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INFLUENCE OF MICROBIAL SURFACTANTS ON BIODEGRADATION OF OIL HYDROCARBONS

It was determined that microbial surfactants (biosurfactants) of various structure have stimulating influence on the biodegradation of oil hydrocarbons in model experiments in soil and water medium. In the process of biodegradation of aromatic hydrocarbon dodecylbenzene lipopeptide have shown the greatest activity, while for pristane, a hydrocarbon of isoparaffin group, rhamnolipid had the highest accelerating efficiency. Stimulating action on bioremediation of soils contaminated with diesel fuel was shown by all investigated biosurfactants.

1. INTRODUCTION

The contamination of the environment with hydrophobic organic substances is a relevant problem during many years. Microbiological methods, used for remediation of soil and water, are usually limited by low solubility of contaminants in water, which reduces their bioavailability to microorganisms. It is well known, that surfactants, products of chemical or microbial synthesis, can have an influence on biodegradation of organic hydrophobic contaminations [1].

Biosurfactants are surface-active compounds, which are produced by microorganisms. They can be found on the surface of microbial cells or can be secreted into extracellular space. Physical-chemical and biological properties stipulate their use in oil and pharmaceutical industry, agriculture, in development of environmentally friendly washing means and in remediation [4,11,15]. The ability of biosurfactant application in stimulation of degradation of oil products in soil and water has great importance [2–3].

Hydrocarbons belong to hardly-degradable substrates due to their lipophilic nature. Bioavailability of oil products is limited by their low solubility in water and tendency towards adsorption in soil.

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It especially concerns polychlorinated and polycyclic aromatic hydrocarbons [5,6,8].
The abilities of biosurfactants to decrease surface and interfacial tension as well as to form micelles [9] are the most important for the process of biodegradation. Theirsignificant characteristic is critical micelle concentration (CMC), above which the molecules of surfactant aggregate by 20–200 [2].
Surfactants are able to disperse non-aqueous phase liquids (NAPL) forming thermodynamically stable emulsions [2,10]. In the developed remediation technologies the efficiency of the biodegradation process is increased as a result of extension of interfacial surface of hydrocarbons via emulsification, which leads to much easier and faster access to particles of oil products to bacteria [7].
Among the surfactants which are used in the process of remediation biological substances (biosurfactants) are of greater priority since they are biodegradable, intoxic i.e. ecologically safe being highly effective. The known biosurfactants belong to anionogenic or nonionic substances. Their hydrophobic part is usually a residue of long-chain fatty acid. It is bound with hydrophilic part (by ether, glycoside or amide bond), which can be mono-, di- or polysaccharide, peptide, phosphate, sulfate, carboxyl etc. The classification of biosurfactants is based on their chemical structure [5].
The rich collection of microorganisms – biosurfactant producers is gathered in Laboratory of Biotechnology, Lviv Department of Physical-Organic Chemistry [11–14]. Among them - strains *Bacillus subtilis* C14, *Rhodococcus erythropolis* AP 25 [13], *Candida lipolytica* Y-917 [14] or *Cryptococcus albidus* Y-78, *Pseudomonas sp*. PS-17 etc.
The aim of this work was the assessment of the ability of the selected biosurfactants to stimulate the biodegradation of oil hydrocarbons.

2. MATERIALS

Hydrocarbons, which were used as a carbon source for microorganisms: *Pristane* (*2,6,10,1,4-tetramethylpentadecane* $C_{19}H_{40}$, M 268,5 (Sigma), *Dodecylbenzene (*$C_{18}H_{30}$, M 226,0, Merck), *Diesel fuel* – commercially available.

Microorganisms

The bacterial strains, isolated from water and soil environment and capable of degrading oil products were used in the biodegradation process:

1. Bacterial association BK, which consists of 4 strains - *Alcaligenes xylosoxidans, Brevibacterium brevis, Serratia marcescens* and *Stenotrophomonas malthophilia.*
2. Bacterial association EK, which consists of 4 strains - *Rhodococcus erythropolis, Rhodococcus maris, Pseudomonas stutzeri* and yeasts *Candida sp.*

Biosurfactants

Biosurfactants, which are used in this study: RL – rhamnolipids, biosurfactants of the strain *Pseudomonas sp*. PS-17; TL - trehalosolipids, biosurfactants of the strain *Rhodococcus erythropolis* 140; PL – lipopeptides, biosurfactants of the strain *Bacillus subtilis*; PD – polysaccharides, biosurfactants of the strain *Bacillus polymixa*.

3. METHODS

Isolation of biosurfactants

The microorganisms where cultivated on the optimized nutrient medium; glycerol, plant oils or n-alkanes (20 g/l) were used as a carbon source [12]. Surface and interfacial tension was determined using method with platinum Wilhelmi plate, the content of surface-active lipids was determined after extraction from cultural broth: rhamnolipids – using orcinol method, trehalosolipids – using antron method, peptidolipids – using method with methylene blue.

Rhamnolipids were isolated from supernatant of cultural broth via acid precipitation (10% HCl to pH=3) and following extraction of the obtained precipitate with Folch mixture (chloroform:isopropanol – 2:1). Trehalosolipids and peptidolipids were isolated with the same extragent from cultural broth. Polysaccharides were isolated from cultural broth via precipitation with 2 volumes of ethanol. The solvent was evaporated under vacuum. The lipids were analyzed via thin layer chromatography.

Determination of influence of biosurfactants on biodegradation of model hydrocarbons

The efficiency of the process of biodegradation of model hydrocarbons, which was carried out with and without addition of biosurfactants, was analyzed. The process was carried out in 100 ml Erlenmayer flasks, which contained 30 ml of mineral nutrition medium with dodecylbenzene (1% weight) or pristane (0,6% weight). Biosurfactants were added in concentration 100 mg/l. 0,2 ml of association BK or association EK in form of suspension in phosphate buffer (OD_{600} =1,0) were added to each sample as inoculum. The mineral medium had the following content: KH_2PO_4 - 1,56 g, Na_2HPO_4 - 2,13 g, $(NH_4)_2SO_4$ - 0,5 g, $MgSO_4 \times 7H_2O$ - 0,2 g, $CaCL_2 \times 2H_2O$ - 0,02 g, distilled water - 1000 ml, microelements. The cultivation was carried out during 14 days at 20°C, 140 rpm. After the end of cultivation the residual hydrocarbons were extracted from the cultural broth using dichloromethane and the degree of reduction of their concentration in the process of biodegradation was determined.

The method of investigation of the remediation on soils

The investigation of the influence of biosurfactants on biodegradation of diesel fuel in soil was conducted in model experiments in vessels. 500 g of soil artificially contaminated with diesel fuel in concentration 16 g/kg of dry mass of soil were placed into each vessel. The content of biogenic soils was maintained at the level C:N:P=100:10:1. The absolute humidity of soil was maintained at the level of 60%. All soil samples were inoculated with the suspension on basis of the association BK before agitation. The number of bacteria in soil after the inoculation amounted to 123×10^5 ml^{-1}. 4 preparations of biosurfactants were used in the investigations: RL – rhamnolipids, TL - trehalosolipids, PL – lipopeptides, PD – polysaccharides. The residual content of diesel fuel in soil after biodegradation was determined using gravimetric method (extraction with dichloromethane), as well as chromatography method. The samples were incubated during 14 and 28 days.

Analysis of hydrocarbons

The identification of diesel oil hydrocarbons was carried out using method GC-MS. The following chromatograms were recorded: TIC and selected fragmentation ions (85 – n-alkanes; 183 - isoprenoids; 82 - alkylocyclohexanes; 91 - alkylbenzenes) and by molecular ions (128 – naphthalene; 142 – methylnaphthalenes).The hydrocarbon type content of the diesel oil was ascertained via liquid chromatography (HPLC).

4. RESULTS

Analysis of the influence of biosurfactants on biodegradation of dodecylbenzene

The influence of 3 biosurfactants on the process of biodegradation of dodecylbenzene with application of 2 associations of microorganisms-destructors was studied. It was ascertained that the addition of biosurfactants to nutrition medium increased the intensity of decomposition of the hydrocarbon when applying both associations (Fig. 1). Biosurfactant RL appeared to be the least effective; its addition practically didn't affect the biodegradation of dodecylbenzene by association BK and slightly affected the biodegradation of dodecylbenzene by association EK.
Biosurfactants Pl and TL increased the effectiveness of degradation on 10% when using association EK and on few percents - when using association BK.

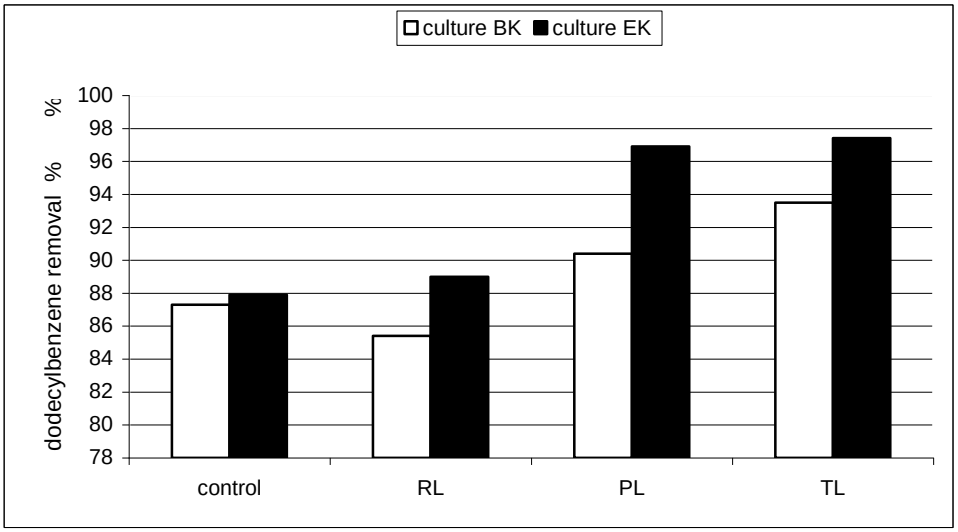

Fig. 1. Reduction of concentration of dodecylbenzene in the process of biodegradation under the influence of biosurfactants

Analysis of the influence of biosurfactants on biodegradation of pristane

The influence of 2 biosurfactants RL and TL on the biodegradation of pristane was investigated. It was determined after the cultivation, that reduction of quantity of substrate in cultural broth made 20,4% (RL) and 15,1% (PL) compared to control. Thus, in case of pristane higher efficiency was determined with rhamnolipid, while for dodecylbenzene, mononuclear aromatic hydrocarbon with long aliphatic side chain, lipopeptide was more effective.

Influence of biosurfactants on biodegradation of diesel fuel in soil

The qualitative analysis have shown, that diesel fuel was a mixture of substances, uppermost hydrocarbons: saturated (paraffinic, naphthenic) - 68,88% weight, aromatic - 30,55% weight (monoaromatic - 22,86%, diaromatic - 6,53%, triaromatic - 1,16%). Polar substances made 0,57% of fuel mass. Hydrocarbons C_8-C_{26}, uppermost n-alkanes as well as isoprenoids (norpristane, pristane, phytane) and naphthenes – alkil-derivatives of cyclohexane (with aliphatic side chain C_2-C_{15}) were found in the diesel

oil. Among the aromatic hydrocarbons were the alkil-derivatives of benzene as well as naphthalene and methylnaphthalenes. Significant changes in the hydrocarbon type content of the samples were observed after the biodegradation process if compared with the input fuel. The content of saturated hydrocarbons, especially aromatic, decreased, while the content of polar substances increased.

The experiments have shown that the speed of destruction was high in all variants and amounted above 50% after 14 days of the incubation (Fig. 2).

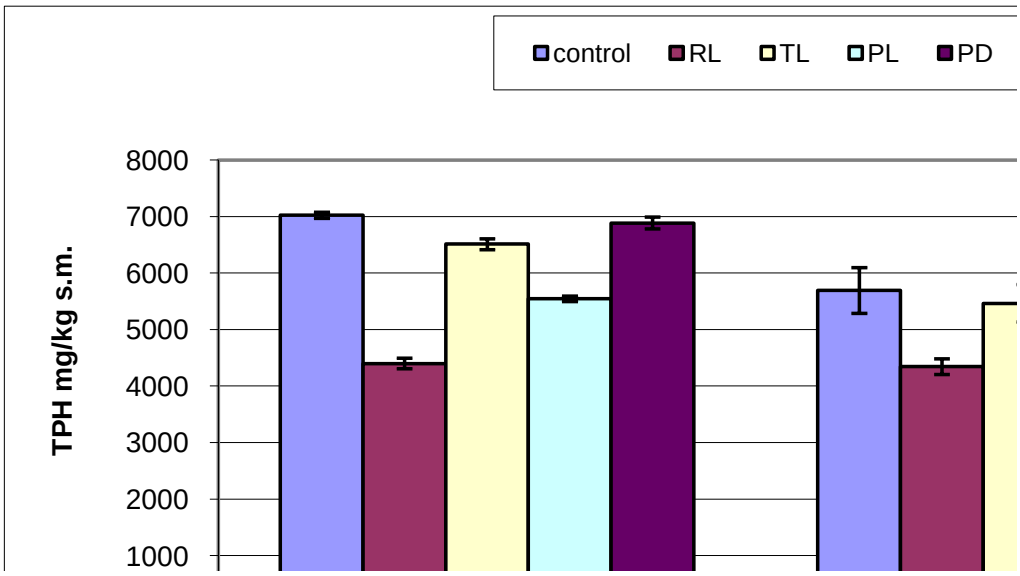

Fig. 2. The content of the residual hydrocarbons in soil after 14 and 28 days of biodegradation

The stimulation of biodegradation was observed in all variants, to which biosurfactants RL and PL were added. The reduction of hydrocarbons content in vessels with addition of these surfactants made 38% and 21% respectively. In the rest variants the stimulation made only several percents. After 28 days of incubation the stimulation of biodegradation was observed when applying all biosurfactants; however for TL and PD it was lower and made 4% and 11% respectively.

The analysis of enzymatic activity of soil in certain vessels indicates the faster behavior of the enzymatic processes in variants with addition of biosurfactants RL and PL.

5. CONCLUSIONS

1. The stimulating influence of biosurfactants on the degradation of oil products of various compositions was ascertained.
2. In the process of biodegradation of pristane, a hydrocarbon of isoparaffin group, rhamnolipid had the highest accelerating efficiency, while for dodecylbenzene, aromatic hydrocarbon with aliphatic chain; the greatest activity was established for lipopeptide.

3. Stimulating action on bioremediation of soils contaminated with diesel oil was shown for all investigated biosurfactants. RL and PL had the greatest efficiency and caused the reduction of hydrocarbon concentration in soil on 38% and 21% respectively.
4. The obtained results create the perspective of wide range of application of biosurfactants in modern bioremediation technologies.

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