

Biosurfactants are useful tools for the bioremediation of contaminated soil: a review

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Abstract

Bioremediation processes are negatively affected by the low aqueous solubility of some contaminants; therefore their bioavailability may be enhanced by the addition of surfactants. These compounds are organic molecules that can be chemically and biologically produced. Surfactants contain both hydrophilic and hydrophobic groups, therefore reducing surface and interfacial tensions of immiscible fluids and increasing the solubility and sorption of hydrophobic organic and inorganic compounds. This article provides an overview of characteristics of natural and synthetic surfactants and the effects of biosurfactants on solubility, sorption and biodegradation of hydrophobic organic contaminants; as well as the effects of biosurfactants on degrader microorganisms as white-rot fungi. Finally, some examples of application of natural surfactants for bioremediation of contaminated soils are shown. In general, this overview indicates the great potential of biosurfactants on the remediation of contaminated sites.

Keywords: Biosurfactants, hydrophobic organic compounds, soil.

1. Introduction

Soil pollution is a consequence of the accumulation of a wide range of chemical compounds generated either by natural or industrial processes. The existence of contaminated sites is an important environmental problem today.

Several strategies involving biological, physico-chemical, and thermal processes have been developed to remediate contaminated sites (Bollag and Bollag, 1995; Vidali, 2001; Rubilar *et al.*, 2011). Methods such as incineration, excavation, landfilling and storage are expensive, sometimes difficult to execute (Vidali, 2001; Jain *et al.*, 2005), inefficient, and often exchange one problem for another (Bollag and Bollag, 1995). Alternatively, biological processes offer several advantages over conventional technologies, because they are often more environmentally friendly, economic and versatile, and they can reduce the concentration and toxicity of a large number of contaminants (Vidali, 2001; Jain *et al.*, 2005). However, these processes are limited by the low water solubility of the contaminants, limiting their availability to microorganisms (Bollag and Bollag, 1995; Volkering *et al.*, 1998).

Bioavailability of a contaminant is largely controlled by its hydrophobicity and ease of desorption from the solid phase of the soil to the aqueous solution (Semple *et al.*, 2003). The molecular structure, concentration and physico-chemical characteristics of the pollutants limit their bioavailability (Volkering *et al.*, 1998; Alexander, 2000). Low bioavailability is also related to the ageing of the pollutants in the soil (Alexander, 1995; Semple *et al.*, 2003). The addition of a surfactant to a contaminated soil can reduce the interfacial tension thus increasing the mass transfer of the contaminants (Mulligan *et al.*, 2001; Gao *et al.*, 2007; Franzetti *et al.*, 2008). In this context, several researchers have shown that various surfactants can enhance desorption (Aronstein *et al.*, 1991; Mata-Sandoval *et al.*, 2002; Xu *et al.*, 2006), solubilization

(Garon *et al.*, 2002; Prak and Pritchard, 2002; Doong and Lei, 2003), biodegradation of organic compounds (Fava and Di Gioia, 2001; Kim *et al.*, 2001), and removal of heavy metals from soil (Dahrazma and Mulligan, 2007; Rufino *et al.*, 2011).

Thus, the aim of this review is to provide an overview of characteristics of natural surfactant (biosurfactant) and synthetic surfactants (surfactant), the effects of biosurfactants on solubility, sorption and biodegradation of hydrophobic organic contaminants. The effects of biosurfactants on degrader microorganisms and white-rot fungi are also presented. Finally, some examples of application of biosurfactants for bioremediation of contaminated soils are shown.

2. Characteristics and properties of the surfactants

The surface activity of surfactants derives from their amphiphilic structure, meaning that their molecules contain both water soluble and water insoluble portions (West and Harwell, 1992). The water solubility of the surfactants is due to the hydrophilic portion (polar group), while the hydrophobic portion (nonpolar chain) tends to concentrate at the air-water interfaces or in the center of micelles, reducing the surface tension of the solution (West and Harwell, 1992; Desai and Banat, 1997; Volkering *et al.*, 1998). Surfactants form aggregates or micelles; this ability confers certain properties such as emulsifying, foaming, dispersing, and the capacity to act as a detergent, making surfactants very versatile chemical compounds. They are applied in several industrial sectors such as the cosmetics, pharmaceuticals and food, petroleum, agrochemical and fertilizer industries, as it has been reviewed by Kosaric (1992), Deleu and Paquot (2004) and Banat *et al.* (2010).

Surfactants are characterized by properties such as critical micelle concentration (CMC), hydrophilic-lipophilic balance (HLB), chemical structure and charge, as well as properties from their origin source (Van Hamme *et al.*, 2006). The surfactants exist as monomers or single molecules at low concentration in aqueous solutions; over the CMC, the surfactant molecules assemble together, forming aggregates. The CMC depends on surfactant structure, composition, temperature, ionic strength, and the

presence and types of organic additives in the solutions (Fuget *et al.*, 2005). At the CMC of surfactant solutions, a drastic change occurs in many physico-chemical properties (surface tension, conductivity, or turbidity) (Figure 1) (Hanna *et al.*, 2005; Zana, 2005). Micelles are capable of dissolving hydrophobic contaminants in their hydrophobic core, which results in an increased apparent aqueous solubility of the pollutants (Edwards *et al.*, 1991; Prak and Pritchard, 2002).

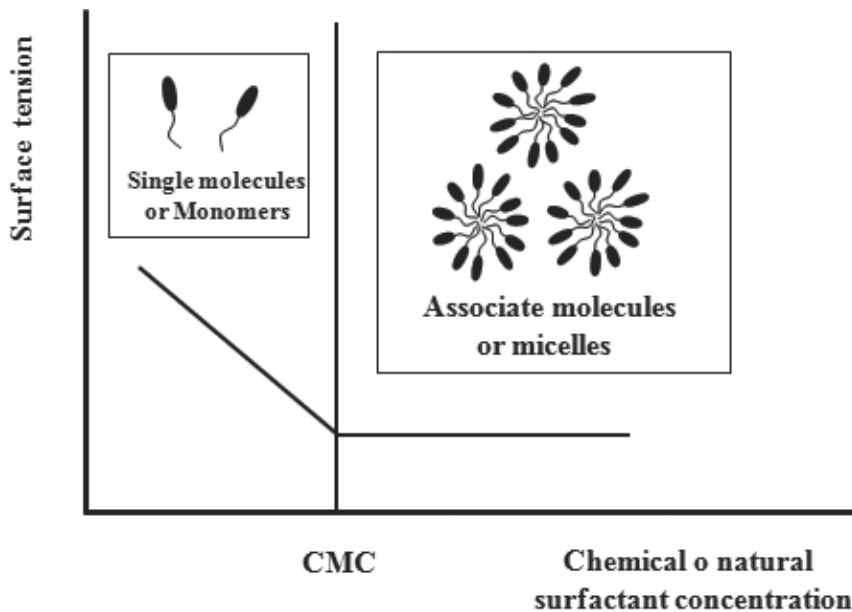


Figure 1. Surface tension as a function of chemical or natural surfactant concentration, CMC represents critical micelle concentration (Patist *et al.*, 2000; Whang *et al.*, 2008).

The HLB number is also an important parameter of the surfactants, describing their physical properties and is specific for each surfactant. This number is determined by the relationship of the hydrophilic and the hydrophobic parts of the surfactant molecule (Tiehm, 1994). This indicates the types of oils that can emulsify them and can be also used to determine their suitability for use. Surfactants with a low HLB are lipophilic where-

as a high HLB is indicative of better water solubility (West and Harwell, 1992; Tiehm, 1994). In terms of the hydrophilic portion, surfactants are classified as anionic (negative charge), cationic (positive charge), zwitterionic (both negative and positive charges), or nonionic (no charge) (West and Harwell, 1992; Volkering *et al.*, 1998). Table 1 shows the CMC and HLB number, as well as charge type, of some surfactants.

Table 1. The charge type, critical micelle concentration (CMC) and hydrophilic-hydrophobic balance number (HLB) of some surfactants, adapted from Doong and Lei (2003).

Surfactant	MW †	Charge type	CMC	HLB	Reference
SDS†	288 g mol ⁻¹	Anionic	8.10 mM	40.0	Eriksson <i>et al.</i> (2002)
Triton X-100	628 g mol ⁻¹	Nonionic	0.31 mM	13.5	Eriksson <i>et al.</i> (2002)
Tween 80	1310 g mol ⁻¹	Nonionic	0.01 mM	15.0	Eriksson <i>et al.</i> (2002)
Brij 35	1198 g mol ⁻¹	Nonionic	0.05 mM	16.9	Yeom <i>et al.</i> (1996), Zhu and Feng (2003)
Igepal CA-720	735 g mol ⁻¹	Nonionic	0.23 mM	14.6	Saichek and Reddy (2004)
Rhamnolipid JBR515	577 g mol ⁻¹	Anionic	0.02 mM	22-24	Xie <i>et al.</i> (2005), Nguyen <i>et al.</i> (2008)
Saponin	1800-2000 Da	Nonionic	87.60 mg/L	-	Soeder <i>et al.</i> (1996), Urum and Pekdemir (2004), Rigano <i>et al.</i> (2009)
Lecithin	773 g mol ⁻¹	Zwitterionic	610.00 mg/L	3-4	Soeder <i>et al.</i> (1996), Bergenstahl and Fontell (1983), Cubero <i>et al.</i> (2002), Aulton (2004)
Tergitol NP-10	683 g mol ⁻¹	Nonionic	0.05 mM	14.0	Laha and Luthy (1991), Mulder <i>et al.</i> (1998)

†: MW = Molecular weight; SDS = Sodium dodecyl sulfate

2. 1 Synthetic surfactants

Surfactants that are produced chemically are known as synthetic surfactants. The hydrophobic chain of these surfactants are paraffins, olefins, alkylbenzenes, alkylphenols and alcohols; the polar group is usually either a sulphate group, a sulphonate group, or a carboxylate group for anionic surfactants, or a quaternary ammonium group for cationic surfactants. For nonionic surfactants the polar groups are poly-

oxyethylenes, sucrose, or polypeptides (Volkering *et al.*, 1998). The most common chemical surfactants are sodium dodecyl sulfate (SDS), Triton X-100 (TX100) and Tween 80 (TW80).

Synthetic surfactants are readily available and of (relatively) low cost, so are extensively utilized in remediation processes of contaminated water or soil. On the other hand, the low yields and high costs that can be incurred in the production of biosurfactants (Deleu and Paquot, 2004; Mukherjee *et al.*, 2006; Banat *et*

al., 2010) have restricted their use. However, considerable attention has been paid to the production and study of biosurfactants, since they offer several advantages over synthetic surfactants: low toxicity, low CMC, biodegradability, ecological acceptability, high selectivity, and specific activity at extreme temperatures, pH, and salinity (West and Harwell, 1992; Desai and Banat, 1997; Kosaric, 2001; Anandaraj and Thivakaran, 2010).

2. 2 Biosurfactants

Natural surfactants or biosurfactants can be produced extracellularly or as part of the cell membrane by a wide variety of microorganisms such as bacteria, fungi, and yeast. Some examples include *Pseudomonas aeruginosa* (produces rhamnolipids), *Bacillus subtilis* (produces a lipopeptide called surfactin) (Ron and Rosenberg, 2001; Mata-Sandoval *et al.*, 2002; Mulligan, 2005), *Nocardia amarae* (Moussa *et al.*, 2006), and *Saccharomyces lipolytica* CCT-0913 (Lima and Alegre, 2009). Most biosurfactants are either anionic or neutral, only a few are cationic, containing amine groups. The hydrophobic part is based on long chain fatty acids, hydroxyl fatty acids or α -alkyl- β -hydroxy fatty acids. The hydrophilic group can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol (Mulligan *et al.*, 2001).

Biosurfactants are grouped mainly by their chemical composition and their microbial origin. The main classes of these compounds include glycolipids; lipopeptides and lipoproteins; fatty acids, phospholipids, and neutral lipids; and polymeric biosurfactants, as has been reviewed by Desai and Banat (1997), Kosaric (2001), Rahman and Gakpe (2008) and Gautam and Tyagi (2006). Besides, biosurfactants can be classified according to their molecular weight into two main classes, low-molecular-weight molecules called

biosurfactants and high-molecular-weight polymers or bioemulsans (Neu, 1996; Rosenberg and Ron, 1999). Biosurfactants lower surface and interfacial tension; this group includes glycolipids, lipopeptides, phospholipids, and proteins. On the other hand, bioemulsans are more effective as emulsion-stabilizing agents, i.e. stabilize oil-in-water; this group includes polymers of polysaccharides, lipoproteins, and particulate surfactants (Neu, 1996; Rosenberg and Ron, 1999; Perfumo *et al.*, 2010). In this context, surfactin and rhamnolipids are low-molecular mass biosurfactants with molecular weight of 1036 and 802 Da, respectively (Mulligan and Gibbs, 1990). Besides, emulsan an extracellular lipopolysaccharide biosurfactant produced by *Acinetobacter calcoaceticus*, is a high-molecular-weight bioemulsifier with an average molecular weight of about 1000 kDa (Kim *et al.*, 1997). Alasan is another bioemulsifier complex produced by *Acinetobacter radioresistens* KA53, with an average molecular weight of 1 MDa (Navon-Venezia *et al.*, 1995).

Biosurfactants can be synthesized using different microorganisms and carbon sources and production is influenced by the composition of the medium and by culture conditions (Desai and Banat, 1997; Franzetti *et al.*, 2009). The carbon sources used for biosurfactant production are hydrocarbons, carbohydrates, vegetable oils and oil wastes, olive oil mill effluent, lactic whey and distiller wastes, starchy substrates, renewable resources, industrial and/or municipal wastewater, under aerobic conditions (Kosaric, 1992; Desai and Banat, 1997; Gautam and Tyagi, 2006). In this context, Franzetti *et al.* (2008) found three new bacterial strains hydrocarbon-degrading *Gordonia* genus. They were isolated from a site chronically contaminated by diesel. These strains were able to grow using a wide range of straight and branched aliphatic hydrocarbons as carbon and energy sources and to produce at least two classes of

surface-active compounds, emulsifying agents and water-soluble substrates. Cell-bound biosurfactants, which reduce surface tension, were produced in hydrocarbons; however their production was lower in water soluble substrates. *Gordonia* sp. BS29 synthesized, and then released extracellularly, bioemulsions during the exponential phase with *n*-hexadecane as carbon and energy source. The production of biosurfactants started in the exponential phase and their concentration increased following linear growth. Calvo *et al.* (2008) isolated *Ochrobactrum anthropi* strain AD2 from the waste water treatment plant of an oil refinery. This bacterium produced exopolysaccharide AD2 (exopolysaccharide emulsifiers) in glucose nutrient broth media with various added hydrocarbons; such as *n*-octane, mineral light and heavy oils and crude oils. In addition, Franzetti *et al.* (2009) studied the cultural factors that affecting the production of the cell-bound biosurfactants by *Gordonia* sp. BS29. Their research evaluated the type and concentration of the carbon source, the concentration of phosphates and sodium chloride, and the interactions among these factors. The results showed that with the optimized cultural conditions a 5-fold increase in the biosurfactant concentration, compared to the un-optimized medium, was obtained. The optimization did not change the number and type of the glycolipid biosurfactants produced by *Gordonia* sp. BS29.

The phytogetic surfactant is another group of biosurfactants, such as saponins and lecithins (Soeder *et al.*, 1996), and humic acids (Conte *et al.*, 2005). The phytogetic surfactants are released from decaying roots, and can be found in considerable amounts in the rhizosphere, where phosphatidylcholine, the most important component of lecithin, is the major phospholipid (Soeder *et al.*, 1996).

Cyclodextrins are another group of substances that enhanced the apparent solubility and biodegradation of

hydrophobic organic compounds (HOCs) in aqueous solutions and contaminated soil (Boyle, 2006). These substances can imitate the function of surfactants since they can form soluble complexes with hydrophobic compounds. Cyclodextrins have a non-polar cavity into which the HOCs partition to form inclusion complexes and a polar exterior that provides the molecule with a relatively high aqueous solubility. Moreover they are of interest in microbial processes because they do not exhibit the toxicity of many synthetic surfactants (Singh *et al.*, 2007).

The most important characteristic of biosurfactants is their environmental acceptability, as they are biodegradable, have lower toxicity than synthetic surfactants, their own specific action, effectiveness at extremes of temperature, pH and salinity, and are ecologically safe, as it has been reviewed by Kosaric (1992) and Desai and Banat (1997). These properties have allowed use of biosurfactants in the remediation of inorganic compounds such as heavy metals (Kosaric, 1992; Zouboulis *et al.*, 2003), and in the remediation of organic compounds such as hydrocarbons (Franzetti *et al.*, 2008). Also, the ability to reduce the interfacial tension of oil in water has allowed applied of biosurfactants for the removal of water from emulsions prior to processing (Mulligan, 2005) and therefore they are applied in oil recovery (Plaza *et al.*, 2008; Abdolhamid *et al.*, 2009). Moreover, natural surfactants have been used in the food processing industry, and the health care and cosmetics industries (Desai and Banat, 1997). The properties of biosurfactants have generated a large number of investigations, which have allowed identification of new microorganism producers of natural surfactants, determination of their structure, finding new sources of carbon and energy, enhancing the production processes, and generating several patents (Shete *et al.*, 2006).

3. Influence of biosurfactants on the bioavailability of hydrophobic organic compounds

The bioavailability of HOCs can be enhanced by biosurfactants through the following mechanisms: emulsification of non-aqueous phase liquid contaminants (Edwards *et al.*, 1991; Volkering *et al.*, 1998; Jiménez Islas *et al.*, 2010), enhancement of the apparent solubility of the pollutants (Edwards *et al.*, 1991; Volkering *et al.*, 1995), and facilitated transport of the pollutants from the solid phase (Yeom *et al.*, 1996; Jiménez Islas *et al.*, 2010). These mechanisms may cause enhanced mass transport and their relative contributions strongly depend on the physical state of the pollutants (Volkering *et al.* 1998). A fourth possible mechanism has been suggested by Tang *et al.*, (1998) and Poeton *et al.* (1999): the biosurfactants help microorganisms adsorb to soil particles occupied by the contaminant, thus decreasing the diffusion path length between the sites of adsorption and the site of bio-uptake by the microorganisms.

In the first mechanism, emulsification of non-aqueous phase liquid contaminants, the biosurfactants can decrease the interfacial tension between and aqueous and non-aqueous phase. This may guide the formation of micro and macro emulsions. This results in an increase in the contact area, enabling improved mass transport of the contaminants to the aqueous phase and in mobilization of sorbed liquid-phase contaminants (Edwards *et al.*, 1991, Volkering *et al.*, 1998).

The second mechanism, enhancement of the apparent solubility of the HOCs, is due to presence of micelles that contain high concentrations of HOCs in the hydrophobic center of the micelles (Edwards *et al.*, 1991, Volkering *et al.*, 1995). Brown (2007) explains the apparent aqueous solubility of the contaminant as the sum of the aqueous (C_{aq}) and micellar (C_{mic}) HOC

concentrations. Edwards *et al.*, (1991) established that micellar-phase HOC concentration can be modeled using a linear partition relationship of the form:

$$C_{mic} = k_{mic} C_{mic} C_{aq} \quad (1)$$

where k_{mic} is the HOC-micelle partition coefficient and S_{mic} is the micelle concentration. The S_{mic} correspond to difference between the total surfactant concentration and their CMC.

Finally, facilitated transport of the contaminants from the solid phase can involve several processes, such as the interaction of contaminants with single biosurfactant molecules, the interaction of surfactants with separate-phase or sorbed hydrocarbons, the mobilization of contaminants by swelling of the organic matrix, and the mobilization of contaminants trapped in soil caused by lowering of the surface tension of the soil particle pore water in soil particles, as it has been reviewed by Volkering *et al.* (1998).

The use of biosurfactants can improve the bioremediation processes by mobilization, solubilization or emulsification (Urum and Pekdemir, 2004; Nguyen *et al.* 2008). The mobilization and solubilization mechanisms are promoted low-molar mass biosurfactants, at below and above the CMC, respectively. Whereas, the emulsification processes is promoted by high-molar mass biosurfactant (Urum and Pekdemir, 2004; Pacwa-Plociniczak *et al.*, 2011).

3.1 Emulsification and solubilization of hydrophobic organic contaminants by biosurfactants

Whang *et al.* (2008) studied the capacity of rhamnolipid and surfactin to reduce surface tension. The biosurfactants were produced by *P. aeruginosa* J4 and *B. subtilis* ATCC 21332, respectively. The results showed that the biosurfactants were able to reduce surface

tension to less than 30 from 72 dynes cm^{-1} with CMC values of 45 and 50 mg L^{-1} for surfactin and rhamnolipid, respectively. Also, the results of diesel dissolution experiments demonstrated that the diesel solubility was enhanced with increased biosurfactant addition.

Most studies of the effect of surfactants on the solubilization of PAHs have been performed under mesophilic conditions. Related to this, Wong *et al.* (2004) evaluated the influence of TW80, TX100 and the biosurfactants produced from *P. aeruginosa* strain P-CG3 and *P. aeruginosa* strain ATCC 9027 on the solubilization of phenanthrene under thermophilic conditions. They found that the surfactants enhanced the solubility of phenanthrene at 50°C. The biosurfactant from P-CG3 was the most effective with a 28-fold increase in apparent solubility of phenanthrene at a concentration of 10 x CMC, compared with the controls (TW80 and TX100).

Franzetti *et al.* (2009) determined that the BS29 bioemulsans, produced by *Gordania* sp. strain BS29, effectively remove crude oil and PAHs from soil. The crude oil removal by BS29 bioemulsans is comparable with rhamnolipid in the same experimental conditions.

Barkay *et al.* (1999) evaluated the effect of alasan in the enhancement of solubilization of polycyclic aromatic hydrocarbons (PAHs). Alasan is a high-molecular-weight bioemulsifier complex of an anionic polysaccharide and proteins. The concentration of solubilized PAHs increased linearly with the addition of the biosurfactant (50 to 500 $\mu\text{g mL}^{-1}$). The apparent aqueous solubilities of PAHs were increased higher than their solubilities without alasan. The results of physicochemical characterization of the solubilization activity suggest that alasan solubilizes PAHs by physical interaction, likely of the hydrophobic nature, and that this interaction is slowly reversible. Moreover, the increase in apparent aqueous solubility of PAHs does not depend on the conformation of alasan and is not affected by the formation of multimolecular ag-

gregates of alasan above its saturation concentration. Also, alasan enhances the biodegradation of PAHs.

Tecon and van der Meer (2010) evaluated the effects of two types of biosurfactants produced by *Pseudomonas* sp. (cyclic lipopeptides and rhamnolipids) on phenanthrene bioavailability. They measured the bioavailability from growth rates on contaminants and from specific induction of a phenanthrene-responsive green fluorescent protein (GFP) reporter in *Burkholderia sartisoli* strain RP037. Their results showed that the co-culturing of strain RP037 with lipopeptide-producing bacterium *Pseudomonas putida* strain PCL1445 enhanced GFP expression, compared to a single culture, but this effect was not significantly different when strain RP037 was co-cultivated with a non-lipopeptide-producing mutant of *P. putida*. The addition of partially purified supernatant extracts from the *P. putida* lipopeptide producer also did not unequivocally enhance phenanthrene bioavailability for strain RP037, compared to controls. In contrast, a 0.1% rhamnolipid solution strongly augmented RP037 growth rates on contaminants and led to a significantly larger proportion of cells in culture with high GFP expression.

3.2 Desorption of hydrophobic organic compounds by biosurfactants

The HOCs in contact with the soil are associated with organic matter by different mechanisms: adsorption and electrostatic and covalent bonding (Alexander, 1995). Adsorption is the most important mode of interaction between soil and HOCs. Adsorption processes of these pollutants in soil occur from complete reversibility to total irreversibility. Prolonged exposure time to the pollutant decreases its bioavailability. The extent of adsorption depends on the properties of the soil (mineral and organic matter content) and of the contaminant (solubility, polarity, molecular structure), as

has been reviewed by Semple *et al.* (2003) and Gevaio *et al.* (2000). Hence, the application of biosurfactant solutions, in soil-water systems, may result in the transfer of HOCs from the soil-sorbed phase to the aqueous phase, allowing mass-transfer processes and biodegradation (Aronstein *et al.*, 1991; Tiehm, 1994; Volkerling *et al.*, 1995; Jiménez Islas *et al.*, 2010).

Biosurfactants can be effective in facilitating desorption of the pollutants from soil as a possible integral part of a biodegradation process (Mata-Sandoval *et al.*, 2002) or in an aqueous soil washing method, where a biological or nonbiological process is subsequently applied to remove the contaminants from the recovered aqueous washing (Singh *et al.*, 2007). Bioemulsifiers from bacteria were able to emulsify n-octane, toluene, xylene, mineral oil and crude oil and they looked promising for remediation application (Toledo *et al.*, 2008). Urum and Pekdemir (2004) evaluated the ability of aqueous biosurfactant solutions for possible applications in washing crude oil contaminated soil. The results showed that the biosurfactants were able to remove significant amounts of crude oil from contaminated soil. Rhamnolipid removed up to 80% oil and lecithin about 42%. In comparison with distilled water washing, crude oil removal from soil using aescin (mixture of saponins), lecithin, saponin and tannin was not effective. These investigators propose that the removal was due to mobilization, caused by the reduction of surface and interfacial tensions. On the other hand, Kang *et al.* (2010) investigated the effectiveness of sophorolipid in washing and biodegradation of hydrocarbons and crude oil in soil on a laboratory scale. The results showed that the addition of this biosurfactant to soil enhanced the washing and the biodegradation of the tested hydrocarbons.

Another important aspect to consider is the sorption of biosurfactants onto soil, a condition that can cause natural surfactant losses, which in turn reduce the performance of the solubilization of hydrophobic

contaminants (Chu, 2003; Zhou and Zhu, 2007), decrease the remediation efficiency and result in an increase in remediation time and costs (Yu *et al.*, 2007; Zhou and Zhu, 2008). At biosurfactant concentrations below the CMC, competitive adsorption of an organic compound by soil and by a biosurfactant in solution may occur, which may cause an increase or a decrease in the desorption of the contaminant from soil, depending on the characteristics of the soil and the organic compound, as has been reviewed by Rodríguez-Cruz *et al.* (2004). Pei *et al.* (2009) examined the effect of biosurfactant on the sorption of phenanthrene onto the original or H₂O₂-treated black loamy soil and red sandy soil. The result showed that organic matter played an important role in phenanthrene sorption onto the soil evaluated. The changes values of partition coefficient suggested that biosurfactant inhibited phenanthrene sorption onto the black loamy soil, however facilitated phenanthrene, sorption onto the red sandy soil. On the other hand, was observed that biosurfactant could also be sorbed onto soils. The maximal sorption capacity of the red sandy soil was 76.9 µg g⁻¹, which was 1.31 times that of black loamy soil. Moreover, biosurfactant was degraded in the two selected soils, and 92% was mineralized after 7 days of incubation. It implied that biosurfactant should be added frequently in remediation process of PAH-contaminated soils. The research of Van Dyke *et al.* (1993). showed that the ability of UG2 rhamnolipid, produced by *P. aeruginosa* UG2, to enhance removal of PAHs into the aqueous phase was affected by the soil type, hydrocarbon equilibration time, and biosurfactant adsorption to soil.

3.3 Influence of biosurfactants on the desorption and solubilization of aged chemicals in soil

Organic compounds freshly added to soils are bound almost exclusively to the soil particle surfaces (ad-

sorption) and their desorption is almost complete after a short period of time (Hatzinger and Alexander, 1995; Alexander, 2000). However, if the time of contact between a pollutant and soil increases, decrease in chemical and biological availability occurs, called “ageing” or “sequestration” (Alexander, 1995; Hatzinger and Alexander, 1995; Semple *et al.*, 2003). In the process of ageing, the interactions between soil and HOCs are affected by: the soil organic matter, both its amount and its nature; inorganic components with particular consideration to pore size and structure; microbial activity; and pollutant concentration, as it has been reviewed by Semple *et al.* (2003).

Over the past few years, numerous researchers have studied the biosurfactant-enhanced desorption of organic contaminants adsorbed onto soil. However, few works reported in the literature have addressed the influence of the time of residence in the soil, or ageing time, of organic pollutants on their desorption in soil-water-surfactant systems. Fava *et al.* (2004) evaluated the effects of soya lecithin on the desorption of PAHs in an aged-contaminated soil and they obtained a faster and more extensive overall removal of PAHs accompanied by a large soil detoxification under slurry-phase conditions. After 150 days of incubation at room temperature, about 60% of the original PAHs was biodegraded. Berselli *et al.* (2004) investigated the effects of TX100 and the biogenic agents: cyclodextrins, humic substances, and rhamnolipids, on the washing of a soil historically contaminated with PAHs. The soil was washed in water with 1% of biogenic agents or TX100 and both the biogenic agents and the synthetic surfactant enhanced the capacity of water to elute organic contaminants from the soil. The biogenic agents sustained the biodegradation of contaminants by enhancing the availability to bacteria; in contrast,

TX100 affected the bioremediation due to their toxic effects on bacterial biomass.

The results of Leonardi *et al.* (2007) showed that the addition of several surfactants (soybean oil, Tween 20, TW80 and olive-mill wastewater) to an aged soil with a negligible amount of the non-bioavailable fraction of PAHs had either a limited or even a negative impact on PAH degradation by *Irpex lacteus* and *Pleurotus ostreatus*.

Fava *et al.* (2003) studied the effects of methyl- β -cyclodextrins on the solubilization of polychlorinated biphenyls (PCB) of two different real, aged contaminated soils in bench-scale reactors. They found that the addition of 0.5 and 0.1% of methyl- β -cyclodextrins increased the concentration of PCBs in the water phase in slurry-phase reactors.

In general, most pesticides used in agriculture are moderately hydrophobic compounds, with complex molecular structures that differ from hydrocarbons in their lower hydrophobicity and in the presence of a polar functional group. These compounds are also strongly adsorbed by soil organic matter and desorption is limited (Rodríguez-Cruz *et al.*, 2004). Their desorption rate decreased with an increase in ageing time. Wattanaphon *et al.* (2008) evaluated the ability of a BS biosurfactant produced by *Burkholderia cenocepacia* BSP3 to enhance pesticide solubilization for further application in environmental remediation. The BS biosurfactant was identified as a glucolipid, having a CMC of 316 mg L⁻¹. Moreover, it lowered the surface tension of deionized water to 25 ± 0.2 mN m⁻¹ and exhibited good emulsion stability. The results showed that the application of the BS biosurfactant to facilitate pesticide solubilization demonstrated that this biosurfactant at concentrations below and above its CMC could enhance the apparent water solubility of methyl parathion, ethyl parathion and trifluralin.

4. Influence of biosurfactants on the degradation of hydrophobic organic contaminants by microorganisms

4.1 Biosurfactant-microorganism interactions

Biosurfactants have the potential to enhance the bioavailability of HOCs in contaminated sites, and therefore enhance the efficiency biodegradation processes. In general, the biosurfactants are considered as low or non-toxic (Desai and Banat, 1997). Soeder *et al.* (1996) showed that the soya lecithin had a lower bacterial toxicity than quillaya saponin. Flasz *et al.* (1998) determined that the synthetic surfactants presented higher toxicity and mutagenic effect, whereas that the natural surfactants were considered slightly non-toxic and non-mutagenic. Boyle (2006) evaluated the effects of various cyclodextrins (Gamma W8, Beta W7 M1.8 and Alpha W6 M1.8) and pentachlorophenol on the radial growth of the white-rot fungus *Trametes hirsute*. The results at pH 4.9 showed that the cyclodextrin Gamma W8 eliminated the inhibitory effects of 10 mg L⁻¹ of pentachlorophenol and partially overcame those at 50 mg L⁻¹. Beta W7 M1.8 also alleviated inhibition, but the effect was less pronounced and Alpha W6 M1.8 had little effect. In control assays, without pentachlorophenol, cyclodextrins did not affect radial growth of *T. hirsute*. On the other hand, Bustamante *et al.* (2011) found that the increase in soya lecithin concentration from 0 to 10 g L⁻¹ caused an increase in mycelia growth of *Anthracoxyllum discolor*, a white-rot fungus isolated from a Chilean forest. However, biosurfactants can be exerting negative effects on the biodegrading microorganisms (Fava and Di Gioia, 2001), bacteria, fungi, algae, and virus, as it has been by Volkering *et al.* (1995), Muthusamy *et al.* (2008), and Banat *et al.* (2010). Some biosurfactants have antibiotic prop-

erties, which can inhibit spore germination, hyphal growth of some fungi. Das *et al.* (2008) showed that a biosurfactant from *Bacillus circulans*, different from surfactin produced from *B. subtilis*, was effective for Gram-negative and Gram-positive pathogenic and semipathogenic microorganisms. In the medical, cosmetics, and pharmaceutical areas, biosurfactant have been used to inhibit bacterial growth, cell lysis, tumor growth, synthesis of cell wall, and to stimulate enzymes, inhibit the adhesion of pathogenic organisms to solid surfaces and the recovery of purified intracellular proteins. More applications are reviewed in the articles published by Muthusamy *et al.* (2008) and Gharaei-Fathabad (2011). The effect of biosurfactants on the microorganisms will depend of factors, such as: biosurfactant concentration and bioavailability, environmental and cultural conditions, charge type of biosurfactants, and characteristic and properties of microorganisms as cellular ultrastructure (Van Hamme *et al.*, 2006).

4.2 Effect of biosurfactants on the degradation of hydrophobic organic pollutants by white-rot fungi

Most of the studies have been directed towards removal of HOCs from soil using biosurfactants and added bacteria or indigenous soil microorganisms; but few studies have addressed the removal of hydrophobic pollutants from soil using biosurfactants and white-rot fungi, which indicates the necessity of evaluating the potential ligninolytic effect of this combination.

White-rot fungi have the ability to degrade a wide range of persistent or toxic environmental contaminants, such as PCBs, PAHs, pentachlorophenol, pesticides, dioxins, and synthetic dyes, among others, which makes them good candidates for use in processes of bioremediation (Barr and Aust, 1994; Pointing, 2001; Eichlerová *et al.*, 2005; Tortella *et al.*, 2005; Ru-

bilar *et al.*, 2008; Rubilar *et al.*, 2011). The potential of white-rot fungi resides in their enzymatic system, which is nonspecific, and secreted into the extracellular environment (Durán and Esposito, 2000). White-rot fungi secrete one or more of the three enzymes that are essential for the degradation of lignin: laccase (Lac), lignin peroxidase (LiP), and Mn dependant peroxidase (MnP) (Reddy, 1995; Pointing, 2001; Eichlerová *et al.*, 2005). These enzymes are secreted during secondary metabolism, as a result of nutrient depletion of carbon or sulphur, or especially, manganese and nitrogen (Hamman *et al.*, 1997; Leung and Pointing, 2002). Other factors are also implicated, such as initial pH of the medium and incubation temperature (Bustamante *et al.*, 2011), the level of aeration and agitation during incubation, the availability of mediator compounds and metals (Leung and Pointing, 2002; Mouso *et al.*, 2003; Cordi *et al.*, 2007), or the presence and concentration of surfactants (Rodríguez Couto *et al.*, 2000; Ürek and Pazarlıoğlu, 2005; Wang *et al.*, 2008). Recently, Bustamante *et al.* (2011) determined that *A. discolor* produced more activity of MnP when soya lecithin was included in the growth medium. Yamanaka *et al.* (2008) found that supplementation of a *Trametes villosa* culture medium with a vegetable oil-surfactant emulsion induced MnP activity and higher Lac activity when copper was added. On the other hand, Zhou *et al.* (2007) showed that white-rot fungi degraded decabromodiphenyl ether (BDE-2009, a widely used flame retardant) and that TW80 and β -cyclodextrin could both increase the biodegradation.

5. Remediation applications

The addition of biosurfactants, bioemulsifiers, and/or biosurfactant-producing microorganisms can be used in soil biodegradation techniques, soil washing, and water and waste treatment (*in situ* and *ex situ*) (Urum and Pekdemir, 2004; Zhou and Zhu, 2008). Biosur-

factants have also been found to be useful for oil spill remediation and for dispersing oil slicks into fine droplets and converting mousse oil into an oil-in-water emulsion (Toledo *et al.*, 2008).

Barkay *et al.* (1999) examined the influence of the bioemulsifier alasan on the biodegradation fates of PAHs. The presence of alasan (500 $\mu\text{g mL}^{-1}$) more than doubled the rate of [^{14}C]fluoranthene mineralization and significantly increased the rate of [^{14}C]phenanthrene mineralization by *Sphingomonas paucimobilis* EPA505. Shin *et al.* (2006) used a rhamnolipid from *Pseudomonas* to remediate soil contaminated with phenanthrene by the combined solubilization-biodegradation process. They reported a high percentage of removal in the solubilization step and a significant decrease of phenanthrene in the soil sample during the biodegradation. From their results, they suggest that the degradation of contaminants by specific species might not be affected by the residual biosurfactants following application of the solubilization process that they would not present negative effects to the environment, and that they could be combined with the biodegradation process to improve the removal efficiency.

Soeder *et al.* (1996) studied the influence of two phytogetic surfactants, quillaya saponin and soya lecithin, on the biodegradation of PAHs. They found that high concentrations of phytogetic surfactants efficiently solubilized phenanthrene and fluoranthene. On the other hand, Fava and Di Gioia (2001) evaluated the effects of soya lecithin on the bioremediation of PCBs in an artificially contaminated soil and, in this work; the soya lecithin enhanced the availability of PCBs, while soya lecithin was also found to be an excellent carbon source for the microorganisms. Similar results were obtained by Fava *et al.* (2004) when studying the influence of soya lecithin on the bioremediation of an aged-PAHs contaminated soil. These results suggest the capacity of a phytogetic surfactant, soya lecithin,

to improve the bioavailability of HOCs in contaminated sites. In addition, they have other properties, such as being nontoxic and biodegradable.

The bioremediation of petroleum is carried out by microorganisms capable of utilizing hydrocarbons as a source of energy and carbon. These microorganisms are ubiquitous in nature and are capable of degrading various types of hydrocarbons, all with low solubility in water. The hydrocarbon-degrading microorganisms generally produce emulsifiers, so biosurfactants help to disperse the oil, increase the surface area for growth, remove the bacteria from the oil droplets after the utilizable hydrocarbon has been depleted (Ron and Rosenberg, 2002), and stimulate the indigenous bacterial population to degrade hydrocarbons at rates higher than those which could be achieved through addition of nutrients alone (Desai and Banat, 1997). Whang *et al.* (2008) investigated the application of a biosurfactant, rhamnolipid and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. Their results in diesel/water batch experiments showed that with the addition of 40 mg L⁻¹ of surfactin, there was significantly enhanced biomass growth as well as increased diesel biodegradation (94%), compared with 40% in batch experiments without surfactin. A concentration of biosurfactant more than 40 mg L⁻¹ decreased both biomass growth and diesel biodegradation. Addition of rhamnolipid to the diesel-water systems from 0 to 80 mg L⁻¹ increased biomass growth and diesel biodegradation. The application of surfactin and rhamnolipid stimulated the indigenous microorganisms for enhanced biodegradation of diesel-contaminated soil. On the other hand, *Ralstonia picketti* and *Alcaligenes piechaudii*, which are producers of biosurfactants, degraded crude oil over 80% in 20 days incubation (Plaza *et al.*, 2008).

Exopolysaccharide AD2 (EPS emulsifier) produced by *O. anthropi* strain AD2 was tested in soil micro-

cosms and experimental biopiles. Also, its efficiency in mixtures with activated sludge from an oil refinery was tested. In soil microcosms the EPS emulsifier together with an oleophilic fertilizer (S200 C) increased the indigenous microbial populations as well as hydrocarbon degradation and therefore decreased the amount of hydrocarbon remaining. Similar effects were obtained in biopile assays amended with EPS emulsifier plus activated sludge (Calvo *et al.*, 2008).

In soil washing, the recovery and reuse of biosurfactants would be the preferred option because of the expense involved in production, although, if they are labile, this may not be an option (Christofi and Ivshina, 2002). Rhamnolipids have been proposed as soil washing agents for an enhanced removal of organic pollutants and metals from soil. A potential limitation to the application of biosurfactants is sorption by soil matrix components. *Pseudomonas* spp. produce rhamnolipids, either in the monorhamnolipid form or, more frequently, as a mixture of the mono- and dirhamnolipid forms. Ochoa-Loza *et al.* (2007) demonstrated that monorhamnolipid sorption on soil matrix components is concentration-dependent, and that the monorhamnolipid form sorbs more strongly alone than when in a mixture of forms. Conte *et al.* (2005) compared the efficiency of a humic acid with that of common surfactants, SDS and TX100, and water in the washing of polluted soil in the contaminated industrial area of a chemical plant. The results showed that the water was unable to fully remove pollutants from the soil, whereas all the organic surfactants revealed similar efficiencies (up to 90%) in the removal of the pollutants from the soils. Hence, the use of solutions of natural humic acids appears to be a better choice for soil washings of highly contaminated soils due to their additional capacity to promote microbial activity, in contrast to chemical surfactants.

Biosurfactants may have applications in metal treatment (Soeder *et al.*, 1996; Zouboulis *et al.*, 2003;

Aşçi et al., 2007). Heavy metals along with other metals and minerals have been released from their natural chemical compounds through industrial activities and processes into rivers, lakes and other surface waters. Since the sediments that have become contaminated below these surface waters have large quantities of water after dredging, dewatering is necessary before using treatment techniques (Dahrazma and Mulligan, 2007). Rhamnolipids, due to their anionic nature, are able to remove metals from soil and ions such as cadmium, copper, lanthanum, lead and zinc due to their complexation ability (Mulligan, 2005). Dahrazma and Mulligan (2007) demonstrated that rhamnolipids have the capacity to remove heavy metals from sediments; the removal was up to 37% of Cu, 13% of Zn, and 27% of Ni, when the biosurfactant was applied in a continuous flow configuration. Rhamnolipids also presented the capacity for enhanced recovery of Cd(II) from kaolin, a soil component (Aşçi et al., 2007).

6. Future prospects

The continuous release of contaminants, organic and inorganic, through either natural or industrial processes, has led to the accumulation and contamination of soils and sediments, surface and groundwater. Several strategies have been studied and developed to decontaminate and restore these sites. However, the efficiency of these processes is limited principally due to low aqueous solubility of contaminants and, therefore, low availability to both physical-chemical processes and microorganism degraders.

The use of biosurfactants is presented as an attractive option because of its versatility, biodegradability, ecological safety and environmental acceptance. However, their high production cost limits their use in bioremediation processes. In this context, it is necessary to evaluate the culturing conditions that optimize their production, assess the economic use of new

substrates, such as those arising from industrial waste, and to evaluate techniques of isolation and purification to make production more economically feasible.

The available information is related to studies under laboratory conditions, and little work has been done on a field scale. Therefore, more efforts are required to evaluate biosurfactant production *in situ* and their effect on the indigenous microorganisms, and to evaluate the efficiency and effectiveness of a bioremediation processes *in situ*.

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