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9. (Bio)surfactant and Bioremediation, Successes and Failures

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Abstract. Application of bioremediation technologies for hydrocarbon contaminated soil is often limited by the presence of high recalcitrant and low bioavailable compounds within the mixture of contaminants. It has been demonstrated that slow release of these compounds from the soil particles into the water phase could represent a rate-limiting factor for bioremediation processes, leading to inability to reach the target of remediation. Due to their surface properties, both chemically synthesised surfactants and microbial produced surfactants (biosurfactants) are used in soil remediation processes to improve removal rate of pollutants in conventional methods. Surfactants are utilised within chemico-physical remediation technologies such as *in situ soil flushing* and *ex situ soil washing* for remediation of unsaturated zone and *pump and treat* technologies for aquifer remediation. However, due the complex interactions between the amphiphilic molecules, the cell surfaces and their abiotic environment, both cases of success and failures have been reported in literature. In this chapter the current knowledge about the natural role of biosurfactants and the effect of (bio)surfactants on the biological systems and abiotic compartments during bioremediation treatments are reviewed.

1. Surface active compounds

1.1. Structures and properties

Surface active agents (bio/surfactants) are amphiphilic molecules with both hydrophilic and hydrophobic moieties, which show a wide range of properties, including

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the lowering of surface and interfacial tension of liquids, and the ability to form micelles and microemulsions between two different phases. The hydrophilic moiety of a surfactant is defined as the "head", while the hydrophobic one is referred to as the "tail" of the molecule which generally consists of a hydrocarbon chain of varying length. Surfactants are classified as anionic, cationic, non-ionic and zwitterionic, according to the ionic charge of the hydrophilic head of the molecule [1]. The most common hydrophobic parts of chemically synthesized surfactants are paraffins, olefins, alkylbenzenes, alkylphenols and alcohols; the hydrophilic part is usually a sulphate, sulphonate or a carboxylate group in anionic surfactants, a quaternary ammonium group in cationic surfactants and polyoxyethylene, sucrose or a polypeptide in nonionic surfactants [2]. An important descriptor of chemico-physical properties of surfactants is related to the balance between their hydrophilic and hydrophobic moieties. Thus, surfactants can also be classified according to their Hydrophile-Lipophile Balance (HLB) [3]. The HLB value indicates whether a surfactant will produce a water-in-oil or oil-in-water emulsion: emulsifiers with a lower HLB value of 3-6 are lipophilic and promote water-in-oil emulsification, while emulsifiers with higher HLB values between 10 and 18 are more hydrophilic and promote oil-in-water emulsions [4]. A classification based on HLB values has been used to evaluate the suitability of different surfactants for various applications. For example, it has been reported that the most successful surfactants in washing oil contaminated soils are those with a HLB value above 10 [2].

As the name suggests and due to their chemico-physical structure, "surfactants" partition preferentially at the interface between phases with different degrees of polarity and hydrogen bonding such as oil/water and air/liquid interfaces. The presence of surfactant molecules at the interfaces results in a reduction of the interfacial tension of the solution. A number of different surfactants, both synthetic and of biological origin, are able to reduce the surface tension of water from 72 mN m^{-1} to 27-30 mN m^{-1} [1,4]. In the presence of a non-aqueous phase liquid (NAPL), the surfactant molecules also aggregate at the liquid-liquid interface, thus reducing the interfacial tension [2].

Another fundamental property of surfactants is the ability to form micelles which is responsible for the excellent detergency and dispersing properties of these compounds. When dissolved in water in very low concentrations, surfactants are present as monomers. In such conditions, the hydrophobic tail, unable to form hydrogen bonding, disrupt the water structure in its vicinity, thus causing an increase in the free energy of the system. At higher concentrations, when this effect is more pronounced, the free energy can be reduced by the aggregation of the surfactant molecules into micelles, where the hydrophobic tails are located in the inner part of the cluster and the hydrophilic heads are exposed to the bulk water phase. The concentration above which the formation of micelles is thermodynamically favoured is called Critical Micelle Concentration (CMC) [5]. The number of molecules necessary to form a micelle generally varies between 50 and 100; this is defined as the aggregation number. As a general rule, the greater the hydrophobicity of the molecules in the aqueous solution, the greater is the aggregation number [6]. CMC is commonly used to measure the efficiency of a surface active agent [4]. The CMC of surfactants in aqueous solution can vary depending on several factors, such as molecule structure, temperature, presence of electrolytes and organic compounds in solution. At soil temperatures, the CMC typically varies between 0.1 and 1 mM [2]. The size of the hydrophobic region of the surfactant is particularly important for the determination of the CMC: in fact the CMC decreases with increasing hydrocarbon chain length, i.e. increasing hydrophobicity. The addition of a CH_2- group to the chain has been shown to decrease the CMC by a factor of 3, according to the Traube's

rule [7]. However, anionic surfactants have higher CMCs than nonionic surfactants even when they share the same hydrophobic group. Electrolytes in solution can reduce the CMC by shielding the electrical repulsion among the hydrophilic heads of the molecules; such effect is more pronounced with anionic and cationic surfactants than with nonionic compounds [5]. At concentrations above the CMC, additional quantities of surfactant in solution will promote the formation of more micelles. The formation of micelles leads to a significant increase in the apparent solubility of hydrophobic organic compounds, even above their water solubility limit, as these compounds can partition into the central core of a micelle. The effect of such a process is the enhancement of mobilization of organic compounds and of their dispersion in solution [8]. This effect is also achieved by the lowering of the interfacial tension between immiscible phases. In fact, this contributes to the creation of additional surfaces, thus improving the contact between different phases [1]. The reduction effect of interfacial tension is particularly relevant when the pollutant is present in soil as a non-aqueous phase liquid. In summary, the main surfactant-mediated mechanisms which may potentially enhance hydrophobic organic compound remediation include the reduction of interfacial tension, micellar solubilization and phase transfer between soil particles and the pseudo-aqueous phase.

1.2. Microbial surface active compounds

Biosurfactants are a wide group of structurally diverse surface active compounds produced by a variety of microorganisms which are mainly classified by their chemical structure and their microbial origin. They are generally composed of a hydrophilic part, consisting of amino acid or peptide anions or cations, mono- or polysaccharides, and a hydrophobic part consisting of saturated, unsaturated or fatty acids [4]. According to a classification proposed by Neu [9], the term “biosurfactants” should be correctly used to identify low-molecular-weight microbial surfactants. In contrast, high-molecular-weight polymers can be collectively defined as bioemulsifiers [10] also otherwise known as bioemulsifiers[11,12]. In fact, the former group includes molecules which efficiently lower surface and interfacial tension, while the latter is composed of amphiphilic and polyphilic polymers which are more effective in stabilizing oil-in-water emulsions but do not lower the surface tension as much. The low-molecular-weight biosurfactants are generally glycolipids, such as rhamnolipids, trehalose lipids, sophorolipids and fructose lipids, or lipopeptides, such as surfactin, gramicidin S and polymixin. The high-molecular-weight bioemulsifiers are amphiphilic or polyphilic polysaccharides, proteins, lipopolysaccharides and lipoproteins [12].

1.2.1. Glycolipids

The best studied microbial surfactants are glycolipids. Among these, the most known compounds are rhamnolipids, trehalolipids and sophorolipids, which are disaccharides combined with long-chain aliphatic acids or hydroxyaliphatic acids.

Rhamnolipids are composed of one or two molecules of rhamnose linked to one or two molecules of β-hydroxydecanoic acid. The hydroxyl group in one of the acids has a glycosidic linkage with the reducing end of the rhamnose disaccharide, while the hydroxyl group of the second acid is involved in ester formation. As one carboxylic group is free, the rhamnolipids are anions above pH 4. Production of rhamnolipids was firstly reported in *Pseudomonas aeruginosa* and was then extensively studied also in other *Pseudomonas* species. Rhamnolipids can lower the surface tension of water to 25-

30 mN m^{-1} and the interfacial tension against *n*-hexadecane to 1 mN m^{-1}; their CMC value range from 10 to 30 mgL^{-1} (see the reviews [4] and [13] for references). Rhamnolipid 1 and rhamnolipid 2 (L-rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate and L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate, respectively) are the main glycolipids produced by *P. aeruginosa* [8] . At present, they are the only microbial surfactants fully commercialized as a mixture for bioremediation purposes.

Trehalolipids are a wide group of glycolipids, constituted by the disaccharide trehalose linked at C-6 and C-6' to mycolic acids, which are long-chain α-branched-β-hydroxy fatty acids. Trehalolipids are produced by a number of different microorganisms, such as *Mycobacterium*, *Nocardia* and *Corynebacterium*. However, the most extensively studied compounds in this class are trehalose dimycolates produced by *Rhodococcus erythropolis*. Trehalolipids produced by different microorganisms differ in their structure, size and degree of saturation. The minimal values for interfacial tension of water against *n*-hexadecane achieved with different trehalolipids range between 1 and 17 mN m^{-1}, while the surface tension is lowered to 25 and 40 mN m^{-1} by trehalose lipids produced by *R. erythropolis* and *Arthrobacter* sp. The CMC for trehalolipids is quite low, about 2 mgL^{-1} [4, 13].

Sophorolipids consist of two glucose molecules linked β-1,2 (sophorose), with 6- and 6'-hydroxyl groups, generally acetylated, linked to a long-chain hydroxy fatty acid. The terminal carboxyl group can be in the lactonic form or hydrolyzed to give an anionic surfactant. They are produced mainly by yeasts, such as *Torulopsis bombicola*, *T. petrophilum*, *T. apicola* and *Candida bogoriensis*. Both lactonic and anionic sophorolipids were demonstrated to lower the interfacial tension of water against *n*-hexadecane or vegetable oils to 1-5 mN m^{-1} over a wide range of pH, temperature and salt concentration [4,13].

1.2.2. Lipopeptides

Most *Bacillus* species synthesize a number of cyclic lipopeptide antibiotics during the early stages of sporulation [14]. For example, *B. polymyxa* produces polymixin, a decapeptide in which amino acids 3-10 form a ring structure, linked to a branched fatty acid [15], while *B. brevis* produces gramicidin S, a cyclic decapeptide consisting of a rigid ring with two positively charged ornitine side-chains on one side and the hydrophobic side-chains of the other residues on the other side [16]. *B. licheniformis* produces a mixture of several lipopeptides acting synergistically; one of these surfactants can lower the interfacial tension between water and *n*-hexadecane to the very low value of 0.36 mN m^{-1} [17]. The most relevant cyclic lipopeptide is surfactin produced by *B. subtilis*, because of its very high activity. Surfactin has a CMC of 25-50 mgL^{-1} and can lower the surface tension of water to 27 mN m^{-1}, while the lowest interfacial tension against *n*-hexadecane is 1 mN m^{-1} [13].

1.2.3. Bioemulsifiers

A wide variety of microorganisms, including some *Archaea*, produce high-molecular-weight polymers having the property to stabilize emulsions. Such polymeric compounds are generally exocellular polysaccharides, proteins, lipopolysaccharides or lipoproteins, in some cases combined in complex mixtures [13]. The best studied bioemulsifiers are those synthesised by various species of *Acinetobacter*. Among these,

the first studied compound was RAG-1 emulsan, produced by *A. calcoaceticus* RAG-1. It is a polyanionic amphiphilic heteropolysaccharide which contains a repeating trisaccharide, with long-chain fatty acids covalently linked through ester bonds. The hydrophobic groups are distributed across the molecule, forming a comb-type polymer. It is different from most of the other bioemulsifiers, since the latter are rather composed by mixtures of hydrophilic and hydrophobic polymers. Emulsan is a very effective emulsifier even at low concentrations, ranging 0.01% to 0.001% [18]. Biodispersan, produced by *A. calcoaceticus* A2, is an extracellular anionic heteropolysaccharide, which can bind to $CaCO_3$ and TiO_2 powder, allowing their dispersion in water [19]. *A. radioresistens* synthesised alasan, consisting in an anionic polysaccharide with covalently bound alanines and a protein component. Its emulsifying activity was shown to increase three-fold when the compound was heated up to 100°C under neutral or alkaline conditions. Such activity enhancement was parallel to conformational modifications of the polymer, as measured by viscosity changes [20]. In addition to emulsifying activity, alasan also lowered surface tension of a 20 mM Tris solution from 69 to 42 mN m^{-1} with a CMC of 200 μg mL^{-1} [21].

In addition to *Acinetobacter* bioemulsifiers, a large number of emulsifying compounds were reported, such as a protein complex from *Methanobacterium thermoautotrophium* [22], a protein-polysaccharide-lipid complex from *Bacillus stearothermophilus* [23] and various polymers from *Pseudomonas* spp. (see [4,13] for references). A number of bioemulsifiers from yeasts are also known, such as the protein-polysaccharide complex liposan from *Candida lipolytica* [24] or mannoprotein by *Saccharomices cerevisiae* [25].

In comparison with low-molecular-weight biosurfactants, bioemulsifiers adhere more strongly to interfaces, even when the water phase is replaced, so that concentrations to be used in bioremediation applications can be low, about 1:50-1:1000 of the water-insoluble phase [10].

2. (Bio)surfactant enhanced bioremediation

In 1997 the enhancing effect of (bio)surfactants on the biodegradation of hydrocarbons was demonstrated [26]. Subsequently, several investigations were published describing the use of (bio)surfactants in different systems and environments (i.e. liquid, slurry and solid phases, soil, water); for references see the reviews [27] and [28]. Also considering the publication bias which probably led to an over-publication of successful applications, the main emerging feature of this large body of literature is the contrasting results reported. In fact, one key point in the application of biosurfactants to environmental remediation is their specificity, due to the fact that different microbial strains produce different molecules. In some cases biosurfactants have enhancing effects on the same producing strain or related organisms. For examples degradation of *n*-hexadecane was stimulated by rhamnolipid in *Pseudomonas aeruginosa*, but not in *Rhodococcus* strains, and the same *P. aeruginosa* was stimulated only by its own rhamnolipid [29]. Nevertheless, in contrast to this study, biosurfactants from *R. erythropolis* strain 3C-9 significantly increased the degradation rate of *n*-hexadecane by two phylogenetically distant strains, *Alcanivorax dieselolei* and *Psychrobacter celer,* in flask tests [30].

Amphiphiles are able to alter the physico-chemical conditions at the interfaces affecting the distribution of the chemicals among the phases. A hydrocarbon contaminated soil contains at least six phases: bacteria, soil particles, water, air, unsoluble

liquid and solid hydrocarbon. The hydrocarbons can be partitioned among different states: solubilised in water phase, ab/adsorbed to soil particle, sorbed to cell surface, as free/unsoluble phase. The addition of (bio)surfactants alters the hydrophobicity of the surfaces, solubilises organic matter and hydrocarbons within the micelles, thus dramatically complicating an already complicate system [2]. For these reasons at the current stage of knowledge, the accurate modelling of the effect of (bio)surfactants addition in bioremediation treatment is not possible and the feasibility has to be evaluated experimentally. However, the understanding of the natural roles of biosurfactants and the interaction with (bio)surfactants and the environment is crucial for our ability to forecast the effects of the addition of amphiphilic molecules, either of biological or chemical origin, on the biodegradation of pollutants. In the following paragraphs the current knowledge about these interactions is reviewed.

2.1. Roles of microbial SACs

Several natural roles have been proposed for microbial amphiphiles due to their different chemical structures and chemico-physical properties. None the less it is still impossible to make any generalization or ability to identify one or more roles common to all microbial surfactants. It can probably only be generally stated that biosurfactants are a common tool by which microorganisms deal with interfacial challenges [28, 31]. The first and main role described is the involvement of biosurfactants in hydrocarbon uptake and access. Microbial surfactants can promote the growth of bacteria on hydrocarbons both increasing the surface area between oil and water by emulsification and increasing pseudosolubility of hydrocarbons by partition into micelles [2, 26]. However, there are some conceptual difficulties in understanding the evolutionary advantages of producing extracellular emulsifying agents, since it is impossible to obtain an oil/water emulsion available only for emulsifier-producing strain in open systems.

Effective interactions with metals have been also described. Rhamnolipids can form a complex with cadmium reducing its cell toxicity [32] while Alasan binds uranium [33]. Lipopetide biosurfactants (i.e. surfactin from *Bacillus* sp. and streptofacin from *Streptomyces tandae)* however are known to be potent antibiotics. A general role in regulating cell attachment to solid and liquid surface has also been proposed. Microorganisms are able to expose outwardly and inwardly the hydrophobic moiety of cell-bound biosurfactants thus increasing and decreasing the surface hydrophobicity, respectively [19, 34].

2.2. Interaction between (bio)surfactant and the environment

Due to their amphiphilic nature, (bio)surfactants can alter phase distribution of contaminants and environmental parameters by different mechanisms [2, 28]: 1) emulsification, 2) micellarization, 3) sorption to soil and 4) desorption of contaminants. These phenomena can be exploited in enhanced bioremediation processes by adding biological and chemical surfactants.

2.2.1. Emulsification

High-molecular-weight biosurfactants have a great potential in stabilizing emulsions between liquid hydrocarbons and water, thus increasing the surface area available for bacterial biodegradation. However they have been rarely tested as enhancers of

hydrocarbon biodegradation in bioremediation and contrasting results are reported in literature. Barkay et al. [21] showed that Alasan produced by *Acinetobacter radioresistens* more than doubled the rate of [^{14}C] fluoranthene mineralization and significantly increased the rate of [^{14}C] phenanthrene mineralization by *Sphingomonas paucimobilis* EPA505; in contrast, Franzetti et al. [35] reported that biosurfactants produced by *Gordonia* sp. strain BS29, while effective in enhancing crude oil and PAH removal by soil washing, were generally not able to increase the rate or extent of their biodegradation.

2.2.2. Micellarization

Above the CMC, a significant fraction of the hydrophobic contaminants partitions in the surfactant micelle cores. In some cases this resulted in a general increase in the bioavailability of contaminants for degrading-microorganisms. Rhamnolipids have been shown to accelerate the biodegradation of hexadecane, octadecane, n-paraffins, creosotes and other hydrocarbon mixtures, and promoted the bioremediation of petroleum sludges when added to contaminated soils at a concentration above the CMC [36, 37, 38, 39]. Pesticide biodegradation was also reported to be promoted by surfactin [40].

In contrast, micelle cores can trap organic contaminants providing a barrier between microorganisms and organic molecules resulting in the latter becoming less bioavailable. Mineralization of hexadecane and phenanthrene was inhibited by Witconol SN70, a nonionic alcohol ethoxylate surfactant [41] while Tween 20, sodium dodecyl sulfonate, tetradecyl trimethyl ammonium bromide, Citrikleen added at concentrations equal or greater than their CMCs, inhibited mineralization of phenanthrene in a soil-water system [42]. In aqueous media, the biodegradation of four PCB congeners by *Pseudomonas* LB-400 was inhibited by Igepal CO-630, a nonionic surfactant, at concentrations above its CMC [42].

2.2.3. Surfactant interactions with sorbed contaminants

In porous materials, such as soils, organic compounds can be strongly ab/adsorbed to particle and trapped in micro and nano pores. This behaviour is known to be the cause of extending remediation times and the difficulties in remediating old contaminations (so called ageing effects). In fact, the mass transfer from ab/adsorbed phase to liquid one is often the process limiting the biodegradation rate [43]. In these cases, (bio)surfactants could reduces surface and interfacial tensions, capillary forces and wettability, while an increase of contact angle reducing the capillary force holding together oil and soil particles. These effect can occur even at concentration below the CMC. Surfactants have been applied to stimulate the dissolution of non-aqueous phase liquids initially present in soils [44], the dissolution of solid contaminants [43], and the desorption and transport of soil-sorbed contaminants [46, 47].

Noordman et al. (2002) [30] investigated the effect of the rhamnolipid biosurfactant on hexadecane degradation in the case of substrate entrapped in small soil pore sizes (6 nm). Even in low mixing conditions, rhamnolipids stimulated the release of entrapped substrates and enhanced uptake by cells.

2.2.4. Surfactant sorption to soil

The CMC of a surfactant measured in presence of soils is normally higher than the CMC measured in water system. This elevated CMC is referred as effective CMC. This

increment is due to the surfactant partitioning onto soil. Losses of surfactant due to sorption need to be considered when selecting surfactant doses for soil/aquifer cleanup operations [48]. The degree of surfactant sorption onto soil depends primarily on the organic carbon fraction of soil and the chemical nature of the surfactant [49]. In some cases the sorption isotherms showed that the higher the concentration of surfactants the higher is the affinity for soil particles. This behaviour is called "cooperative adsorption"; it means that surfactant molecules show more affinity for sorbed molecules of surfactant than for soil. In this kind of isotherms it is not possible to detect an asymptotic value of sorbed compounds. This characteristic could limit the utilisation of these surfactants in bioremediation technologies because high amount of surfactant could be required to obtain necessary water concentration and it is likely that an unacceptable percentage of surfactant accumulates in soil. This type of behaviour was reported both for ethoxylated non-ionic surfactants [50, 51] and for sorbitan derivates [35]. Moreover, the higher the organic content of soil, the greater is the surfactant dose required for contaminant solubilization. Furthermore, in addition to reducing micelle formation, surfactant sorption also increases soil organic carbon content with implications on the partitioning behaviour of target hydrophobic organic compounds [52].

2.3. Interaction between (bio)surfactants and microbial cells

2.3.1. Regulation of adhesion-deadhesion of microorganisms to and from hydrocarbons

The proposed role for microbial SACs is in the regulation of the adhesion-deadhesion of microorganisms to and from hydrocarbons. The exploitation of this natural role consists in the addition of surfactants to increase the hydrophobicity of degrading microorganisms which allows cells to access to hydrophobic substrates more easily [53]. Al-Tahhan et al. [54] demonstrated that sub-CMC levels of rhamnolipids caused the release of LPS by *Pseudomonas* spp.. This phenomenon rendered the cell surface more hydrophobic allowing a more efficient uptake of hexadecane. Normann et al. [30] demonstrated that rhamnolipid by *P. aeruginosa* UG2 stimulated the degradation of hexadecane by the same organism facilitating the hydrocarbon uptake. This rhamnolipid did not to the same extent stimulate the biodegradation of hexadecane by four other strains (*A. lwoffii* RAG1, *Rhodococcus erythropolis* DSM 43066, *R. erythropolis* ATCC 19558, and strain BCG112), nor was degradation of hexadecane stimulated by addition of their own biosurfactants. More recently, Zhang et al. [55] studied the adsorption of dirhamnolipid biosurfactants on cells of *P. aeruginosa*, *B. subtilis*, and *Candida lipolytica*. Their results showed that the adsorption was specific to the microorganisms and depended on the physiological status of their cells. Furthermore, the biosurfactant adsorption caused the cell surface hydrophobicity to change depending on both the rhamnolipid concentrations and the cell physiological conditions. The effect of exogenous rhamnolipids on cell surface composition of *P. aeruginosa* NBIMCC 1390 was recently studied by Sotirova et al. [56]. They showed that above CMC, the rhamnolipid caused reduction of total cellular LPS content of 22%, which can be associated with an increase in cell hydrophobicity to 31% while at concentrations below CMC it did not affect the LPS component of the bacterial outer membrane but caused changes in outer membrane protein composition. Cases of inhibition of microbial degradation due to surfactant-induced change in surface hydrophobicity have also been reported. Chen et al. [57] observed that low concentration (0.09 CMD) of Triton X-100 inhibited the growth on solid anthracene of a *Mycobacterium* sp. strain and a

Pseudomonas sp. strain. The causes of inhibition were believed to be the sorption of the surfactant onto both microbial cell surfaces and anthracene particles.

2.3.2. Surfactant toxicity

Surface active compounds themselves can represent a contamination when introduced in the environment. Toxicity of surfactants could be both toward the whole ecosystems or the degrading microorganisms, thus inhibiting pollutant biodegradation [2].

Disruption of cellular membranes by interaction with lipid components and reactions of surfactant molecules with proteins that are essential to the functioning of the cell are reported as one of the main toxicity mechanisms of surfactants [58]. For these reasons, the toxicity of such compounds should always be assessed, especially when an *in situ* application is planned. A step-wise procedure has been proposed for the selection of biosurfactants to enhance diesel biodegradation considering the toxicity other than the physical – chemical properties and the influence of the surfactants on the biodegradation rate. The toxicity was firstly estimated by QSAR model and then experimentally tested [59].

Among the chemical synthesised surfactants, non ionic surfactants are considered less toxic and biodegradable than anionic and cationic ones; furthermore, the use of alkyl phenol plyethoxylates is discouraged because its biodegradation leads to formation of alkyl phenol, more toxic and persistent than parent compounds [60]. The use of alkyl polyethoxylates and sorbitan derivates is preferred [48, 59]. Biologically produced surfactants are naturally occurred molecules, and the use of these surfactants in bioremediation processes is more acceptable because of their lower toxicity and higher biodegradability [3]. Munstermann et al. [61] verified that trehalose tetraester from *Rhodococcus erythropolis* were less toxic to *Vibrio fischeri* (acute Microtox® toxicity test) than a number of synthetic surfactants and bioremediation formulations. Ivshina et al. [62] found that a *R. ruber* glycolipid complex was the least toxic agent of all (bio)surfactants cited by Munstermann, having an IC_{50} more than 10 times higher than its CMC value. In particular, it was 100-1000 times less toxic than synthetic surfactants. Furthermore, glycolipids produced by *Rhodococcus* sp. strain 413A was reported to be 50% less toxic than Tween 80 in naphthalene solubilisation tests [63]. Also the toxicity of (bio)surfactants on microorganisms is strain-dependent. Shin et al. [64] reported inhibition of phenanthrene biodegradation due to rhamnolipid addition for two phenanthrene-degrading bacterial strains (*Sphingomonas* sp. strain 3Y and *Paenibacillus* sp strain 4-3). The biosurfactant itself showed significant toxic effects towards strain 3Y, but was nontoxic toward strain 4-3. The authors explains this behaviour combining the inhibitory and toxicity results with regard to the biodegradation. In the biodegradation experiments, the toxicity of rhamnolipid itself was mainly responsible for the inhibitory effect on strain 3Y, whereas the toxicity of solubilized phenanthrene or the increased toxicity of rhamnolipid in the presence of solubilized phenanthrene could have resulted in the inhibitory effect noted in the case of strain 4-3.

2.3.3. Surfactant biodegradation

Both positive and negative effect on biodegradation of pollutants have been described when using (bio)surfactant. The most common reported negative effects occurred in the cases where (bio)surfactants provided a more easily degradable carbon source alternative to the contaminants [59, 65]. Surfactant intermediates can also be more toxic than parent compounds as reported for nonil phenol ethoxylates [60]. Biodegradability of

(bio)surfactants can also affect both the persistence of the molecules in soil. This can be considered as a positive or a negative effect taking into consideration the undesirable high residual concentration of surfactants remaining in the soil after treatment and the cost associated with the replacement of degraded surfactant. Microbial surfactants have been demonstrate to be generally more biodegradable than synthetic ones and within chemical surfactants non-ionic surfactants showed less recalcitrance. Zeng et al. [66] compared the biodegradability of some chemical surfactants with rhamnolipids. The results showed that CTAB, Triton X-100 and SDS have different degrees of biodegradability and toxicity. Rhamnolipid showed no toxicity and could be degraded by *Bacillus subtilis* and compost microorganisms, while it could not be utilized by its producing bacterium *Pseudomonas aeruginosa*. It is also worth noting that also for all these aspects contrasting results are reported in the literature. In 2006 Franzetti et al. [49] reported complete mineralization of Tween 80 using mixed soil bacterial population while in 2009 Frank and colleagues [67] reported only primary degradation of this surfactant with mixed cultures. As noted by the authors, these results illustrate the problem of using different soil samples which naturally contain different indigenous microbial communities.

3. Conclusions

The use of (bio)surfactants as an additive in bioremediation applications to soil and groundwater contaminated by insoluble organic pollutants can potentially increase the biodegradation rate and reduce contaminant minimum concentration. This is due to their ability to enhance the pseudosolubilisation and emulsification of the immiscible fractions of the contaminants, thus enhancing their bioavailability to degrading microorganisms. However, together with many successful applications, several cases of no effect or even inhibition of biodegradation upon use of (bio)surfactant have been reported in literature. This is mainly due the complex interactions taking place between the amphiphilic molecules, the cell surface and the abiotic environment. Despite most of these possible interactions discussed above, a more detailed understanding of the natural roles of biosurfactants and the effects of (bio)surfactants on biological and abiotic compartments is necessary to allow considering them as a reliable technology to ensure enhancing bioremediation.

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