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MICROORGANISMS' SURFACE ACTIVE SUBSTANCES ROLE IN HYDROCARBONS BIODEGRADATION

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Abstract. *Existing data and publications regarding oil, hydrocarbon biodegradation, metabolism, and bioremediation were analyzed. Search of hydrocarbon degrading bacteria which are producers of biosurfactants was provided, types of microbial surfactants and their physiological role were analyzed and ordered. The study of factors affecting the surface active properties of producers' cultures was done.*

Keywords: biodegradation, biosurfactants, hydrocarbons, petroleum products.

Introduction

Petroleum products powerful negative impact 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.

The analysis of studies and published works

There were analyzed existing data and publications regarding oil, hydrocarbon degradation, metabolism, and bioremediation, with especial attention paid to works of last decade. Materials concerning types of microbial surfactants, their physiological role and major producing species were analyzed and ordered with response to further practical implementation.

Purpose of the research is search of hydrocarbon degrading bacteria which are producers of biosurfactants, the study of factors affecting the surface active properties of producers' cultures.

Types of microbial surfactants

Surfactants include compounds capable of surface and interfacial tension reducing. This is a large class of different chemical nature substances, 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, leading to surface active properties. 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:

– the lower toxicity, or lack thereof;

– high biodegradability by natural microorganisms;

– activity in a wide medium acidity/salinity range;

– possibility of their microbial synthesis from relatively cheap substrates, including polluting compounds.

According to the chemical nature biosurfactants are heterogeneous compounds, As a rule, the polar components are peptides or glycanes and non-polar components are various lipids. BioSAS refer to the following major groups [1].

A) glycolipids are 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 are 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 are fatty acids, which 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 are 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 [2] classify microbial surfactants into two groups according to the exhibited surface active properties type:

– liquid medium surface tension reducing (biosurfactants);

– emulsification ability (bioemulsifiesrs).

The low-molecular bioSAS, as a rule, are referred to the first group, and high-molecular ones – to the second. Usually, biosurfactants may possess emulsifying properties, whereas bioemulsifiers do not reduce the medium surface tension.

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, the most common part of which are petroleum products. Nevertheless, the group of microorganisms is 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 [3], although it is possible at surfactant adding to the medium. The responsible for bioSAS synthesis genes expression activates uptake of hydrocarbons by bacteria [4].

There are three major pathways of water insoluble compounds uptake [5]:

1) lipophilic cell wall channels filled with hydrophobic substance of polysaccharide nature and with high affinity for hydrocarbons formation;

2) SAS, hydrocarbons emulsifying and solubilizing compounds synthesis;

3) the hydrophobic cell wall on the lipophilic compounds basis formation provides direct contact with the hydrocarbon molecules.

The first way is a 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 do 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 [4; 6], some strains of the *Rhodococcus*, *Gordonia* genera, *Candida* yeast [5]. Cell-associated biosurfactants are less common and characteristic primarily 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 [5]. However, having more hydrophilic shell 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.

Culture conditions effect on hydrocarbon degrading bacteria bioSAS production

Biosurfactants production depends on the following conditions:

– carbon source nature in medium, its hydrophilicity degree;

– nitrogen source nature, carbon/nitrogen ratio in medium;

– 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 [7]:

1) induction is the hydrocarbons appearance in the medium causes increased glycolipids synthesis by *Rhodococcus, Pseudomonas, Torulopsis* spp.;

2) repression is 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 [6; 8]. However, bacteria biosurfactants synthesis occurs also at the surface growth on solid media, both hydrocarbons supplied and limited [7].

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 bioSAS. Some microorganisms' ability to reduce surface tension and emulsifying activity occur only during their growth on hydrocarbon substrates [2].

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 [8]. 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 the beginning from the initial growth phase to the stationary phase or later cultural development stages.

Conclusions

The microbial bioSAS synthesis from hydrocarbons has important practical significance for the 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.

Although extensive investigations have been carried out regarding the hydrocarbon biodegradation, these studies have been not exhausted. Nevertheless, the effectiveness of this technology has only rarely been convincingly demonstrated, and in the case of commercial bioremediation stressing on biosurfactants production, 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 oil pollutants biological degradation under different conditions.

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