholipids, fatty acids, and neutral lipids. This diversity promotes many advantages compared to synthetic surfactants, thus making biosurfactants the most natural choice for technological advances associated with sustainable development. Such advantages include fermentative production viability by using renewable resources, effectiveness in small concentrations even under extreme conditions, selective and specific potential for several applications, lower toxicity, higher biodegradability, and better stability to physicochemical variations. Despite their benefits, biosurfactants are not widely used because of the high production costs. Hence, cost-effective substrates, optimized cultivation conditions, and mutant lineage development are imperative to make these biomolecules an economically competitive product to propose a widespread replacement of synthetic surfactants.

2.1 Introduction

Petroleum spills, including oil-based products, can cause considerable damage to the environment, generating an enormous public concern. Oil pollution demands fast and cost-effective solutions. It is estimated that about 0.40% of global oil

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production eventually reaches the oceans (Banat et al. 2000). Several accidents with oil spills in recent years have continuously shown the environmental damage from hydrocarbons (Montagnolli and Bidoia 2012).

The major oil spill sources can be traced down to all stages of petroleum processing (exploration, refining, and transport). Accidents are prone to occur in marine tanks, complex bed drilling, marine fleets, refineries, and associated leaks (El-Tarabily 2002; Mille et al. 2006). In Brazil, many accidents involving petroleum, including derivatives such as gasoline and jet fuel, have caused serious environmental problems. In 1998, 1.200 m^3 of diesel oil were released due to pipeline corrosion underneath a considerable area below the city of Cubatão-SP. Pipeline corrosion was also responsible for 1.300 m^3 of hydrocarbons spilled in Guanabara Bay in Rio de Janeiro, which had a history of being contaminated constantly by oil spills (Benincasa 2007).

The lubricating oils are an important petroleum-based product, reaching about 60% of petrochemical products. Its importance is mostly industrial but also due to the massive automobile applications (Mang and Gosalia 2017). However, this high consumption also means a serious potential for environmental problems (Rahman et al. 2002). Lubricants often leak chronically from storage tanks (Lopes et al. 2008). Thus, continuous and prolonged release sets up the scenario for a long-term and highly persistent pollution regarding lubricant oils, leading to the contamination of soils and groundwaters (Chavan and Mukherji 2008). In addition, used lubricant oil suffers structural changes caused by high pressures and temperatures inside automobile engines that affect the biodegradability (Lopes and Bidoia 2009).

The persistence of petroleum hydrocarbons in the environment depends on several factors, such as chemical structure, concentration, and dispersion (Atlas 1981; Mille et al. 2006; Prosser et al. 2016; Duan et al. 2017). Physicochemical treatments are applied in the event of the oil spill; however, these treatments are very expensive, and even more strategy might be necessary depending on the chemical agents chosen as dispersants or catalysts (Elanchezhiyan et al. 2016; Grote et al. 2018).

Alternatively, the conventional physical treatments can separate soil and contaminants without destroying or chemically modifying the oils. Most of the hydrocarbons are absorbed in the soil matrix, reducing the removal efficiency of any treatment. Biological processes, on the other hand, are promising clean decontamination technologies, as they combine simplicity and cost-effectiveness. Thus, among many novel strategies, bioremediation emerges as the least aggressive and the most suitable method for keeping the ecological balance (Montagnolli and Bidoia 2012).

The decrease of contaminant concentrations by biodegradation or other treatment does not always indicate a decreased toxicity. The incomplete degradation and consequent formation of toxic intermediate compounds (metabolites) can result in increased toxicity during remediation processes (Tamada et al. 2012). Therefore, the combination of chemical analysis and ecotoxicity assays is recommended to elucidate the risks associated with contamination. In this way, a detailed monitoring is crucial for better bioremediation strategies and the establishment of environmental safety standards (Al-Mutairi et al. 2008).

2.2 Bioremediation of Areas Impacted by Petroleum Hydrocarbon

The microbial ability to use petroleum hydrocarbons as a carbon source was first demonstrated by Zobell (1946), who also revealed that these organisms were widely distributed in nature. The author described 100 bacterial species, belonging to 30 genera capable of metabolizing petroleum. Later, Bartha and Atlas (1977) expanded this list into another 22 bacterial genera, 14 fungi, and 1 alga. Any efficient bioremediation proposal should demonstrate that the contaminant removal is primarily due to biodegradation rates. In other words, the biodegradation must be higher than natural attenuation degree of decontamination. Among the many difficulties in the development of bioremediation methods is the reliability of laboratory-scale experiments in comparison to field results (Juhasz et al. 2000; Simpanen et al. 2016).

There are also several strategies for improving natural attenuation processes. Many of them are extremely cost-effective compared to physical and chemical treatments. The most common approaches in bioremediation are biostimulation, bio-aeration, bioaugmentation, land farming, composting, and phytoremediation (Wu et al. 2016; Agnello et al. 2016). The insertion of nutrients (biostimulation) and oxygen (bio-aeration) favors microbial metabolism when using pollutants as substrates (Seklemova et al. 2001). Another commonly used procedure known as bioaugmentation is the inoculation of an enriched microbial consortium in soil (Richard and Vogel 1999; Barathi and Vasudevan 2001; Agnello et al. 2016). Montagnolli et al. (2009) obtained biodegradability datasets of lubricating oils using respirometric flasks. Kinetic models have been applied to biodegradation curves, which demonstrated the rate of biodegradation of automotive lubricating oils compared to vegetable oils. It was observed that petroleum degradation tends to decrease slower and last longer. However, the influence of other factors in biodegradation had not been determined. To better establish the optimal conditions of biodegradation, it is important to know the key features of an impacted area, such as residual oil concentration, density of degrading microorganisms, and microbial biodegradation potential.

Generally, an accelerated biodegradation of hydrocarbons depends on the presence of specific microorganisms. In addition, the composition of the microbial population is directly affected by the environmental conditions and the type of hydrocarbons (Admon et al. 2001). However, these indigenous communities lack species with proper enzymatic mechanisms necessary for a rapid biodegradation, which results in long-term biodegradation processes if not bioremediated (Díaz-Ramírez et al. 2008).

In this regard, the bioremediation of soils contaminated with mixed hydrocarbons from petroleum sources is a challenge due to the poor bioavailability and complex chemical composition of these compounds (Lee et al. 2008; Sabate et al. 2004; Yu et al. 2018). The hydrocarbon concentration and the presence of oxidative metabolites with potential risks to the environment can also remain after treatment (Pagnout et al. 2006).

The chromatographic profile of lubricating oils (Lladó et al. 2012), for example, shows a considerable fraction of a nondegradable complex mixture (unresolved complex mixture, UCM). In fact, little is known about the composition of the UCM, although the major components of oils are characterized by their high resistance to biodegradation (Wang and Fingas 2003; Wang et al. 2016). Most of the petroleum-based products are composed of branched and cyclic aliphatic and aromatic compounds, including polycyclic aromatic hydrocarbons (PAH) (Nievas et al. 2008).

The PAHs are often found in the environment due to atmospheric deposition originated from natural sources such as burning biomass and volcanic activity or artificial sources including burned fuels and many environmental accidents from the petrochemical industry (Lors et al. 2010). The release of PAHs represents a great concern due to their toxic, mutagenic, and carcinogenic properties (Martins et al. 2013). Although these aromatic molecules can undergo chemical oxidation, photolysis, adsorption, and volatilization, however, the microbial degradation of PAHs is, in most cases, the main alternative of soil treatment (Yu et al. 2018, Liu et al. 2017) .

Several studies on petroleum hydrocarbon biodegradation have been adopting different methodologies (Bidoia et al. 2010; Cerqueira et al. 2014; Zhang et al. 2016), but they all indicate that degradation occurs at least in some specific fractions of these substances. There is no general rule in petroleum biodegradation patterns, as most cases shown with preferential remediation of the lighter compounds were observed, whereas, in other studies, it was directed toward the heavier hydrocarbons (Huang et al. 2004).

In aquatic environments, the biodegradation of pollutants is limited and depends on the bioavailability of nutrients (such as nitrogen and phosphorus) required for the onset of microbial growth. The use of soluble salts containing these elements is an effective way to recreate and optimize the biodegradation under laboratory conditions. Field results are often not the same, as in situ treatments yield unsatisfactory stirring of the medium as well as a much reduced dissolution of salts. As a viable alternative, biosurfactants can be associated with the nutrient solution. This is importantly aimed toward petroleum pollution, because the oil is emulsified by the action of biosurfactants and thus provides a rapid microbial growth (Thavasi et al. 2011; Bezza and Chirwa 2015a, b; Mnif et al. 2017).

2.3 Surfactants

Surfactants are amphipathic molecules, i.e., compounds which have polar (hydrophobic) and nonpolar portions (hydrophilic), shifting solubility of other molecules in aqueous solutions based on polarity. These molecules act on the water-oil interface, thus forming micelles in various shapes and sizes (Van Hamme et al. 2006). Surfactants are an important class of chemicals widely used in modern industry (Develter and Lauryssen 2010; Franzetti et al. 2010). In 2007, chemical surfactant production reached about 10 million tons (Van Bogaert et al. 2007). In this context, the market share is led by cleaning detergents (up to 50% of surfactant production), generating a 60-billion-dollar revenue in 2004 (Scheibel 2004). It is known that almost all commercially available surfactants are now chemically synthesized from petroleum.

The conventional chemical surfactants derived from petroleum are subject to the availability of fossil fuels and pose potential threats to the environment due to their recalcitrant nature (Makkar and Rockne 2003; Aparna et al. 2012). Approximately, 0.57 tons of petrochemical intermediates are consumed for each ton of surfactant produced (Patel et al. 1999). By projecting these values to the global production of surfactants, it is estimated that 7.40 million tons of petrochemicals are destined annually for the production of surfactants (Reznik et al. 2010). Thus, there is a trend toward eco-friendly technologies mobilizing the search for novel biodegradable compounds and renewable substrates, including industrial waste (Marchant and Banat 2012; Sasayama et al. 2018).

2.4 Biosurfactants

The advances in surfactant technologies are within the scope of many biotechnological types of researches. There was a subtle increase in the number of patents involving biosurfactants at the beginning of this century. More than 70% of these were reported between 2000 and 2010. In contrast, most of the patent registrations for chemical surfactants were performed in the 1900s, with a sharp drop after 2000 (Müller et al. 2012).

Biosurfactants are a natural choice as substitutes to synthetic surfactants because they have several advantages, such as (1) viable fermentative production using renewable resources; (2) effectiveness in small quantities, even under extreme conditions; (3) selective and specific potential for various applications; (4) low toxicity; (5) high biodegradability; and (6) stability to pH, salinity, and temperature variations (Abdel-Mawgoud et al. 2010; Banat et al. 2000; Cameotra and Makkar 2010; Lovaglio et al. 2011; Hazra et al. 2011; Zhao et al. 2017).

The biosurfactants are produced by microorganisms to increase cellular access to hydrophobic substrates. This facilitates the metabolism and promotes the development of biomass, hence increasing biodegradation (Bordoloi and Konwar 2009; Singh et al. 2007). The major advantage of biosurfactants compared to synthetic surfactants lies in their structural diversity and environmental acceptability. Biosurfactants are biodegradable, biocompatible, and less toxic with higher specificity, the possibility of in situ production. They can be produced from renewable substrates and organic residues (Mulligan 2009). A wide range of biosurfactants are potential to apply in various industrial approaches: food, petrochemical, mining, agriculture, cosmetics, pharmaceutical, textile, leather, construction, dyes, and chemicals (Araújo et al. 2016; Ferreira et al. 2017). In addition, these molecules have the ability to decrease surface and interfacial tension. These properties can promote microbial growth, aid microbial enhanced oil recovery (MEOR) procedures in drilling oil wells, and facilitate bioremediation of pollutants (Zhao et al. 2017). Thus, biosurfactants are a multifunctional material and an important alternative to replace compounds and chemical processes (Silva et al. 2017).

Typically, biosurfactants have hydrophilic structures (amino acids, peptides, mono-/disaccharides, and/or polysaccharides) and hydrophobic structures (saturated and/or unsaturated fatty acids) in their molecules (Smyth et al. 2010; Shao et al. 2015). Biosurfactants are classified by their chemical structure, and this composition depends on the producing microorganisms, the substrate, and the conditions of the fermentation process (Cameotra and Makkar 2004; Nitschke et al. 2005a; Singh et al. 2007; Makkar and Cameotra 1999; Nitschke and Pastore 2006).

Among the various microorganisms able to produce biosurfactants, bacteria belonging to the genus *Pseudomonas* are often the most promising group. They are able to synthesize biosurfactants known as rhamnolipids. These molecules are glycolipids containing fatty acid groups linked to a rhamnose. The lipid portion is composed of β-hydroxydecanoic acid (Benincasa et al. 2004; Mulligan 2009; Abdel-Mawgoud et al. 2010). These different types of rhamnolipids slightly differ on their chemical structures and surface activities. The production of each homolog depends on the nutrient availability, the environmental conditions, and the biosynthesis capabilities of the specific *P. aeruginosa* strain (Oluwaseun et al. 2017; Mondal et al. 2017).

Rhamnolipids are considered as the most promising biosurfactant class in terms of industrial production, due to their physicochemical characteristics, outstandingly high productivity, and deep understanding of the rhamnolipid production (Müller et al. 2012).

2.5 Biological Function of Biosurfactants

Biosurfactants reduce surface tension or emulsify hydrophobic substrates (Diaz de Rienzo et al. 2016). However, the biological function of the biosurfactants into the cell is beyond just solubilizing substrates. From the ecological point of view, biosurfactants provide advantages to surfactant-producing microorganisms in relation to other organisms that do not have such capacity. Biosurfactants also have different chemical structures and are produced by different microorganisms (Mulligan 2009; Abdel-Mawgoud et al. 2010, Banat et al. 2010). These biosurfactants are described in the literature as molecules with antimicrobial and antiviral activities (Plaza et al. 2013, Remichkova et al. 2014). This ability benefits ecological interactions (e.g., competition).

There is a quorum sensing mechanism that controls the genes *rhl* and *pqs* responsible to produce rhamnolipids (Pearson et al. 1997; Dusane et al. 2010). Quorum sensing ("sufficient amount" in Latin) is a mechanism for assessing population density through molecular signals to activate a response that requires a certain population density (Madigan et al. 2015). Rhamnolipids are also important for the formation of water channels in the biofilm. These channels provide the homogenous flow of nutrients and oxygen in the biofilm, in addition to allowing the release of metabolites. Davey et al. (2003) silenced the rhl gene of *Pseudomonas aeruginosa*, which caused the blockade of rhamnolipid production and prevented the formation of water channels in the biofilm.

Due to the multiple functions of these biosurfactants, their applicability was observed in activities such as the recovery of areas contaminated with hydrophobic compounds (Amani et al. 2013), emulsifiers in cosmetics and the pharmaceutical industry (Ferreira et al. 2017; Bhadoriya et al. 2013), and phytopathogen control (Ongena and Jacques 2008; D'aes et al. 2010, Falardeau et al. 2013). However, the cost of producing these biomolecules today is high, resulting in unfeasible applications. In comparison with synthetic surfactants with an average cost around $ 1 to $ 3 per kg, rhamnolipids cost $ 20 to $ 25 per kg (Chong and Li 2017). To overcome these challenges, many strategies are being adopted to increase productivity and reduce costs. These costs are mainly related to the costly carbon source required for the biosurfactant production and also to extraction and purification processes (Chong and Li 2017).

2.6 Production of Biosurfactants from Alternative Substrates

At the beginning of scientific interest in biosurfactants around 1980 (Syldatk and Wagner 1987), only pure hydrocarbons were used as carbon sources for their production (Fish et al. 1982; Hisatsuka et al. 1971; Itoh and Suzuki 1972; Syldatk et al. 1985). This consequently raised the biosurfactant market value to an unfeasible acceptance scenario. Despite its many advantages over synthetic chemical surfactants, the biosurfactants still have economic obstacles to overcome in the large-scale process, including a drastic reduction in production costs. In fact, there are efforts in the recent decades that focused on minimizing biosurfactant production costs to promote commercial acceptance (Mukherjee et al. 2006; Costa et al. 2008; Nitschke et al. 2011; Heryani and Putra 2017).

Currently, biosurfactants commercialized in the USA are more expensive than synthetic surfactants (Rosenberg and Ron 1997; Maier and Soberon-Chavez 2000; Makkar et al. 2011). In this context, alternative strategies have been adopted to establish a competitive price. Among the strategies are the development of genetically modified microorganisms toward better yields during the fermentation process (Dobler et al. 2016; Du et al. 2017), use of more cost-effective raw materials for biosurfactant production, and the development of economically viable production processes on a large scale (Mukherjee et al. 2006; Hazra et al. 2011; Makkar et al. 2011).

The use of agro-industrial waste becomes an economically interesting strategy since the raw material accounts for about 10–30% of the total cost in this biotechnological process (Makkar and Cameotra 1999; Mukherjee et al. 2006; Makkar et al. 2011). There are many alternative substrates currently proposed for the production of biosurfactants: residues generated by the vegetable oil manufacturing (peanut, coconut, corn, olive, soybean), cooking oils (sunflower, olive, soy), vegetable processing waste (potato, barley, cashew, cassava, wheat), sugar cane molasses, whey, peat, oily waste from oil refineries, lignocellulosic waste (fruit peels, corncobs), and glycerol from biodiesel production (Desai and Banat 1997; Nitschke et al. 2004; Benincasa 2007; Moldes et al. 2007; Barros et al. 2008; Thavasi et al. 2008; Monteiro et al. 2009; Rocha et al. 2009; Thavasi et al. 2011; Aparna et al. 2012; Cruz et al. 2017; Meneses et al. 2017; Rane et al. 2017). These compounds are known to exhibit high levels of carbohydrates and lipid, both required for the growth of microorganisms and the biosynthesis of biosurfactants (Nitschke et al. 2005b; Nee' Nigam and Pandey 2009; Benincasa 2007; Diaz et al. 2018).

Many microbial genera proved to be able to produce biosurfactants from these residues – *Azotobacter*, *Bacillus*, *Brevibacterium*, *Burkholderia*, *Corynebacterium*, *Flavobacterium*, *Lactobacillus*, *Micrococcus*, *Nocardiopsis*, *Pseudomonas*, *Pseudoxanthomonas*, *Rhodococcus*, *Tsukamurella*, *Candida*, *Pseudozyma*, and *Trichosporon* (Boudour et al. 2004; Thavasi et al. 2008; Monteiro et al. 2009; Thavasi et al. 2011).

Agricultural residues result in lower production costs and a much smaller volume of compounds released into the environment. By successfully developing effective ways of producing surfactants, the environmental impact of surfactant industry may become smaller. Moreover, there is a sustainable gain by recycling waste (Mulligan 2009; Accorsini et al. 2012).

2.7 Conclusion

Microbial ability to use petroleum hydrocarbons as a carbon source enables an alternative treatment based on bioremediation. Moreover, biotechnological strategies are able to improve natural attenuation processes, and they present cost-effectiveness compared to physicochemical treatments. Generally, an accelerated hydrocarbon biodegradation depends on the presence of specific microorganisms and/or bioavailability of pollutant compounds. Thus, environmental contamination by petroleum derivatives presents many hydrophobic molecules, and microbial metabolism can be enhanced by using tensioactive compounds. In this context, biosurfactants are demonstrated to act on water-oil interface thus forming micelles that raise bioavailability of hydrocarbons to biological treatment. This facilitates the metabolism and promotes the development of biomass, hence increasing biodegradation. Therefore, these biocompounds produced by microorganisms increase cellular access to hydrophobic substrates, and they are a natural choice as substitutes for synthetic surfactants. The advantages of biosurfactant application are currently based on technological advancement associated with sustainable development. Biosurfactants still have economic obstacles to overcome in the large-scale process, including a drastic reduction in production costs. In fact, there are efforts in the recent decades that focused on minimizing biosurfactant production costs to promote commercial acceptance. Hence, the use of agro-industrial waste as a substrate for fermentative processes becomes an economically interesting strategy. There are many alternative substrates currently proposed for the production of biosurfactants, and also many microbial genera proved to be able to produce biosurfactants from these residues.

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