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THEORETICAL SUBSTANTIATION OF THE PETROLEUM HYDROCARBONS DESTRUCTION BY SPECIFIC MICROFLORA USING ANAEROBIC DIGESTATE

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Abstract. Implementation of the proposed approach to bioremediation of oil-contaminated soils provides an intensification of the hydrocarbons degradation process biologically using a digestate as a bio-stimulant, contributes to the production of an ecologically safe substrate, and excludes its toxicity to living organisms due to the degradation of petroleum hydrocarbons.

Key words: biodegradation, biodestructors, digestate, oil-destructive bacteria, oil contaminated soil.

1. Introduction

Soil pollution with oil and oil products provokes a number of changes in soil properties, which leads to a decrease in fertility and quality in general. Decontamination of hydrocarbons is efficiently and environmentally safe to carry out by a biological method, however, depending on the level of pollutants, biodegradation is relatively delayed in time, which is associated with the gradual development of a specific aboriginal microflora, which has oil-oxidizing properties. The peculiarities of the development of specific bacteria and fungi when oil enters the soil have been sufficiently studied, so Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Planctomycetes and Proteobacteria were the dominant phyla among all the contaminated soil samples [1], phylum Proteobacteria, including genera *Acinetobacter*, *Marinobacter*, *Pseudomonas*, *Bacilli* and *Clostridia* were mostly common in desert oil polluted soils [2], *Gammaproteobacteria* including *Acinetobacter radioresistens*, *Acinetobacter calcoaceticus* (grew on fluorene and phenanthrene), *Pseudomonas stutzeri* and *Pseudomonas chloritidismutans* (grew on fluorene and naphthalene) had additionally a copper or cadmium tolerance [3]. A lot of bacteria strains are identified in oil-polluted soils, nevertheless not all of them obtain a

high capacity to degrade these chemicals. Al-Awadhi et al. claimed in their research [4] that different bacteria respond to certain hydrocarbons influence as follows: good growth of *Bacillus infantis* on all the individual aliphatic and aromatic hydrocarbons, good growth on the n-alkanes C9–C21 and fair growth on the n-alkanes C22–C40 and the three aromatic hydrocarbons of *Dietzia maris*, *Kocuria flavus*, *Microbacterium marinilacus*, *Nocardia pneumoniae* and *Pseudomonas pachastrella*, good growth on the medium chain n-alkanes C15–C20, but only fair growth on the alkanes C9–C14 and C21–C40 as well as the three aromatics of *Echinicola vietnamensis*, *Alcanivorax jadensis*, *Mycobacterium wolinskyi* and *Stappia kahanamokuae*. *Bacillus flexus* and *Ochrobactrum anthropi* have a high ability to a phenanthrene and anthracene degradation [5].

The isolated bacterial species of 8 genera: *Achromobacter*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Lysinibacillus*, *Ochrobactrum*, *Proteus*, and *Pusillimonas* were reported to participate in polycyclic aromatic hydrocarbon (PAH), at that amongst the PAH detected in the polluted samples before remediation, dibenz[g,h,i]anthracene, ideno[1,2,3-c,d]perylene, and benzo[g,h,i]perylene were not detected during, and after the remediation [6]. *Rhodococcus* sp. A has been reported to degrade 65.27±5.63 % of the crude oil in 9 days, and almost all components including pristane, phytane, and other long-chain substrates were largely decomposed [7].

Microorganisms could use oil hydrocarbons as a carbon source in the metabolic energy exchange and associated ability to produce specific substances biosurfactants allowing organic xenobiotics to penetrate through the cell wall into the cell after emulsification, the most common of them are monorhamnolipid (MRL) and dirhamnolipid (DML) often produced by *Pseudomonas aeruginosa* [8, 9] and their proportion

such as the ratio of MRL and DRL can change emulsification index and antimicrobial property to Gram-negative bacteria, the more MRL the higher emulsification potential [10].

The most effective result can be achieved when using a consortium of microorganisms specializing in the degradation of organic substances of a given composition and a certain chemical nature. A mixed culture of microalgae *Synechocystis* sp. and indigenous bacteria *Pseudomonas* *indoxyladons*, and *Bacillus* *benzoevorans* were successfully applied in the remediation of different concentrations of high molecular weight four ring Polynuclear Aromatic Hydrocarbon Pyrene [11]. A consortium of two *Bacillus* strains, namely *Bacillus pumilus* KS2 and *Bacillus cereus* R2 has been reported to have the highest hydrocarbon degradation rate (68.12 % of total petroleum hydrocarbon – TPH) achieved at the third week of incubation related to the produced biosurfactants rhamnolipid [12], consortium of *Bacillus cereus* and *Pseudomonas putida* has shown a biodegradation percentage of 80–90 % after 49 days [13], moreover, individual strain *Bacillus cereus* has been reported to be a good kerosene degrader related to the production of the specific bio-surfactant with appropriate surface hydrophobicity trait (60.67 ± 1.53 %) and foaming percentage of 31.33 ± 0.58 % [14], nevertheless *Pseudomonas putida* has shown higher efficiency in the germinating and growth ability compared with *Bacillus cereus* due to the effect of crude oil on the growth by pot culture experiments [15].

Synergistic effect was reported between bacteria *Mycobacterium hyalinum* and Filamentous Fungi *Cladosporium* resulted in almost complete degradation of diesel oil, achieving a total diesel removal of 99 % over 5 days of treatment [16]. Soil is a complex system that comprises an organomineral complex, liquid and gas phases, has a buffer capacity, and acts as a sorbent, destroyer and neutralizer of most pollutants. At the same time, the processes of self-healing and self-purification in the soil are very slow, which requires the use of additional funds. This task is partially solved with the help of bio-augmentation, bio-stimulation and the use of biological products in implementing the In-situ method (without soil removal), which contributes to the intensification of the metabolism of the native microflora [17].

The question of studying the mechanisms and ways of biotransformation of petroleum products at the cellular level remains insufficiently studied, which would allow to use an appropriate bio-stimulant and create an effective bacteria consortium which became the subject of present study.

2. Theoretical part

2.1 Biochemical processes of petroleum hydrocarbon metabolism

When oil enters the soil, an uneven dynamics of enzymatic activity is noted: an increase in the number of specific enzymes (catalase, peroxidase, polyphenol oxidase) and carbon dioxide emission for 3 days, provided the dose of oil is not more than 5 %, initial inhibition of enzymes at an oil concentration exceeding 5 %. For ordinary chernozem, self-purification is possible, as evidenced by an increase in these parameters 3 months after pollution, regardless of the dose of oil, and an intensification of the humification process, the nature of which has not been studied [18]. The enzymatic activity of the soil depends on the oil content in the soil. Research [19] has shown that the concentration of oil up to 10 % has a positive effect on catalase, dehydrogenase and urease activity; up to 17 % – for phosphatase and lipolytic activity. The oil content in the soil above the shown values inhibits the activity of all types of enzymes. On the other hand, it was found that in petroleum-contaminated soil the activity of urease, protease, invertase, and dehydrogenase significantly decreased in microflora, whereas polyphenol oxidase activity significantly increased, which is explained by the intensification of oil destructive processes and evident toxicity for microorganisms [20]. Spent engine oil caused a slight change in soil pH relative to the control and a significant decrease in catalase activity in contrast to a significant increase observed in dehydrogenase activity [21]. Urease activity plays a very important role in the mineralisation of nitrogen compounds. Monitoring of the soil urease activity is considered to be a good indicator of the mineralisation potential of organic nitrogen compounds in the soil.

Bioremediation involves indigenous strains of oil-destructive bacteria, lower fungi and unicellular algae. Hydrocarbon-oxidizing microorganisms are heterotrophic aerobes, which use petroleum hydrocarbons as a source of carbon and energy [22]. To absorb the hydrophobic substrate and the decomposition of organic substances, these organisms produce a complex of special enzymes. In connection with the hydrophobic nature of substances transported to the cytoplasm of cells through the cytoplasmic membrane through passive or active transport, enzymes of the oxygenase group are synthesized. With hydrophilic compounds, the oxidation processes are carried out with the help of dehydrogenases (Fig. 1). According to the authors' results, the dehydrogenase produced by *Bacillus* sp. strain X6 showed the highest biodegradation rate at the level of 50 % in comparison with other enzymes such as lipase and catechol 2,3-dioxygenase [23].

The use of bio-surfactants is of special interest in the biotechnological process of crude oil destruction. Oil-oxidizing strains of microorganisms produce bio-surfactants necessary for dispersing petroleum products and increasing the selective permeability of the membrane, which facilitates the flow of hydrocarbons into the cytoplasm from the external environment. Extracellular lipopolysaccharide biosurfactant produced by *Acinetobacter calcoaceticus*, *Acinetobacter radioresistens* KA53 are a high-molecular-weight bioemulsifier (1000 kDa and 1MDa) [24]. The processes of bioremediation are enhanced due to the application of

bio-surfactants by means of emulsification (improved by high molar mass), solubilization and mobilization (promoted low-molar mass) [25]. A number of bacteria and yeast yielded a vast amount of phospholipids and fatty acids surfactants when growing on n-alkanes through microbial oxidations.

The basic principle of involvement of bio-surfactant (rhamnolipid) produced by *Pseudomonas* sp in the uptake of hydrocarbons and main interactions between bacteria cells and bio-surfactant molecules are shown in Fig. 2 [26].

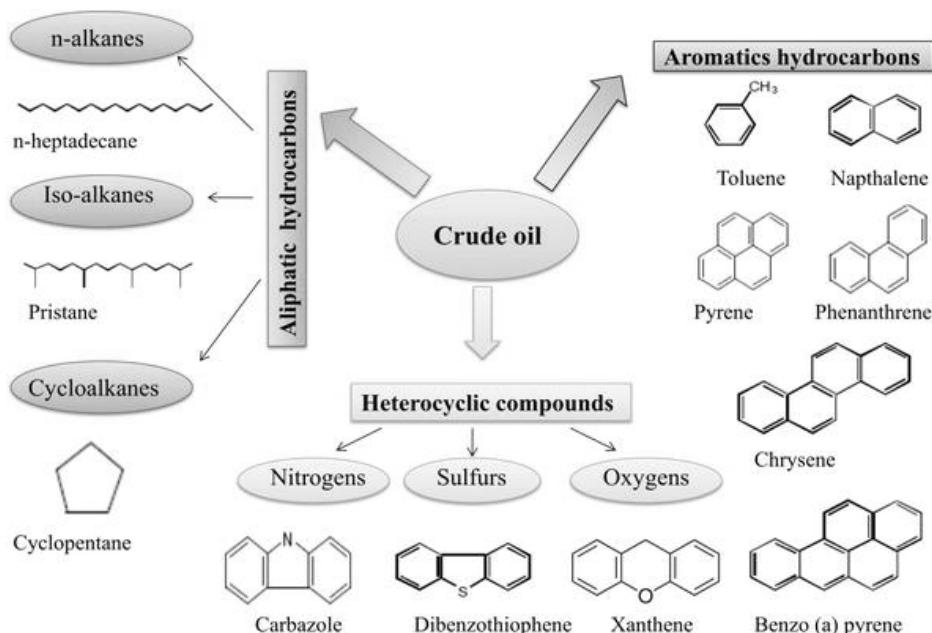


Fig. 1. Different classes of hydrocarbon

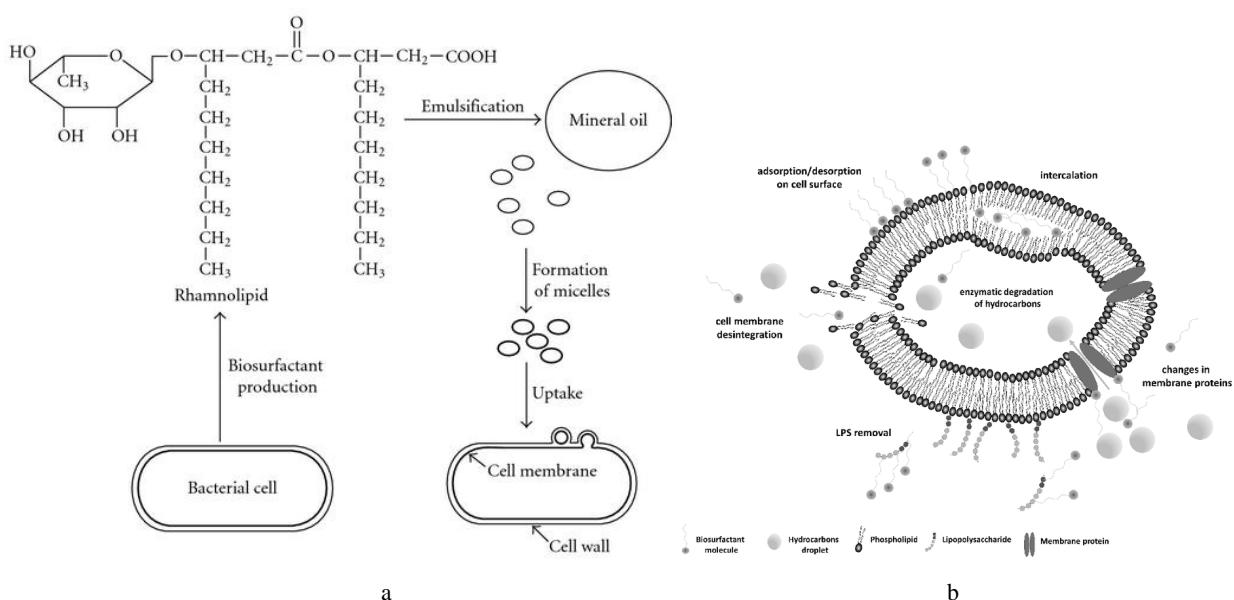


Fig. 2. Involvement of biosurfactant (rhamnolipid) produced by *Pseudomonas* sp in the uptake of hydrocarbons (a) and main interactions between bacteria cells and biosurfactants molecules (b)

2.2 The impact of biostimulation on the process of hydrocarbons destruction

To intensify the process of biodegradation of substances and eliminate oil pollution of soils, it is proposed to use bio-augmentation and bio-stimulation, since the supply of soil with nutrients is an important factor that determines the intensity of decomposition of oil and oil products, contributes to an increase in the biological activity of the soil in terms of respiration rate and the number of microorganisms.

Oil causes a change in the fractional composition of humus, expressed in a decrease in the concentration of humic acids and an increase in the proportion of non-hydrolyzing residue. The formation of the potential certain system, which has a high redox capacity, provides oxidizing conditions conducive to the activity of specific bacteria and the degradation of oil in an aerobic environment.

Soleimani et al. in their research [27] stated that bacterial enrichment and supplement of nutrients must be the most efficient treatment in removal of soil TPH (50–62 %) compared to other treatment (addition of surfactant, hydrogen peroxide, molasses and planting). In particular, TPH concentrations decreased significantly ($p < 0.05$) in bacterial enrichment and with the addition of nutrients and treatment with microbial enrichment and nutrient addition (nitrogen and phosphorous were added to the soils to reach the: C:N:P = 100:10:1).

The high reduction of crude oil (94.54 %) during bioremediation was observed in bio-augmented soil treated with palm bunch ash with microbial counts of 9.90×10^3 to 2.5×10^8 cfu/g, while low reduction potential for contaminated soil without surfactant treatment was proved [28]. Combination of bio-stimulation and bio-augmentation approaches has good practical application and high results of respiration activities and oil mineralization. Moreover, hydrocarbon degradation rate depends on different abiotic parameters such as temperature, salinity, pH, aeration and moisture, nutrient content. Moistening and aeration have been reported to be the main factors influencing microbial biomass, while implementation of biochar and introduction of microbes were the main factors influencing microbial respiration in the case of *Pseudomonas aeruginosa* or *Acinetobacter radioresistens* immobilized on biochar [29]. Fluctuations in temperature and salinity of desert soils directly influence the activity and diversity of microorganisms even with bio-stimulation, performed by adding NH₄Cl and NaH₂PO₄ as N and P sources. Oil destruction efficiency has a positive correlation with temperature: the highest oil mineralization rate was observed at

50 °C, and at 30–50 °C the microbial community in the oil-polluted soils became more diverse due to the detection of new bacterial groups. Instead of this, oil mineralization is decreasing with salinity increasing to 7 % [30]. Bio-stimulation treatment (C:N:P ratio was adjusted to 100:15:3) and combination of bio-augmentation and bio-stimulation treatment (106 CFU/g soil of the consortium with nutrient addition) had a significant positive influence on the level of soil dehydrogenase activity, which increased markedly when the initial crude oil load was increased from 10 to 30 g/kg, nevertheless salinity acted as a stable inhibitor of enzymatic activity, and hence the hydrocarbons destruction by *Pseudomonas aeruginosa* consortium and the process of bioremediation [31]. Organic fertilizers and/or agricultural waste are becoming widespread in addition to inorganic nutrient sources. High efficiency was confirmed by chicken and pig manure as a source of nitrogen and phosphorus, as well as other substances necessary for the intensification of oil-destructive microorganism's metabolism. For instance, application of rice husk and chicken manure with rice husk in a ratio of 3:1 as bio-stimulant showed high results in hydrocarbons degradation [32]. The amendment of single poultry manure (poultry droppings) in the mass concentration of 5–25 % was successfully used for remediation of the crude-oil contaminated soils. Concentrations of TPH and PAH generally decreased with increasing rate of poultry manure application related to the manure capacity to change pH of contaminated soil, so rate 25 g of poultry droppings per 100 g of soil is optimal for pH value increasing to the neutral range (6.3–6.4). Lower and higher concentrations of manure are not effective for the bioremediation process due to the high or low acidity respectively [33].

2.3 Bio-information databases application

The question of studying the mechanisms and ways of biotransformation of petroleum products at the cellular level remains an insufficiently studied one, which would allow us to create the necessary strains of bacteria by genetic engineering methods. Efficiency of bioremediation is mainly determined by such abiotic factors as temperature, nutrients, chemical composition of petroleum hydrocarbons, solubility, bioavailability, physical and chemical properties of the soil, oxygen, soil moisture, acidity and alkalinity.

The paper [34] indicates that large metabolic pathway databases useful for genome analysis and metabolic engineering have been developed by MetaCyc and KEGG. Comparison of this two databases has

identified differences in chemical compounds, reactions and pathways sets, for example KEGG contains pathways for xenobiotic degradation not found in MetaCyc. The research was subject to metabolic pathways of aromatic hydrocarbons degradation by specific bacteria, with the determination of the significant enzymes responsible for the effectiveness of this process. It was used a specific research database KEGG (Kyoto Encyclopedia of Genes and Genomes) to achieve this goal. KEGG (<http://www.kegg.jp/> or <http://www.genome.jp/kegg/>) is a database resource for understanding high-level functions and utilities of the biological system from genomic and molecular-level information. It is a computer

representation of the biological system, consisting of molecular building blocks of genes and proteins (genomic information) and chemical substances (chemical information) that are integrated with the knowledge on molecular wiring diagrams of interaction, reaction and relation networks (systems information) [35]. KEGG allows to create microbiological consortium of certain strains of bacteria to improve hydrocarbon degradation process performance and increase biochemical reaction speed. Consortium participation of *Acinetobacter baumannii* 1B, *Pseudomonas putida* F701 and *Basillus sp.* X6 in degradation of aromatic compounds is submitted as a flowchart (Fig. 3).

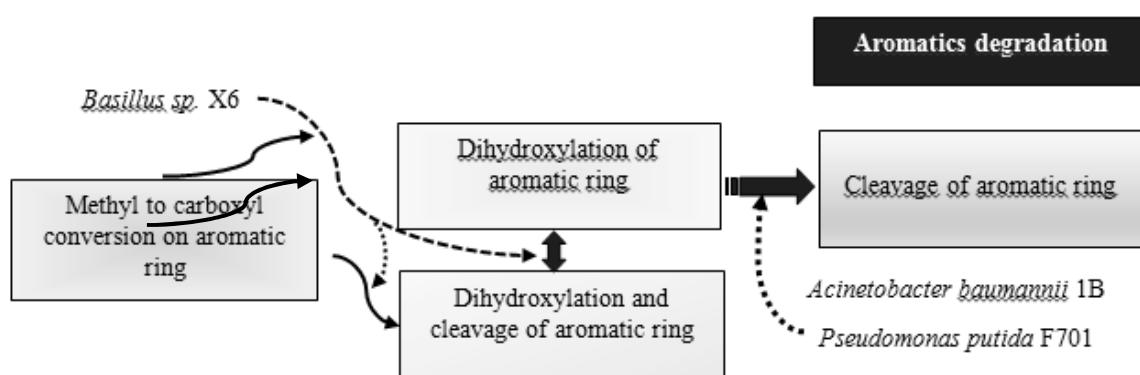


Fig. 3. Flowchart of degradation of aromatic compounds by bacteria consortium

Thus, different ratios of species and genera in an association or consortium are possible as a complex of specific ecological-trophic groups of microorganisms. The most rational implementation of the metabolic pathway in the association of microorganisms allows to improve the purification efficiency of oil-contaminated components of the environment.

3. Results and Discussion

3.1. The results of the digestate application as biostimulant

Numerous research results testify different biostimulation efficiency and biodegradation rate constant in the case of organic and inorganic fertilizers addition. Studied treatment can be placed in sequence in order of decreasing efficiency in the hydrocarbons biodegradation as follows: a combination (inorganic fertilizer and cow dung) > inorganic fertilizer used singly > cow dung used singly > combination (cow dung and palm kernel husk ash) > and finally palm kernel husk ash used singly at 2, 4 % crude oil contamination, and inorganic fertilizer preceding before

a combination (inorganic fertilizer and cow dung) at 6 % crude oil contamination [36]. Moreover, another research was indicated the greatest reduction of TPH (96.6–97.3 % vs 80.4–95.9 %) in the case of poultry waste strains using and for soil amended with poultry waste [37].

One of the challenging aspects of the biogas production, is the destiny of the leftovers after digestion in anaerobic reactors, also known as digestate, that is commonly used as biofertilizer. Digestate is largely stabilised after sufficient anaerobic digestion and can be used as high-quality fertilizer. With the appropriate application rate, the nutrients contained in the feedstock, such as nitrogen, phosphorus, potassium, sulphur etc. and other micronutrients will cover the nutrient demand for plant growth and obviously for enzyme activity of hydrocarbon oxidative bacteria. The addition of nitrogen with digestate to the soil initiates many processes, such as mineralization, immobilization, nitrification and denitrification, as well as leaching and evaporation, which depend not only on the intrinsic properties of the digestate, such as the content and form of nitrogen introduced, but also on soil properties and weather conditions, mainly temperature, precipitation and application technology. The rate of mineralization and

nitrification depends mainly on the content of organic matter in the soil and to some extent on its textural properties. The higher N content of digestate compared to compost is a consequence of the N concentration effect, since the hydrocarbon sources are decomposed to CO₂ and CH₄, and N is retained during anaerobic fermentation.

Single research [38] indicated high efficiency of digestate as a potential nutrient and microbial seeding for bioremediation of petroleum hydrocarbon contaminated soils. Addition of digestate together with saw dust decreased aggregates size and improved soil respiration. Addition of digestate has significantly increased initial level of alkB genes in the treated soils that was remained high until the end of the treatment. Digestate together with bulking agent indicated the highest TPH removal or digestate together with immobilized bacteria. This study proved great potential of digestate as a nutrient and bacteria source for soil bioremediation.

In addition, digestate as a fermented mass contains anaerobic bacteria, which contribute to the mineralization of organic substances and their conversion into a mineral form available to plants, and due to the addition of phosphogypsum and the development of the necessary association of

microorganisms, heavy metals are immobilized and has a confirmed practical agroecological value in the case of application in as a fertilizer. The combination of anaerobic microorganisms with aerobic microorganisms in consortia will create conditions for a more efficient course of humification processes, therefore, along with a biostimulant, compost animal and poultry waste and straw are introduced with a recommended application rate of 5–8 %, which act as sorbents and disintegrants.

3.2. Assessment of the bacteria consortium capacity in hydrocarbons degradation

Cyclic and aromatic hydrocarbons are very difficult to bacterial oxidation, but strains of microorganisms that include these substances in metabolic processes are known today. The developed databases such as KEGG allow the construction of metabolic pathways for various organic substances, including those for oil and oil products, indicating the types of bacteria and enzymes involved. Fig. 4 shows a scheme for the conversion of benzoic acid as a result of bacterial biodegradation with the strains *Acinetobacter baumannii* 1B, *Pseudomonas putida* F701 and others (http://www.kegg.jp/dbget-bin/www_bget?ko:K05549).

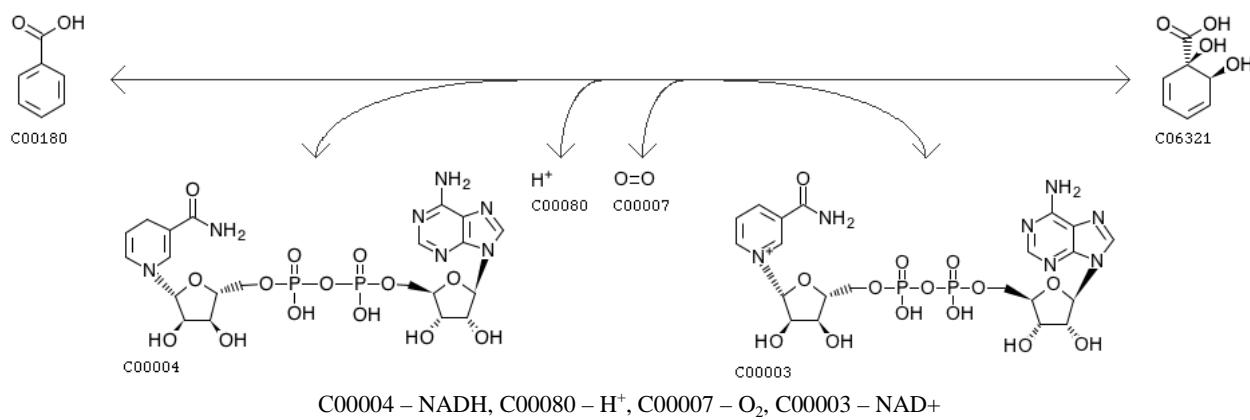


Fig. 4. Scheme for converting benzoic acid (C00180) to 1,6-dihydroxycyclohexa-2,4-diene-1-carboxylate (CO6321) C00004 – NADH, C00080 – H⁺, C00007 – O₂, C00003 – NAD⁺

The analysis of metabolic charts of aromatic hydrocarbons transformation indicates a variety of their degradation ways and connection with other chains, which causes the involvement of several enzymatic systems in the reaction of one substance transformation (Fig. 5 and Table 1). This information was obtained from the KEGG database that allows to select the necessary strains of microorganisms to create a consortium, the effectiveness of which is determined by

the production of enzymes that act not only on the initial substances, but also on the intermediate ones, i.e. oxidation products of petroleum hydrocarbons.

For example, in the oxidation of benzoic acid, enzymes are involved: 1,2-dioxygenase benzoate; benzoate of hydroxylase; benzoic hydroxylase; oxidoreductase of oxygen, which belong to the class of oxidoreductases acting on paired donors with the inclusion or reduction of molecular oxygen. Such

reactions are referred to as NADH or NADPH-dependent. After carrying out reclamation measures to improve the quality and physicochemical properties of oil-contaminated soil, favorable conditions are created (soil structure, nutrient content, pH value, redox potential) to maintain the vital activity of oil-destructive bacteria. The use of special biological products based on a scientifically based consortium of microorganisms *Pseudoxanthomonas spadix* BD-a59,

Rhodococcus jostii RHA1, *Rhodococcus aetherivorans* IcdP1, *Pseudomonas putida* ND6, *Pseudomonas stutzeri* 19SMN4, *Pseudomonas fluorescens* UK4, *Acinetobacter lactucae* OTEC-02, *Bacillus cereus* F837/76.7.9 provides activation of vital processes of aboriginal microflora, in particular increase of speed of a metabolism.

All of mentioned above strains have a capacity to produce specific enzymes involved in aromatic hydrocarbons degradation (Table 2).

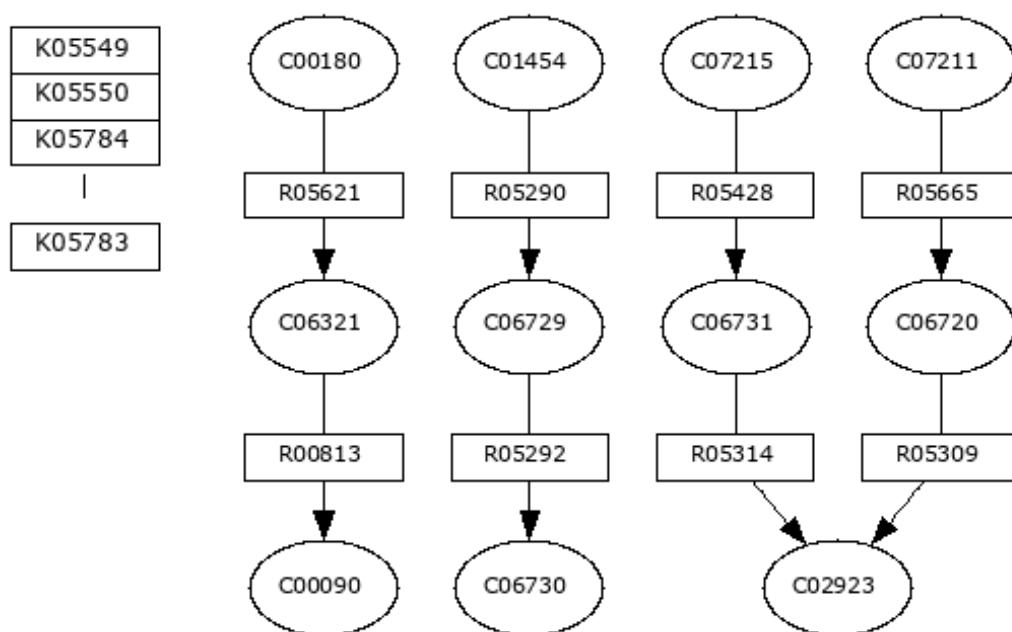


Fig. 5. Dihydroxylation of aromatic ring, type 1a (dioxygenase and decarboxylating dehydrogenase reactions)

Description of enzyme and compound name of Fig. 5

Table 1

KO	Name
<u>K05549</u>	benA-xylX; benzoate/toluate 1,2-dioxygenase subunit alpha [EC: <u>1.14.12.10</u> <u>1.14.12.-</u>]
<u>K05550</u>	benB-xylY; benzoate/toluate 1,2-dioxygenase subunit beta [EC: <u>1.14.12.10</u> <u>1.14.12.-</u>]
<u>K05784</u>	benC-xylZ; benzoate/toluate 1,2-dioxygenase reductase component [EC: <u>1.18.1.-</u>]
<u>K05783</u>	benD-xylL; dihydroxycyclohexadiene carboxylate dehydrogenase [EC: <u>1.3.1.25</u> <u>1.3.1.-</u>]
Compound	Name
<u>C00180</u>	Benzoate
<u>C06321</u>	(1R,6S)-1,6-Dihydroxycyclohexa-2,4-diene-1-carboxylate
<u>C00090</u>	Catechol
<u>C01454</u>	Toluate
<u>C06729</u>	cis-1,2-Dihydroxy-4-methylcyclohexa-3,5-diene-1-carboxylate
<u>C06730</u>	4-Methylcatechol
<u>C07215</u>	o-Toluate
<u>C06731</u>	1,2-Dihydroxy-6-methylcyclohexa-3,5-dienecarboxylate
<u>C02923</u>	2,3-Dihydroxytoluene
<u>C07211</u>	m-Methylbenzoate
<u>C06720</u>	1,6-Dihydroxy-5-methylcyclohexa-2,4-dienecarboxylate

Table 2

**Modules, enzymes and bacteria strains
of polycyclic aromatic hydrocarbon degradation**

Bacteria strains	Enzyme		Degradation	
	Class	Name	Type	Pathway
<i>Rhodococcus jostii</i> RHA1	Lyases	4-hydroxy-2-oxovalerate aldolase	Catechol meta-cleavage	catechol => acetyl-CoA / 4-methylcatechol => propanoyl-CoA
<i>Bacillus cereus</i> F837/76	Isomerases	4-oxalocrotonate <u>tautomerase</u>		
<i>Pseudomonas putida</i> ND6	Hydrolases	2-hydroxymuconate-semialdehyde hydrolase		
	Oxido-reductases	naphthalene 1,2-dioxygenase, naphthalene cis-dihydrodiol dehydrogenase, 1,2-dihydroxy-naphthalene dioxygenase, salicylaldehyde dehydrogenase,	Naphthalene degradation,	naphthalene => salicylate
	Isomerases	2-hydroxychromene-2-carboxylate isomerase		
	Lyases	1,2-dihydroxybenzyl pyruvate aldolase		
	Oxido-reductases	salicylate 1-hydroxylase; salicylate monooxygenase	Phenanthrene degradation	Salicylate => catechol
	Lyases	catechol 1,2- <u>dioxygenase</u> , 2-oxo-3-hexenedioate <u>decarboxylase</u> , 2-oxopent-4-enoate <u>hydratase</u> , 4-hydroxy-2-oxovalerate <u>aldolase</u> , acetaldehyde <u>dehydrogenase</u>	Catechol meta-cleavage	catechol => acetyl-CoA / 4-methylcatechol => propanoyl-CoA
<i>Pseudomonas fluorescens</i> UK4	Oxido-reductases	benzene 1,2-dioxygenase; dihydroxycyclohexadiene carboxylate dehydrogenase	Benzoate degradation	benzoate => catechol / methylbenzoate => methylcatechol
<i>Pseudoxanthomonas spadix</i> BD-a59	Oxido-reductases	xylene monooxygenase	Xylene degradation	xylene => methylbenzoate
		benzaldehyde dehydrogenase II	Toluene degradation	toluene => benzoate
		aryl-alcohol dehydrogenase	Xylene degradation Toluene degradation	xylene => methylbenzoate toluene => benzoate

Species identification of hydrocarbon oxidative microorganisms is carried out by molecular biology methods: the gene sequence of 16S rRNA and multisubstrate testing using the GENIII Microplate system (BioLog). Community, isolated from the highly productive soil, which includes three strains: *Pseudomonas stutzeri*, *Achromobacter insolitus*, *Achromobacter xylosoxidans*, is capable of utilizing

diesel fuel as the only source of nutrition, at a concentration of up to 12 %, retains destructive properties with a salt content of up to 6 % and is capable of utilizing fuel oil, vacuum gas, hexane, phenol, toluene.

The bacterial consortium of mentioned above bacteria strains was created in three variants, for paraffins, cycloalkanes and aromatic hydrocarbons respectively as shown in Fig. 6.

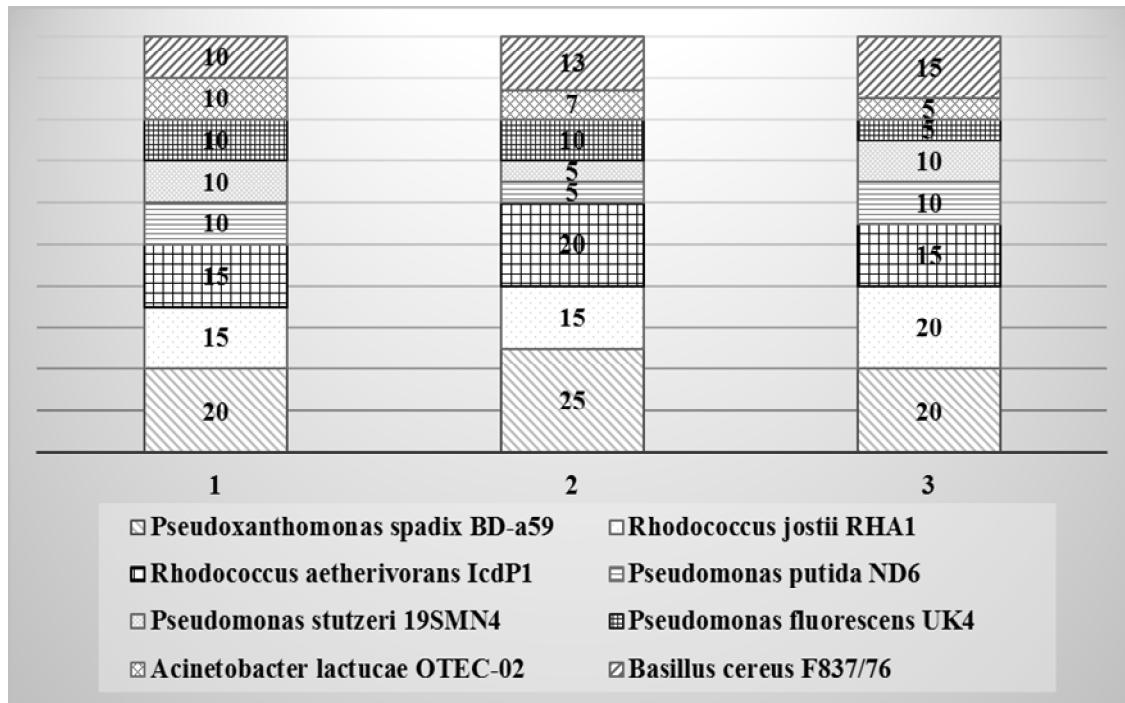


Fig. 6. The diagram of ecological-trophic groups involved in biodegradation of paraffins (1), cycloalkanes (2), aromatic hydrocarbons (3)

Bacillus sp. and *Pseudomonas* sp., namely strains of *Bacillus subtilis* DM-04 and *Pseudomonas aeruginosa* M and NM were able to degrade the oil substrates and grow in the medium with oil as the sole carbon source [39].

Strains *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Acinetobacter venetianus*, *Klebsiella oxytoca* are able to grow using crude oil as a carbon source. Some strains could degrade over 50 % of the 1 % crude oil concentration in 7 days. The majority of the strains had the ability to adapt to extreme environments, including high temperatures, alkaline environments and high salinity environments [40]. The presence of the aromatic ring hydroxylating dioxygenase genes makes it possible for *Pseudomonas* spp. [41] to participate in polycyclic aromatic hydrocarbons (naphthalene, dibenzothiophene, acenaphthylene, fluorene, phenanthrene and anthracene) degradation.

Based on the culture-morphological signs, antibiotic resistance markers and using the method of genomic fingerprints, it was the first time to trace the destiny of introduced microorganisms-oil destructors in an open environment and to show their survival and competitiveness. The selective ability of microorganisms-destructors of strains of *Rhodococcus* sp. X25, *Rhodococcus* sp. X5, *Rhodococcus* sp. S25, *Rhodococcus* sp. S26 and *Pseudomonas* sp. 142NF (pNF142) with respect to the degradation of individual oil fractions, which must be taken into account in the selection of

effective strains in the compilation of microorganism-destructor associations as the basis of biologics for purification from oil contamination [42, 43].

For reclamation of oil-contaminated soil and providing it with the physicochemical properties necessary for the effective course of metabolism by bacteria, which will be additionally introduced to accelerate the destruction of petroleum hydrocarbons, a sorbent and a disintegrant, a biostimulant and a buffer stabilizer are used. For this purpose, phosphogypsum is first introduced as a buffer stabilizer and ameliorants at the rate of up to 7 kg/ton of soil, provides an equalization of the redox potential and additionally is a source of phosphorus, calcium, sulfur, etc. After that, it is recommended to add digestate after anaerobic digestion of organic waste, which acts as a biostimulant, since it contains the required amount of mobile forms of potassium, phosphorus and nitrogen.

Conclusion

The article deals with the actual scientific and practical problem of biodegradation of oil and oil products by specialized strains of microorganisms. Biotechnological method of oil-polluted soil decontamination become more and more popular and useful for nowadays due to its advantages and positive features over physical and chemical techniques. The main methods of intensification of oil contaminated soils bioremediation, including

bioaugmentation and biostimulation, have been analyzed. A high efficiency of petroleum hydrocarbons degradation by different bacteria strains is explained by the capacity of specific living being to include these substances in their metabolic cell processes. Numerical studies show that arenas, naphthenic, paraffin are available practically for the entire indigenous microflora. It was determined that *Acinetobacter*, *Pseudomonas*, *Enterobacter*, *Cronobacter*, *Stenotrophomonas*, *Achromobacter*, *Ochrobactrum*, *Paenibacillus*, *Bacillus*, *Microbacterium*, *Curtobacterium* and *Sphingobacterium* belong to the group of the most productive bacteria in this context.

The results of the studies indicate that certain microorganisms produce enzymes, for example oxygenase and dehydrogenases, that accelerate the decomposition reaction of hydrocarbons; lipase and catechol 2,3-dioxygenase are less effective in this case. According to the mechanism of oil biodestruction biosurfactants are produced by *Bacillus salmala*, *Candida lipolytica*, *Pseudomonas aeruginosa*, and *Saccharomyces lipolytica*.

Aliphatic hydrocarbons are most easily amenable to biooxidation, aromatic hydrocarbons such as phenanthrene, anthracene and others, on the contrary, are very heavily involved in biodegradation processes due to the strength of the benzene ring. For today the construction of metabolic pathways for various organic substances is simplified by using different databases, for instance, Kyoto Encyclopedia of Genes and Genomes as it was showed in this paper.

It was proposed to carry out soil treatment with phosphogypsum as a buffer stabilizer and digestate as biostimulants for bioremediation of oil-contaminated soils, as well as to introduce a developed consortium of oil-oxidizing bacteria, which consists of the following strains: *Pseudoxanthomonas spadix BD-a59*, *Rhodococcus jostii RHA1*, *Rhodococcus aetherivorans IcdP1*, *Pseudomonas putida ND6*, *Pseudomonas stutzeri 19SMN4*, *Pseudomonas fluorescens UK4*, *Acinetobacter lactucae OTEC-02*, *Bacillus cereus F837/76.7.9*.

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