

THE ROLE OF BIOSURFACTANTS IN SOIL REMEDIATION

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Summary. Biosurfactants are promising compounds in the process of soil remediation because of their natural origin and amphiphilic structure as well as beneficial physicochemical and technological properties. They are capable to remove toxic substances (heavy metals, crude oil, hydrocarbons and their derivatives) from contaminated soil using various mechanisms of action, such as mobilization, solubilization, complexation, emulsification. Moreover, natural surfactants cooperate with microorganisms in the process of bioremediation and increase the bioavailability of organic pollutants for their cells. They also affect the microbial cell surface properties and the cellular phospholipid membrane and in this way they can enhance the intracellular transport leading to the increase in biodegradation rate. In this paper, the origin, structures and resulting properties of biosurfactants were described to understand their mechanisms of action in soil remediation, especially in removing of organic pollutants and heavy metals. The examples of their practical application in soil bioremediation were also presented.

Key words: biosurfactants, biodegradation, soil remediation, bioremediation

INTRODUCTION

By definition, the ecosystem consists of mutually co-existing and interacting biocenosis and biotope. Human interference in natural ecosystems results in water, soil and air pollution. Anthropogenic pollutants are usually the result of industrial and agriculture activities as well as improper disposal of waste. Because soil has very divers structure (a mixture of organic matter, minerals, gases, liquids and organisms) it is a very significant

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source of contaminants due to its ability to bind various types of chemicals. A large variety of organic and inorganic contaminants and chemicals can exist in soil in different forms and concentrations. Lead, mercury, arsenic, copper, zinc, nickel, polyaromatic hydrocarbons and herbicides are examples of the most common soil pollutants. Remediation means removal, destruction or transformation of contaminants to less harmful chemicals [Agamuthu et al. 2013]. Due to the large variety of soil contaminants, a number of its remediation methods have been developed. Soil cleaning can be carried out *in-situ* (at the site where it is occurring – ‘soil flushing’) or *ex-situ* (after the excavation of contaminated soil – ‘soil washing’) using techniques such as solidification, stabilization, vapor extraction as well as electrochemical methods and thermal or biological treatment [Khan et al. 2018]. Choosing an appropriate soil remediation method requires the knowledge of its nature, composition, sorption/desorption potential, physicochemical properties, microflora as well as information on the properties of contaminants. It should be also mentioned that traditional remediation techniques are very expensive and less costly alternatives are sought for them.

Bioremediation is of special interest in term of soil cleaning because it is a cost-effective solution in removing numerous pollutants from contaminated sites [Agamuthu et al. 2013]. It is a process in which living organisms (typically bacteria or fungi) are used to transform environmental contaminants into less toxic chemicals. Bioremediation belongs to the group of ‘clean up’ technologies [Lombi and Hamon 2005].

In the process of bioremediation not only microorganisms can be involved but also different metabolites which they produced, especially surface active agents. Surfactants of natural origin have great potential to remove different pollutants from contaminated sites. These compounds significantly reduce surface/interfacial tension, form aggregates at very low concentration, as well as they have solubilizing, emulsifying, wetting and foaming properties which can be successfully used in the process of bioremediation to decontaminate soil. Moreover, they are evolutionarily adapted to cooperation with microorganisms during their uptake of organic compounds.

In order to understand the mechanisms of biosurfactants action in soil bioremediation, which are associated with the complex interactions between microorganisms, biosurfactant, pollutant and soil, it is necessary to have knowledge of their origin, chemical structure and the resulting properties.

BIOSURFACTANTS – ORIGIN, STRUCTURE AND PROPERTIES

Biosurfactants are surface active agents of natural origin. Microbial surfactants can be extracted extracellularly as secondary metabolites of microorganisms (bacteria, yeasts and fungi) [Araújo et al. 2019], produced on their cell surface as well as obtained by bio-transformation using isolated enzymes or whole microbial cells [De et al. 2015]. The best known microbial biosurfactants with their typical producers are listed in Table 1.

Natural surfactants can be also isolated from products of animal or plant origin (e.g. lecithin from eggs, corn, soybean or rapeseed) or can be produced in the human body (e.g. lung surfactants, mucin).

Table 1. Type and microbial origin of biosurfactants [De et al. 2015].

Tabela 1. Rodzaj i mikrobiologiczne pochodzenie biosurfaktantów [De et al. 2015].

Type of biosurfactant Rodzaj biosurfaktantu	Synthesis microorganisms Mikroorganizmy syntetyzujące
Rhamnolipids Ramnolipidy	<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas sp.</i>
Sophorose lipids Soforolipidy	<i>Candida bombicola</i> , <i>Candida apicola</i>
Trehalose lipids Trehalolipidy	<i>Rhodococcus erythropolis</i> , <i>Mycobacterium sp.</i>
Surfactin Surfaktyna	<i>Bacillus subtilis</i>
Emulsan	<i>Acinetobacter calcoaceticus</i>
Liposan	<i>Candida lipolytica</i>

Biosurfactants like chemically synthesized surface active agents have amphiphilic structure. They are made up of hydrophilic moiety (“head”), typically mono-, oligo- or polysaccharide, amino acid, cyclic peptide, carbohydrate or phosphate group, carboxylic acid or alcohol, and hydrophobic moiety (“tail”) of long-chain fatty acids, hydroxy fatty acids or α -alkyl- β -hydroxy fatty acids [Mulligan 2009].

Microbial surfactants are produced as a mixture of several homologues therefore their properties depend on the quantitative and qualitative composition of the mixture. Moreover, both biosurfactant yield and composition are affected by their growth conditions.

According to the chemical structure microbial surfactants can be divided into [Vijayakumar and Saravanan 2015, Geetha et al. 2018]:

- glycolipids (e.g. rhamnolipids, sophorolipids, trehalolipids) are carbohydrates linked to long-chain aliphatic acids or hydroxyl aliphatic acids by an ester group;
- lipopeptides and lipoproteins (e.g. surfactin, lichenysin, iturin, viscosin) are lipids linked to a polypeptide chain;
- fatty acids (e.g. corynomuolic acids), phospholipids (e.g. phosphatidylethanolamine) and neutral lipids;
- polymeric biosurfactants (e.g. emulsan, liposan, alasan, biodispersan) are especially polysaccharide-protein complexes, and particulate biosurfactants (e.g. *Yarrowia sp.*).

The examples of chemical structure of selected biosurfactants belonging to three different groups are presented in Figure 1.

Biosurfactants were also divided by Rosenberg and Ron [1999] into two groups according to their molecular weight. Low-molecular mass compounds (< 1500 Da) including glycolipids, phospholipids and lipopeptides and high-molecular mass compounds including amphiphathic polysaccharides, proteins, lipopolysaccharides and lipoproteins (or complex mixtures of these biopolymers). Low-molecular weight compounds are in-

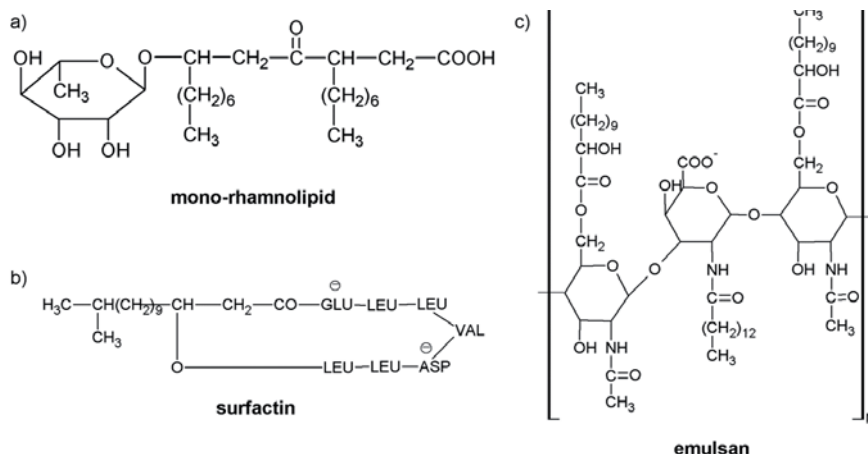


Fig. 1. Chemical structure of selected biosurfactants: a) mono-rhamnolipid (mono-RL), b) surfactin c) emulsan.

Rys. 1. Budowa chemiczna wybranych biosurfaktantów a) mono-ramnolipid (mono-RL), b) surfaktyna, c) emulsan.

vestigated to be better reducers of surface and interfacial tension when high-molecular weight compounds are more effective in stabilizing oil-in-water emulsions.

Natural surfactants are of a special interest because of their unique properties and several advantages over their chemical counterparts. Microbial surfactants have higher surface and interfacial activity, lower values of critical micelle concentration (CMC) and they are more effective as emulsifiers, stabilizers, dispersants or foaming and wetting agents than those chemically synthesized. It is also important that microbial biosurfactants as metabolites of bacteria living in various environments have developed the ability to function effectively even in extreme environmental conditions. In turn, due to their natural origin they are also biodegradable and environmentally biocompatible as well as non-toxic [De et al. 2015]. Moreover, it is possible to produce them *in situ* at contaminated sites.

Biosurfactants even at low concentration reduce surface tension of water as well as water/oil interfacial tension to a significant extent e.g. rhamnolipid can reduce the surface tension of water from 72 to 25–30 mN·m⁻¹ and water/oil interfacial tension from 43 to < 1 mN·m⁻¹ [Lang and Wullbrandt 1999] or surfactin to 27 mN·m⁻¹ and 1 mN·m⁻¹, respectively [Cooper et al. 1981]. At concentration above their CMC they solutions have a minimum value of surface tension and biosurfactants start to associate to form micelles, bilayers and vesicles [Pacwa-Płociniczak et al. 2011]. The CMC value of a given biosurfactant can vary widely depending on the composition of its isolated mixture. Biosurfactants can form aggregates at low concentration among others CMC of rhamnolipids is in the range of 10 to 200 mg·dm⁻³ [Müller et al. 2012] or sophorolipids from 40 to 100 mg·dm⁻³ [Joshi-Navare et al. 2013].

Their tendency to adsorb and to form aggregates is the prerequisite for a broad range of surfactants activities to take place such as emulsifying, solubilizing, foaming, wetting.

These properties make biosurfactants useful in remediation and bioremediation technologies of both organic and heavy metals contaminants. The effectiveness of environmental remediation using natural surfactants depends on the selection of appropriate compounds for this purpose due to high selectivity of their action and specificity of hydrophilic-hydrophobic properties.

BIOREMEDIATION OF WATER INSOLUBLE SUBSTRATES

Hydrophobic organic substances such as crude oil and its derivatives, polycyclic aromatic hydrocarbons, polychlorinated biphenyls or halogenated hydrocarbons are ones of the most common pollutants produced by anthropogenic activities and found in soil. Their relatively low water solubility, high solid-water distribution ratios, high adsorption onto solid particles and tendency to form droplets at these particles are factors limiting desorption and bioavailability of hydrophobic substances for microorganisms [Barkay et al. 1999, Pacwa-Płociniczak et al. 2011].

Hydrocarbon-degrading bacterial populations are often capable to produce substances showing surface active properties. These metabolites are involved in the interaction of microbial cells with surfaces as well as substrates [Perfumo et al. 2010]. In the process of hydrophobic compounds bioremediation biosurfactants present three modes of action depending on their molecular weight and concentration.

The mobilization is characteristic for low-molecular weight surfactants at concentration below their CMC. Because water/oil interfacial tension is very high it is a factor limiting the rate of biomass increasing on hydrocarbons. As mentioned, biosurfactants have the ability to reduce interfacial tension by their adsorption at the interface which causes more interaction and mixing of dissimilar phases. Moreover, by reducing surface/interfacial tension surfactants increase the surface area of water insoluble substrates and in consequence their mobility and bioavailability.

Low-molecular mass biosurfactants are very effective in lowering surface/interfacial tension but high-molecular polymeric surfactants are much less active at the interface of different phases. Thus, the main role of polymeric biosurfactants (bioemulsifiers) even at low concentration is to form stable emulsions of immiscible liquids. The emulsifying properties of surfactants depend on their structure. Especially in the case of non-ionic surfactants, the ratio of their hydrophilic to hydrophobic part expressed as the hydrophilic-lipophilic balance (HLB) allows to predict the main properties of surfactants. The compounds of HLB from 4 to 6 are typical O/W emulsifiers while these of HLB in the range from 8 to 18 are W/O emulsifiers [Müller et al. 2012]. The role of biosurfactants in the emulsification process is lowering the oil/water interfacial tension, which facilitates the breakdown of oil phase into tiny droplets and provides a larger surface area for degrading microbes [Banat et al. 2010]. Ionic surfactants form stable emulsions because of the electrostatic repulsion between oil droplets surrounded by surfactants molecules of the same charge. In contrast to low-molecular mass biosurfactants, the main stabilization mechanism in the case of polymeric biosurfactants is the formation of a steric barrier between oil droplets. The molecules of bioemulsifiers because of their complex structure

res with high number of reactive groups can bind tightly to dispersed organic substances preventing them from merging together [Uzoigwe et al. 2015].

The third mechanism of biosurfactants action in bioremediation is solubilization. Low-molecular mass compounds even at low concentration (but above their CMC) enhance the biodegradability of hydrophobic compounds also by forming micelles. The hydrophobic cores of micelles create apolar medium in which organic substances can dissolve. When hydrocarbons become incorporated within the hydrophobic core of micelles it enhances their dispersion into the aqueous phase and hence bioavailability of poorly-soluble substrates for uptake by bacterial cells. In turn, in the case of high-molecular mass bioemulsifiers, especially polymeric biosurfactants, the formation of multimolecular aggregates practically does not affect solubilization of organic compounds. Polymeric biosurfactants solubilizes them by slowly reversible physical interactions, probably of hydrophobic nature [Barkay et al. 1999].

Moreover, biosurfactants can also act on the bacterial cells modifying their outer membrane structure and surface properties to facilitate the adsorption of organic matter. Biosurfactants can increase the hydrophobicity of microbial cells. Al-Tahhan and co-workers [2000], on the example of rhamnolipid produced by *Pseudomonas spp.*, stated that it could be the result of a release of lipopolysaccharides (LPS) from bacterial cells surface which enhances the affinity of cells for poorly-soluble substrates and facilitate their uptake [Al-Tahhan et al. 2000]. In turn, Sotirova et al. [2009] reported that rhamnolipids only after exceeding their CMC cause the reduction of total cellular LPS content but at low concentration (below CMC) not affect the LPS in bacterial cell surface structure but change the composition of outer membrane proteins (OMP).

Additionally, molecules of biosurfactants can penetrate the cellular phospholipid membrane and change its permeability and fluidity. Biosurfactants enhance the uptake of pollutants increasing the permeability of the bacterial membrane through:

- adsorption at the outer leaflet,
- movement to the inner membrane
- intercalation between the phospholipid bilayer.

Natural surfactants can alter membranes fluidity by reducing the ratio of saturated to unsaturated fatty acids in the lipid bilayer. The greater the amount of unsaturated fatty acids in the membrane the greater is its fluidity, which results in better transport of organic compounds through the microbial cell membranes [Kaczorek et al. 2018]. Such modifications have been observed by several authors for rhamnolipid [Sánchez et al. 2010, Kaczorek and Olszanowski 2011, Lin et al. 2017] – the best known microbial biosurfactant.

METALS REMEDIATION

The soil contamination by heavy metals is a serious problem for the life and health of living organisms include human because of their potential toxicity, reactivity and mobility in the soil. A number of technologies are currently used to remove potentially toxic metals cations such as Pb^{2+} , Zn^{2+} , Cr^{3+} , Cd^{2+} and Hg^{2+} [Miller 1995] from soil and bioremedia-

tion is one of them. However, in contrast to bioremediation of organic substances, metals cannot be biodegraded. Another difference in their remediation is fact that organics usually occur in the soil in the form of neutral molecules and heavy metals as cations. Such anionic biosurfactants as e.g. rhamnolipids with carboxylic group or surfactin with two negative charges on the aspartate and the glutamate residues are cable of binding metals. But, when removing heavy metals from the soil, it is especially important to select the proper complexing agent due to the selectivity of biosurfactants to metals present both in soil solution and those associated with solid soil particles [Miller 1995].

As with the bioremediation of hydrophobic substances, there are differences between the mechanism of action of low and high-molecular weight surfactants in metals remediation. Bacterial exopolysaccharides (e.g. emulsan) have been shown to bind different metals. But such complexation is less effective than complexation by low-molecular mass biosurfactants probably due to the large molecule size of extracellular bioemulsifiers [Miller 1995].

Biosurfactants can enhance the mobility of heavy metals in two different ways – by lowering interfacial tension and by forming micelles [Wang and Mulligan 2004]. Initially, at low concentration ZPC molecules adsorb at metal–soil and soil–water interface because of their amphiphilic structure. In this way, they improve soil wettability as well as reduce interfacial tension and thus the strength of binding metal cations to soil particles. Next, metal ions can be complexed by negatively charged biosurfactant molecules that are more easily removed from contaminated sites because the strength of the metal-biosurfactant bond is stronger than metal-soil particle. Metal ions are bound to oppositely charged ions or replace the same charged ions (electrostatic interactions or ion exchange) [Rufino et al. 2011]. At the end desorption of the metal from soil particles and its complexation by the biosurfactant micelles is observed [Mulligan 2017]. Forming the micelles stabilize metal-biosurfactant complexes.

BIOSURFACTANTS IN SOIL REMEDIATION – EXAMPLES

In the literature there are many papers confirming the high efficiency of biosurfactant-mediated biodegradation of different water non-soluble substrates such as e.g. polycyclic aromatic hydrocarbons. Desari et al. [2014] examined naphthalene degradation with and without of biosurfactant produced by *Pseudomonas aeruginosa* isolated from oil-contaminated soil. They showed that *Pseudomonas* secreted biosurfactant which reduces surface tension of water, forms stable emulsion with hexadecane and kerosene as well as enhances the biodegradation of naphthalene. In turn, Congiu and Ortega-Calvo [2014] investigated the effect of rhamnolipid on biodegradation of (14)C-labeled phenanthrene and pyrene under desorption-limiting conditions. They showed that RL caused solubilization and enhanced biodegradation of tested PAHs but enhancement was negatively influenced by experimental conditions that caused an enrichment of slow desorption fractions. However, the decline in bioavailability caused by aging on sorbed (14) C-pyrene was partially reversed by RL, which enhanced mineralization of the aged compound [Congiu and Ortega-Calvo 2014].

Yao et al. [2017] studied the effect of rhamnolipid addition on desorption of phenanthrene in soil during aging process under diurnal and seasonal freeze-thawing cycles. They stated that biosurfactant in this case caused an increase in desorption efficiency of phenanthrene as well as that its supplementation minimized the sequestration of PAH [Kaczorek et al. 2018].

Barkay and co-workers [1999] studied the effect of a polymeric biosurfactant, alasan produced by *Acinetobacter radioresistens* KA53 on the solubilization and biodegradation of polyaromatic hydrocarbons (phenanthrene, fluoranthene and pyrene). They observed that the apparent aqueous solubility of tested PAHs was increased 6.6-fold (for phenanthrene), 25.76.6-fold (for fluoranthene) and 19.8-fold (for pyren) in the presence of 0.5 g of bioemulsifier. Moreover, alasan significantly increased the rate of [^{14}C]fluoranthene and [^{14}C]phenanthrene mineralization by *Sphingomonas paucimobilis* EPA505.

The microbial degradation of phenanthrene was also tested by Schippers et al. [2000] in the presence of other biosurfactants – low-molecular weight sophorolipids synthesized by *Candida bombicola*. They observed a reduction to 71% of the detectable amount of tested PAH within 4 days in soil suspension without any biotic influence. When experiments were done with *Sphingomonas yanoikuyae* sophorolipids increased the bioavailability of the phenanthrene and enhanced its biodegradation. Moreover, authors proved that surfactant addition reduced the residual pollutant concentration and increased the maximum degradation rate.

Franzetti et al. [2009] used *Gordonia sp.* BS29 isolated from diesel-contaminated soil and its metabolite (extracellular bioemulsan) in both, bioremediation of soils contaminated by aliphatic and aromatic hydrocarbons as well as washing of soils contaminated by crude oil, polycyclic aromatic hydrocarbons and heavy metals. The obtained results showed that bioemulsan is promising washing agent for remediation of soil from crude oil and PAH but only slightly enhances the biodegradation of recalcitrant branched hydrocarbons

Wang and Mulligan [2004] successfully used JBR425 (rhamnolipids mixture) to remove cadmium and nickel from contaminated soil. They compared metal removal efficiencies by surfactant solutions and by their foams and proved that foam technology is more effective in metal removal than conventional soil flushing by surfactant solutions.

Mulligan et al. [1999] used surfactin to remove heavy metals from contaminated soil and sediments. They showed that series of five washings of the soil with 0.25% surfactin (with 1% NaOH) were required to remove 70% of the cooper and 22% of the zinc compared to water alone that removed less than 1% of Cu and Zn.

Juwarkar et al. [2007] examined biosurfactant technology for remediation of cadmium (Cd) and lead (Pb) contaminated soils. They used rhamnolipid produced by *Pseudomonas aeruginosa* strain BS2 in their research and found that di-rhamnolipid effectively removed not only the leachable (freely available) fraction of cadium and lead but also the bound metals (di-RL removed 92% of Cd and 88% of Pb after 36 hours of leaching). Moreover, they discovered that tested biosurfactant selectively favored the mobilization of metals (Cd > Pb) as well as that reduces the toxicity of heavy metals to soil microflora.

The latest research indicates that it is possible to produce low-cost biosurfactants that will effectively remove heavy metals from the soil. Sarubbo et al. [2018] used the yeast *Candida guilliermondii* UCP 0992 grown in low cost medium for producing the biosur-

factant. They obtained the anionic ZPC (composed of 12.4% of lipids, 52.3% of proteins and 10% of carbohydrates) which reduced the surface tension of water from 72.0 to 28.0 $\text{mN}\cdot\text{m}^{-1}$ at concentration of 0.42%. Crud biosurfactant (cell-free broth) was able to remove 98.9% of zinc, 89.3% of iron and 89.1% of lead from soil coming from a battery industry.

CONCLUSIONS

Biosurfactants application for the remediation of contaminated soil are promising due to their high surface/interface activity, very low values of critical micelle concentration, solubilizing, emulsifying and foaming activities as well as high effectiveness in enhancing biodegradation. Natural surfactants increase the bioavailability of hydrophobic compounds (biosurfactant-enhanced bioremediation) as well as they have affinity for inorganic compounds such as heavy metals, which is very important in term of their removing from the soil. Therefore, biosurfactants are promising agents for soil remediation and bioremediation technologies. But the selection of a suitable biosurfactant for specific environmental applications is crucial especially due to their high selectivity.

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ROLA BIOSURFAKTANTÓW W REMEDIACJI GLEBY

Streszczenie. Biosurfaktanty są obiecującymi związkami w procesie remediacji gleby ze względu na ich naturalne pochodzenie i amfifilową strukturę, a także korzystne właściwości fizykochemiczne i technologiczne. Są w stanie usunąć toksyczne substancje (metale ciężkie, ropę naftową, węglowodory i ich pochodne) z zanieczyszczonej gleby przy wykorzystaniu różnych mechanizmów działania, takich jak mobilizacja, solubilizacja, kompleksowanie, emulgacja. Ponadto naturalne związki powierzchniowo czynne współpracują z mikroorganizmami w procesie bioremediacji i zwiększają biodostępność zanieczyszczeń organicznych dla ich komórek. Wpływają również na właściwości powierzchniowe komórek mikroorganizmów oraz komórkowe błony fosfolipidowe i dzięki temu mogą zwiększać transport wewnątrzkomórkowy, prowadząc do wzrostu szybkości biodegradacji. W niniejszym artykule opisano pochodzenie, struktury i wynikające z nich właściwości biosurfaktantów, aby zrozumieć ich mechanizmy działania w procesie remediacji gleby, zwłaszcza w usuwaniu zanieczyszczeń organicznych i metali ciężkich. Przedstawiono również przykłady ich praktycznego zastosowania w bioremediacji gleby.

Słowa kluczowe: biosurfaktanty, biodegradacja, remediacja gleby, bioremediacja