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Microbial Biosurfactants Role in Oil Products Biodegradation

O. L. Matvyeyeva[1,*], O.A. Vasylchenko[2], O. R. Aliieva[1]

[1]Department of ecology, Institute of environmental safety, National Aviation University
[2]Department of biotechnology, Institute of environmental safety, National Aviation University
*Corresponding author: aliyeva_oks@mail.ru

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Abstract Biosurfactants have a wide-range of applications due to their unique properties like specificity, low toxicity and relative ease of preparation. These properties hold promise for biosurfactants to increase the degree of microbial destruction of petroleum hydrocarbons, which promotes the effective remediation of the petroleum pollution. This article presents a review of available research data and publications regarding hydrocarbon biodegradation, oil-degrading bacteria metabolism, and application of biosurfactants to these processes. Hydrocarbon degrading bacteria producing biosurfactants and their consortia search was provided, types of microbial surfactants and their physiological role were described and ordered. Range of factors affecting the surface active properties of producers' cultures was studied. The results are promising enough to continue the quest for enhancement of hydrocarbon biodegradation with biosurfactants application to look forward for biosurfactants as a solution of petroleum pollution problem.

Keywords: *biodegradation, biosurfactants, hydrocarbons, petroleum products*

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1. Introduction

Powerful negative impact of petroleum products on the atmosphere, hydrosphere, and the soil cover of the Earth is caused by several factors: an active and growing use of hydrocarbon resources in all sectors of human activity; oil producing, transporting, processing and consuming enterprises wide spreading; characteristic physical, chemical and toxic hydrocarbons properties contributing to their low biodegradability in natural ecosystems.

In order to eliminate hydrocarbon contamination a set of measures is used at present time. Applied mechanical treatment, physical and chemical contaminating petroleum products processing are among them. However, bioremediation is the only method that allows for complete transforming hydrocarbon pollutants into harmless compounds in a natural way. The key mechanism of any bioremediation technology is oil-degrading microorganisms use. These destructors can utilize petroleum products as single carbon and energy source, converting them into microbial biomass, carbon dioxide and other metabolites. Strong limiting factor in microbial degradation of petroleum hydrocarbons is their hydrophobic nature. A promising way to resolve this difficulty may be microbial surfactants (biosurfactants) application.

Previous publications [1,2,3,4,5] regarding oil, hydrocarbon degradation, its metabolism, and bioremediation in general have already investigated and shown a perspective of biosurfactants application to stimulate oil degrading processes.

Indian scientists Aparna A., Srinikethan G., Smitha H. [5] have shown that addition of 50 mg of biosurfactant per 100 ml of the basal salt medium containing 1% crude oil significantly enhanced the crude oil degradation indicating that the organism utilized crude oil as carbon source. Earlier Zhang Y. and Miller R.M. [6] described how dirhamnolipids enhanced hexadecane degradation by seven different *Pseudomonas* strains.

One more promising result was achieved by Obayori *et al.* [7] investigated the biodegradative properties of biosurfactant produced by *Pseudomonas sp. LP1* strain on crude oil and diesel. They reported 92.34% degradation of crude oil and 95.29% removal of diesel oil. Biodegradative properties of biosurfactant producing *Brevibacterium sp. PDM-3* strain were tested by Reddy *et al.* [8]. They reported that this strain could degrade 93.92% of the phenanthrene and also had ability to degrade other polyaromatic hydrocarbons such as anthracene and fluorene.

Kang *et al.* [9] used sophorolipid in studies on biodegradation of aliphatic and aromatic hydrocarbons and Iranian light, crude oil under laboratory conditions. Addition of this biosurfactant to soil increased also biodegradation of tested hydrocarbons with the rate of degradation ranging from 85% to 97% of the total amount of hydrocarbons.

Thus, the capability of biosurfactants and biosurfactant-producing bacterial strains to enhance organic contaminants' availability and biodegradation rates was reported by many authors and this makes the use of biosurfactants a promising method that can improve bioremediation effectiveness of hydrocarbon contaminated environments.

2. Types of Microbial Surfactants

Surfactants include compounds capable of surface and interfacial tension reducing. This is a large class of substances with different chemical nature, a common feature of which is oil and water sensitive structure of the constituent molecules. Due to this structure, molecules of these substances are able to concentrate on the interface (Figure 1), leading to surface active properties.

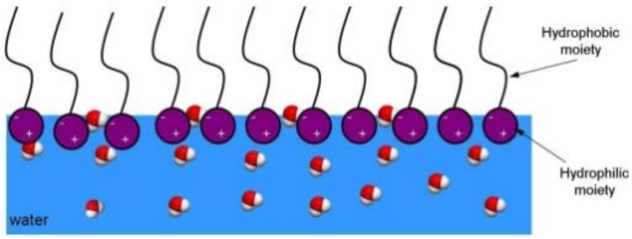

Figure 1. Accumulation of biosurfactants at the interface between liquid and air

Surface active agents of biogenic, particularly microbial, origin referred to biological surface active agents (bioSAS). According to their surface active characteristics they are usually close to the synthetic surfactants, but stand out with next properties:

1. the lower toxicity, or lack thereof;
2. high biodegradability by natural microorganisms;
3 activity in a wide medium acidity/salinity range;
4. possibility of their microbial synthesis from relatively cheap substrates, including polluting compounds.

According to the chemical nature biosurfactants are heterogeneous compounds. The polar components are peptides or glycanes and non-polar components are various lipids. BioSAS refer to the following major groups [3].

A) glycolipids - the most common group, low molecular weight compounds consisting of carbohydrate and lipid components. Synthesized by representatives of the *Rhodococcus, Nocardia, Pseudomonas, Candida* genera.

B) lipopeptides - low molecular weight compounds consisting of a peptide (protein) and lipid parts, synthesized by some pseudomonades and yeasts, the most famous producers are members of the *Bacillus* genus, synthesizing a wide range of lipopeptide biosurfactants.

C) fatty acids and phospholipids - fatty acids are formed at alkanes oxidation and may be released into the environment by *Rhodococcus* sp. Phospholipids are synthesized by representatives of the *Acinetobacter* and *Corinebacterium* genera.

D) polysaccharides - extracellular polymeric compounds with powerful emulsifying properties. Such biosurfactants formed by *Xanthomonas campestris, Acinetobacter radioresistens, Acinetobacter venetianus* are widely known.

All biosurfactants can be divided into two major groups - low (glycolipids, lipopeptides) and high (extracellular polymers) molecular weight surfactants, which differ in physiology and surface active properties.

Some authors [10] classify microbial surfactants into two groups according to the exhibited surface active properties type:

1. liquid medium surface tension reducing (biosurfactants);
2. emulsification ability (bioemulsifiesrs).

The low-molecular bioSAS, as a rule, are referred to the first group, and high-molecular ones – to the second (Figure 2).

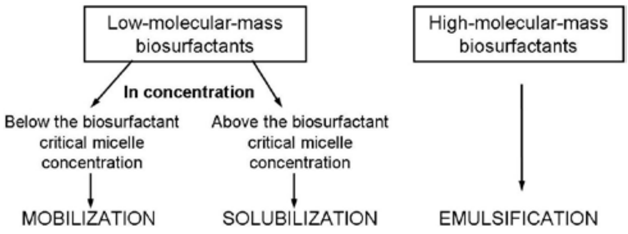

Figure 2. Mechanisms of hydrocarbon removal by biosurfactants depending on their molecular mass and concentration [11,12]

The mobilization mechanism occurs at concentrations below the biosurfactant critical micelle concentration (CMC). At such concentrations, biosurfactants reduce the surface and interfacial tension between air/water and soil/water systems. Due to the reduction of the interfacial force, contact of biosurfactants with soil/oil system increases the contact angle and reduces the capillary force holding oil and soil together. In turn, above the biosurfactant CMC the solubilization process takes place. At these concentrations biosurfactant molecules associate to form micelles, which dramatically increase the solubility of oil [12]. The hydrophobic ends of biosurfactant molecules connect together inside the micelle while the hydrophilic ends are exposed to the aqueous phase on the exterior. Consequently, the interior of a micelle creates an environment compatible for hydrophobic organic molecules. The process of incorporation of these molecules into a micelle is known as solubilization.

3. Petroleum Degrading Microorganisms

Nowadays it is known, that hydrocarbons in the environment can be decomposed mainly by bacteria, algae, yeast and fungi. Despite the fact that these organisms in terrestrial and aquatic ecosystems are ubiquitous, number of heterotrophic microorganisms that can utilize hydrocarbons is very variable: from 6% to 82% of soil fungi, from 0.13 to 50% of soil bacteria, from 0,003 to 100% of marine bacteria [13]. Some microorganisms can metabolize only a limited number of hydrocarbon substrates. A composition of different microorganisms having all possible enzymatic pathways can more efficiently decompose a complex mixture of hydrocarbons in soil, fresh and sea water than single microorganisms can. Thus complete mineralization of substrate can be achieved [14].

Bacteria that utilize gaseous hydrocarbons, particularly propane, are representatives of the genera *Corynebacterium, Mycobacterium, Nocardia, Rhodococcus*. Microorganisms that can use butane as the unique source of carbon and energy are *Arthrobacter, Brevibacterium, Pseudomonas spp.* [15].

Strains capable to utilize polycyclic aromatic hydrocarbons are *Beijerenckia sp., Pseudomonas spp. (P. paucimobilis, P. fluorescens, P. putida*), *Alcaligenes denitrificans WW1, Mycobacterium spp.* (e.g. *M. flavescens), Rhodococcus spp.* (e.g. *R. rhodnii*), *Athrobacter sp., Aeromonas sp.*, sea *Cyanobacteria, Streptomyces flavovirens, Synechococcus sp*. [16,17].

Fungi *Penicillium* and *Polisporum* can grow on nutrient agar medium with crude oil heavy fractions. *Penicillium and Mortierella* sp. can be used for conversion of crude oil high molecular fractions [18].

The most important bacteria in hydrocarbons biodegradation in soils and sea water are *Achromobacter, Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Flavobacterium, Nocardia, Pseudomonas spp.* and *Corynebacterium sp*. Among sea water yeasts and fungi that decompose hydrocarbons the most significant are *Aureobasidium, Candida, Rhodotorula, Sporobolomyces spp.*, species obtained from soils are *Trichoderma* and *Mortierella spp*. [19-24].

B. subtilis was proved to be a better hydrocarbon degrader than the other isolates [25]. In another study, strains were isolated from petroleum polluted soil and identified as *Pseudomonas pseudoalcaligenes, Bacillus firmus, Bacillus alvei, Penicillium funiculosum, Aspergillus sydowii and Rhizopus sp*., and they removed 79%, 80%, 68%, 86%, 81% and 67% of total petroleum hydrocarbon. Genus *Stenotrophomonas, Bacillus, Brevibacillus, Nocardiodes* and *Pseudomonas* were used in combination and give a degradation rate of 67% after only 12 days of treatment [26]. It has been proved that the mixed consortium effectiveness was significantly superior to that obtained by individual strains [15,27,28,29,30].

A number of listed microorganisms in addition to their oil-degrading properties also produce biosurfactants. Examples of biosurfactants and their producers are depicted in Table 1.

Table 1. Classification of biosurfactants and their use in remediation of hydrocarbon contaminated sites

Class of Biosurfact	Microorganism	Application	Ref.
Rhamnolipids	*Pseudomonas aeruginosa, Pseudomonas* sp.	Enhancement of the degradation and dispersion of different classes of hydrocarbons; emulsification of hydrocarbons and vegetable oils	[31,32,33]
Trehalolipids	*Mycobacterium tuberculosis, Rhodococcus erythropolis, Arthrobacter* sp., *Nocardia* sp., *Corynebacterium* sp.	Enhancement of the bioavailability of hydrocarbons	[34]
Sophorolipids	*Torulopsis bombicola, Torulopsis petrophilum, Torulopsis apicola*	Recovery of hydrocarbons from dregs and muds; enhancement of oil recovery	[32,35,36]
Trehalolipids	*Mycobacterium tuberculosis, Rhodococcus erythropolis, Arthrobacter* sp., *Nocardia* sp., *Corynebacterium* sp.	Enhancement of the bioavailability of hydrocarbons	[34]
Corynomycolic acid	*Corynebacterium lepus*	Enhancement of bitumen recovery	[37]
Surfactin	*Bacillus subtilis*	Enhancement of the biodegradation of hydrocarbons	[38]
Lichenysin	*Bacillus licheniformis*	Enhancement of oil recovery	[39]
Emulsan	*Acinetobacter calcoaceticus* RAG-1	Stabilization of the hydrocarbon-in-water emulsions	[40]
Alasan	*Acinetobacter radioresistens* KA-53		[41]
Liposan	*Candida lipolytica*	Stabilization of hydrocarbon-in-water emulsions	[42]
Mannoprotein	*Saccharomyces cerevisiae*		[43]

4. Biosurfactants Physiological Role

The physiological role of biosurfactants in microorganisms is to facilitate the contact of bacterial cells with hydrophobic substrates. Surfaces of most bacteria are hydrophilic, this allows them to effectively interact with the water-soluble compounds and ensure the normal operation of membrane-bound enzyme systems. However, it is difficult to contact with hydrophobic substrates such as petroleum products. Nevertheless, the group of microorganisms capable of assimilating oil hydrocarbons as a source of carbon and energy is very extensive. As the hydrocarbon-enzyme systems are localized in the cytoplasm of the bacteria, the ability of a strain to assimilate hydrocarbons depends primarily on the ability to absorb hydrophobic substrate. The absorption process may limit the oxidation process. Thus microbial hydrocarbons degradation may stop after the emulsifying activity loss [44], although it is possible at surfactant adding to the medium. The responsible for bioSAS synthesis genes expression activates bacterial uptake hydrocarbons [25].

There are three major pathways of water insoluble compounds uptake [45]:

1. lipophilic cell wall channels filled with hydrophobic substance of polysaccharide nature and have high affinity for hydrocarbons formation;
2. SAS, hydrocarbons emulsifying and solubilizing compounds synthesis;
3. hydrophobic cell wall on the lipophilic compounds basis formation provides direct contact with the hydrocarbon molecules.

The first way is characteristic to yeast and some *Arthrobacter* genus representatives. The second and third, based on the bioSAS production, are typical for the most oxidizing microorganisms.

Different hydrocarbons interaction pathways and the corresponding bioSAS types are characteristic to different taxonomic groups of organisms, reflecting the different environmental strategies and are important for their use in biotechnological applications. Intracellular bioSAS not appear to play a physiological role in the absorption of compounds from the environment as well as evolved only after bacteria death. The most widely studied biosurfactant type is extracellular bioSAS that synthesized by broad range of microorganisms: *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Arthrobacter*, *Serratia* genera bacteria [25,45], some strains of the *Rhodococcus*, *Gordonia* genera, *Candida* yeast [46]. Cell-associated biosurfactants are less common and characteristic mostly for actinobacteria representatives.

The key extracellular biosurfactants function is to modify the hydrophobic hydrocarbons and translating them into hydrophilic cell surface accepted form. This is achieved by hydrocarbons dispersion in the aquatic medium.

Rhodococcus sp. have high affinity to hydrocarbons by the cell wall hydrophobicity, its relatively high thickness, larger bacteria cell sizes, which together provide effective passive diffusion and hydrocarbons accumulation in the cell. However, they have low levels of endogenous respiration, slow switching to a new substrate. *Pseudomonas* sp. have high endogenous metabolism activity, easy switch to new carbon sources, rapidly growing [46]. However, having more hydrophilic cell cover and low affinity for hydrocarbons absorption, they are forced to synthesize bioemulsifiers. This fact leads to additional carbon and energy costs. *Pseudomonas* sp. is not able to accumulate an intracellular carbon pool in the form of lipids, this causes reduction in their metabolism rate when nutrients are exhausted.

5. Culture Conditions Effect on Hydrocarbon Degrading Bacteria BioSAS Production

Biosurfactants production depends on the following conditions:

1. carbon source nature in medium, its hydrophilicity degree;
2. nitrogen source nature, carbon/nitrogen ratio in medium;
3. culture growth phase.

Temperature, pH, medium aeration, cultivation method, metal cations and antibiotics presence in medium also have effect.

In the bioSAS production regulation three mechanisms are distinguished [25]: 1) induction - the hydrocarbons appearance in the medium causes increased glycolipids synthesis by *Rhodococcus, Pseudomonas, Torulopsis spp.*; 2) repression - change to the carbohydrates or organic acids inhibits the bioSAS synthesis by *Arthrobacter*, *Acinetobacter sp.*; 3) regulation of nitrogen and multivalent cations presence in medium.

In the vast majority of studies the biosurfactant production by submerged microorganisms cultivation in a liquid medium was investigated [1,46]. However, bacterial biosurfactants synthesis occurs also at the surface growth on solid media, both hydrocarbons supplied and limited [47]. This phenomenon is interesting especially because soil bacteria occur not primarily in suspension but attached to the particles surfaces.

Carbon source in medium, especially its hydrophilic or hydrophobic nature, has a crucial role for the surfactants synthesis. It can induce or repress the synthesis of biosurfactants. Some microorganisms' ability to reduce surface tension and emulsifying activity occur only during their growth on hydrocarbon substrates [1].

Nitrogen concentration in the medium, its nature and nitrogen/carbon ratio play an important role in the bioSAS production. Nitrogen limitation is a factor stimulating bacteria biosurfactants production.

Biosurfactant synthesis and activity also depend on temperature, pH and aeration of the medium, its salinity, the presence of antibiotics and metal ions [4]. Temperature stress causes an increased trehalose synthesis by *Rhodococcus sp.* Their cultivation on hydrocarbon media at the hydrophobic and/or aromatic antibiotics presence leads to an increase in cellular lipid content. Hydrophilic antibiotics do not promote additional lipids production.

One of the factors determining the surface active liquid cultures properties at different phases of growth is the bioSAS synthesis kinetics type. Depending on this type culture surfactant production activity maximum may occur beginning from the initial growth phase to the stationary phase or later cultural development stages.

6. Conclusions

The microbial biosurfactants synthesis and their application have an important practical significance for a range of petroleum pollutants bioremediation. It may be concluded that microbial degradation can be considered as a key component in the cleanup strategy for petroleum hydrocarbon remediation due to their biodegradability and low toxicity.

Although extensive investigations have been carried out regarding hydrocarbon biodegradation, these studies have been not exhausted. Nevertheless, the effectiveness of this technology has only rarely been convincingly demonstrated. In case of commercial bioremediation associated with biosurfactants application, the literature is lacking in supportive evidence of success. Much need still exist for the optimization of the process conditions for more efficient application of biological degradation of oil pollutants under different conditions. The commercial success of biosurfactants is also still limited by their high production cost.

Little is known about the potential of biosurfactant production by microorganisms *in situ*. Most of the described studies were done under laboratory conditions. Remediation systems with only one type of the contaminant have been studied to gain a basic understanding. More information is required concerning the structures of biosurfactants, their interaction with soil and contaminants and scale up and cost effective biosurfactant production [48].

Although the biosurfactants are thought to be ecofriendly, some experiments indicated that under certain circumstances they can be toxic to the environment [49]. Nevertheless, careful and controlled use of these surface active molecules will surely help in the enhanced clean up of the hydrocarbon environmental pollutants and provide us with a clean environment.

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