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Consortium of Hydrocarbon-Oxidizing Microorganisms as a Basis for a Biological Product for Treating Petroleum Industry Waste in Southern Kazakhstan

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Abstract

In order to design a new biological product for treating oil-polluted soil in the arid climate of Kazakhstan, conducted an extensive search for promising oil-oxidizing bacteria that were well resistant to various adverse environmental factors. Strains of petroleum product decomposers Rhodococcus erythropolis DP 304-B7 and Micrococcus varians N 313-S14 could serve as a basis for a biological product. The fact that the studied strains lacked virulence, toxicity, toxigenicity, and ability to invade internal organs of laboratory animals was indicative of their nonpathogenic nature and the possibility of using them in natural conditions.

Keywords: bioremediation, microflora, soil, petroleum

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INTRODUCTION

Oil and petroleum products are one of the main and largest pollutants of the environment. About 50 million tons of oil and petroleum products are lost annually during extraction, transportation, storage, and use (Al–Saleh et al. 2009). Emergency and chronic oil spills rapidly deteriorate the productivity of land and degrade the landscape.

Since the current level of development of the petroleum industry prevents one from excluding its negative impact on the environment, it is necessary to develop methods and technologies for restoring soil that was polluted with hydrocarbons (Païssé et al. 2010). The restoration of oil-polluted land is a relevant environmental problem. Hydrocarbons are some of the most hazardous, quickly spreading, and slowly degrading, in natural conditions, pollutants (Kuyukina and Ivshina 2010).

Oil is a complex substance that consists of more than 1000 organic compounds of various classes and inorganic admixtures, while certain microorganisms metabolize only a limited range of hydrocarbon substrates (Ruggeri et al. 2009), which is why different bacterial groups are required to biodegrade clean oil (Chowdhury et al. 2012). Oil can biodegrade in aerobic or anaerobic conditions. Aerobic biodegradation of oil occurs faster than anaerobic biodegradation does. Aerobic biodegradation of oil is optimal in terms of practical bioremediation of oil-polluted regions. Microorganisms that are polluted with oil in natural environments include many actinobacteria of the *Rhodococcus* and *Gordonia* genera and bacteria of the *Acinetobacter* genus (Alvarez et al. 2013). The active assimilation of various classes of hydrocarbons by these microorganisms is explained by their unique structure and metabolic organization, including the ability to adjust the hydrophobic-hydrophilic balance of the cellular surface and the synthesis of surface active agents, which is accompanied by the emergence of new physiological properties (Achuba and Okoh 2014, Juhasz and Naidu 2000, Robrock et al. 2011).

The problem of removing oil pollution from the environment is the fact that the natural restoration of the polluted environment is a lengthy process, which is not always easy to achieve, for instance, if a 5 g/kg level of pollution lasts from two to 30 years and more (García–Martínez et al. 2010, Keil et al. 2011). In Kazakh regions, the rate of these processes is even lower, since the oil composition contains high-molecular paraffin compounds. The difficulties in bioremediation in Kazakhstan come from the lack of

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regulatory documents that would establish the maximum permissible concentration of petroleum products in soil and water. Furthermore, the difference in the physical and chemical characteristics of soils, increased salt content, and high summer and aridity of the climate present serious limitations for bioremediation.

One of the difficulties in the development of bioremediation strategies for oil-polluted territories is the obtainment of new strains that are capable of removing a wide range of pollutants, resisting multiple environmental factors, and being effective in both laboratory and industrial conditions (Hamamura et al. 2008).

There are many techniques and methods to remove oil pollution and their choice in each particular case is individual and dependent on natural and climatic conditions. It is important to take into consideration the complex structure of oil, which is why the most promising techniques for removing these substances are biological technologies that use several species of microorganisms to decompose oil. Other promising techniques include the microbiological method of oil pollution removal based on the stimulation of growth and activity of natural microorganisms (biostimulation), the addition of sectioned decomposer microorganisms to the soil (bioaugmentation), which were isolated from the probable habitats of their spread – oil-polluted soil from different climate zones (Janbandhu and Fulekar 2011, Kirimura et al. 2002).

Thus, the purpose of this study was to find active strains of oil-decomposing microorganisms. To that end, the objectives of the study were as follows:

- To search for and select natural oil-oxidizing oil-decomposing microorganisms for the conditions of Southern Kazakhstan.
- To isolate from the oil-polluted surface the microorganisms capable of recovering oil and to identify them.
- To study the properties of the most promising oil-decomposing bacteria that constitute a consortium, which are beneficial to the bioremediation of polluted land in laboratory conditions.
- To study the impact of agricultural and technical measures on the effectiveness of oil-polluted soil treatment after the use of promising oil-decomposing bacteria.

MATERIALS AND METHODS

Soil

In laboratory experiments, the soil selected in the industrial area of Petro Kazakhstan Oil Products Limited Liability Partnership (PKOP) is a typical medium-loamy gray soil with a varying content of petroleum products. The study used the typical gray soils, typical for the territory of Southern Kazakhstan. The humic upper part of the typical gray soils is divided into two horizons: humic (A) and transitional (B1). Beneath them lies the illuvial carbonate horizon (Bk), gradually turning into the matrix (C). Humus layer in most cases represented by fulvate composition (humate-fulvate composition is most clearly expressed only in the dark and meadow gray soils). The content of humus in these soils is 1.6-1.8%, total nitrogen N (Kjeldahl) is 0.146% P_2O_5 rolling 38 mg / kg soil. For the physico-chemical properties of gray soils the following signs: low absorbing capacity (9-10 mg. eq. in light gray soils, 12-15 - for typical and up to 18-20 mg. eq. in the dark gray soils), an alkaline reaction and base saturation. The amount of exchange ions K^+ and Na^+ is about 2-5% of the capacity. Selected soil samples were placed in plastic bags (Carter and Gregorich 2007, Kiran and Udiwal 2010).

Oil and Petroleum Products

The objects of this research were oil, diesel fuel, fuel oil, and sludge. Oil was as follows: pour point of 100°C, content of silica gel resins was 19.2%; carboides – 5.82%; asphaltenes – 5.4%; wax – 7.5%; sulfur – 0.064%. At a temperature of 2000°C, the density was 0.850 g/cm3. Diesel fuel fractions were low-sulfur, acidity was less than 3%. At a temperature of 2000°C, the density was 0.804 g/cm3. The high content of n-alkanes (24%) can be regarded as a promising raw material for the production of liquid paraffin. Fuel oil is characterized by an actual freezing point between 25-420°C. At a temperature of 200°C, the density was 0.890-0.899 g/cm°. Viscosity at 80 0°C did not exceed than 16, while the sulfur content did not exceed 0.5%, and solids did not exceed 1%. Slurry-like waste profile of the top layer comprises oil products (3.1%), solids (21.43%), and water (75.47%); middle layer profile comprises oil products (5.55%), solids (25.7%), and water (68.75%); lower layer profile comprises oil products (5.17%), solids (33.33%), and water (61.5%). Oil products from an oil sludge sample have the following characteristics (defined in %): asphalt – 4.87, pitch – 16.0, ash content – 0.2, sulfur content – 0.64, carbon content – 4.0. Grain size trends of solid isolates (defined in %) are as follows: +0.074 mm – 6.6; -0.074+0.044 mm – 18.4; -0.044 mm

– 75.0. Rheological analysis of oil sludge revealed that they stay in a free-running state as long as moisture content is above 80%. With its drop to 50-75%, oil sludge becomes as thick as a paste. At even lower humidity, oil sludge turns into breakable plates.

The soil physico-chemical parameters were analysed. Particle size analysis was carried out using the hydrometer method (Bouyocos 1951). Soil texture was sandy loam. Soil pH was determined using a pH meter. The temperature of the soil samples was determined using a mercury thermometer. The electrical conductivity of the soil suspension was measured using the electronic digital conductivity meter. Total nitrogen was determined by kiedahl digestion and steam distillation method (Sankaram 1996). Available phosphorous was determined by the method of Available micro nutrients were determined by the method of Olsen, S.R.et al. (Olsen 1954).

Microorganisms

Samples were collected at a depth within 5cm from the surface of the soil. They were collected in sterile polythene bags and tightly packed. They were then carefully transferred to the laboratory for analysis and stored at 4°C aseptically before processing. **:** Isolation and enumeration of bacteria were performed by soil dilution plate technique using mineral salts agar media. One gram of dried soil was dissolved in 9ml of distilled water and agitated vigorously. Different aqueous dilutions, 10, 15, 20 of the suspension were applied on to plates and 20ml of melted medium at around 50°C was added to it. Enumeration of different isolates was carried out. Selected colonies of bacteria were transferred from mixed culture of the plates on to respective agar plates and incubated at 37°C for 24 hours. Plates containing pure cultures were stored at 4°C until for the examinations**.** Heterotrophic bacteria isolates were obtained according to the Koch's method, by plating onto a peptone-meat extract agar (PMEA), which composition in grams per liter of water was as follows: pancreatic fishmeal hydrolysate – 24.0; NaCl – 4.0; microbiological agar – 12.0±2.0. Hydrocarbon-oxidizing bacteria were obtained using the same method, but on mineral salts medium (MSM) (g/L), composed of NH_4NO_3 – 1.0; K_2HPO_4 – 1.0; KH_2PO_4 – 1.0; $MgSO_4 \cdot 7H_2O$ – 0.2; $CaCl_2$ – 0.02; $FeCl_2$ – 2 drops (Koch, 1881). For carbon nutrition, oil products were used in a variety of fractions.

Characterisation of Isolates

Each isolate was examined many times for its size, shape, margin, consistency, opacity, elevation, pigmentation, Gram reaction and cell morphology as described by Cowan (1974). The isolates were identified through observation of hyphal growth on agar plates and microscopic observation on cell dimensions. Yeast were classified by the typical glossy surface of their colonies on agar plates, negative response to the Gram stain and microscopic observation of cell dimensions. Only bacterial isolates were used in screening studies Taxonomic analysis. Diagnostic bacteria properties used include motility, production of catalase, indole, urease, oxidative fermentation of sugars, methyl red test, voges proskauer test and citrate utilization test. Antibiotic sensitivity test was also performed and the inhibitory zones were measured. Petroleum products of different fractions were used in the carbon nutrition of microorganisms.

The two selected strains R. erythropolis DP 304-B7 and M. luteus B1Ag8G were used to develop a prototype of the biological product under the code name Peroil, which was a powder or paste consisting of microorganism cells with hydrocarbon-oxidizing activity and a concentration of at least 100,000,000 cells per 1 gram of product, nutrient medium residue, and filler. The biological product was based on a freeze-dehydrated biomass of microorganisms with powdered milk as a filler, which was prepared using a freeze-dryer of the Microbiology and Virus Science Institute. The preparation of biological products included the following stages: growth of the microorganism biomass; sublimation drying using protective media with a final content of microorganisms of 10^8-10^{10} cells per gram of dry substance in the form of powder. The water suspension that was prepared using dry preparation underwent preliminary activation. To that end, it was aerated at a temperature of 23-32°C for four-six hours; at lower temperatures, the holding time was 16-20 hours. The biomass of these microorganism strains, which was used in bioremediation, was prepared in an industrial 1000.0 m^3 aeration tank.

The biological product was studied based on its ability to reclaim soil polluted with fuel oil (the content whereof was 47,300.0 mg/kg). In addition, for reclamation purposes, the biological product was added to the soil at 1:100 and 1:1000 ratios, with a view to comparing the biomass suspension of hydrocarbon-oxidizing microorganism that were cultivated in an aeration tank. The experiment lasted two months. In order to activate the microflora, we used agricultural techniques: spudding, addition of sawdust (to supply oxygen to the microorganisms), and addition of nitric phosphate fertilizers to narrow down the C:N ratio.

The use of sawdust is justified by the fact that the density of grey soil at the plant exceeds 2.2 kg/m3, while the addition of sawdust destructures the soil and reduces its density to 1.5-1.7 kg/m3.

Determination of Petroleum Products

Oil products were defined by a fluorimetric method on the analyzer of liquid "Flyuorat®-02"9. The method is based on their extraction of oil products from a soil sample 10 by hexane or a chloride methylene (chloroform ISO 11916-2:2013), purification of extract with method of a columnar chromatography with the subsequent measurement of intensity of fluorescence of the cleared extract. Oil products were analyzed for hydrocarbons by selective dissolution, thin-layer and column-adsorption chromatography. The dried chloroform residue was first treated with hydrocarbons (C5-C8) to isolate paraffin-naphthenic compounds, then with a hexane/benzene mixture (9:1) to isolate monocyclic aromatic compounds, and then with the same mixture again, but with the 8:2 mix ratio, to isolate bicyclic aromatic compounds. Whatever remained was treated with benzene and an alcohol-benzene mixture (1:1). Alumina-filled columns for chromatography were used in order to separate oil products by grades. The purity of separated samples was ensured through the thin layer chromatography performed on "Sylufol" silica gel plates. Analysis was carried out using a double-beam Specord 75JR spectrophotometer (400-4000 cm^{-1}).

Bioremediation

The effect of the isolated hydrocarbon-oxidizing microorganisms on the qualitative composition of oil and petroleum products was studied in laboratory conditions on real soils polluted with oil, diesel fuel, and fuel oil, which were sampled from the industrial area of PKOP LLC. At that, the consortium of strains *Rhodococcus erythropolis* DP 304-B7 and *Micrococcus* variants N313-S14 was added, together with sawdust and amorphous, to the oil-polluted soil sampled on plot A, 2 kg of which were placed in a glass desiccator. To determine the resistance of microorganisms to OH, them were cultured on a sterile medium with 2% added oil. The incubation was carried out at room temperature for 12 days. Bacterial strain were then determined for media with different concentrations of oil. Their activity was evaluated based on oil consumption and cell growth rate. Aromatic fuel oil hydrocarbons were obtained via adsorption. The experiments were repeated three times.

Statistical Analysis of Results

Experiments were carried out five times in repetition; the standard deviation was $0.95 > P > 0.80$. Statistical treatment was performed using the Microsoft Excel statistical software package on a Pentium-IV PC. The arithmetic mean was determined based on the number of measurements in general diagnostic group (Montgomery 2009).

RESULTS AND DISCUSSION

We conducted a preliminary assessment of the state of soil pollution and the abundance of microorganisms in the soil in the territory of the PetroKazakhstan Oil Products LLC oil refinery, which was sampled from different horizons. The content of petroleum products in the soil of the industrial zone can range from 1.0 to 25.0 grams per 100 grams of soil. The concentration of petroleum products and the base quantity of heterotrophic microflora in soil samples that were taken from different horizons showed that their highest concentration was found in the upper layer (10-20 cm layer), which is explained by favorable ecological factors for this group of strains. Contrary to this, on plots polluted with diesel fuel (mostly containing maltenes), both the content of the petroleum product and the titer of microorganisms were the highest in the 0-10 cm layer. With an increase in sampling depth to 20-30 cm and 30-40 cm, the titer of heterotrophic microorganisms dropped by one-two times. It was found that the autochthonous microflora in the native soil was dominated by bacteria, the count where of reached three-five million cells/g. The determination of the percentage of hydrocarbon-oxidizing microorganisms in the total composition of the soil microflora found that about 30% of the microorganisms were capable of using oil hydrocarbons as the only source of carbon and energy.

Optimum pH for Bacterial Growth

It was found that all strains grew with a 1.0% NaCl concentration in the medium while maintaining a high decomposing activity. The investigation of the effect of the initial pH level of the medium on the hydrocarbon-decomposing activity showed that the highest degree of decomposition was in the pH 6.0-8.0 range. The similar optimum pH range (7.0-8.0) for growth with Rhodococcus sp were presented in (Arif et al. 2012). When the pH level of the medium was reduced (4.0), hydrocarbon decomposition dropped to 17.3%; when the level was increased to 10.0, the substrate almost ceased being assimilated.

Table 1. Bacteria Growth in Liquid MSM with Petroleum Hydrocarbons added in Concentration of 5.0% Vol, %

№	Bacterial Strain	Generic Assignment	Petroleum Hydrocarbons					
			hexadecane	benzene	naphthalene	diesel oil	oil	oil residue
1	D 304-A2	Rhodococcus	85	75	*90*	*88*	*70*	*0*
2	DP 304-B7	Rhodococcus	95	78	*75*	*92*	*95*	*10*
3	B1Ag16G	Micrococcus	90	25	75	*20*	*65*	*10*
4	B1Ag8G	Micrococcus	87	*95*	*90*	*92*	*95*	*10*
5	B1Ag6G	Micrococcus	85	*35*	*60*	*55*	*10*	*0*
6	KH 301-7	Micrococcus	83	*25*	*58*	*83*	*10*	*0*
7	G 311/1	Bacillus	85	15	*10*	*60*	*10*	*0*
8	NP 301-9	Bacillus	85	*20*	*12*	*65*	*0*	*0*
9	NP 301-12	Bacterium	83	*25*	*68*	*15*	*10*	*0*
10	N 313-S14	Micrococcus	90	85	85	95	*35*	*10*
11	M 311/1-A1	Bacterium	83	*70*	*65*	*50*	*15*	*0*
12	MP 311/1-V4	Pseudomonas	85	*15*	*0*	*16*	*20*	*0*
13	M 314-3	Pseudomonas	85	*25*	*10*	*35*	*12*	*10*
14	M 314-6	Rhodococcus	90	*85*	*45*	*40*	*15*	*0*

Note – 81-100% – intensive growth, 51-80% – good growth, 31-50% – moderate growth, 10-30% – weak growth, 0 – no growth

Bacterial Isolates by Growth Speed

Isolated strains of microorganisms obtained from various oil-polluted sites, which assimilated various fractions of petroleum products, underwent screening. Active oil-destructing bacteria from different samples were tested for adaptation to high concentrations of oil products, and the most resistant ones were selected for further study. Organic compounds found in oil and oil products were analyzed by culture on a liquid mineral medium, in which hexadecane, benzene, naphthalene, diesel oil, oil, oil residue may be added as a source of carbon. Oil products were screened in liquid MSM after being added to it in the following volume concentrations, vol%: 1.0; 3.0; 5.0. Since oil-contaminated material gave us 255 isolates, we had to apply rapid screening method intended for detecting active strains. By this definition, we settled on visualization, which criteria such as oil film disappearance from the medium surface and turbidity in the nutrient medium indicate together the features bacterial growth in experimental media. At that, we addressed the following gradation scale: 81-100% – intensive growth, 51-80% – good growth, 31-50% – moderate growth, 10-30% – weak growth, 0 – no growth. Bacteria growth profile in MSM with oil products added in concentration of 5.0% vol is presented in **Table 1**.

Thus, 10 out of 14 tested bacterial cultures were good in destructing oil film on the mineral medium surface. These 10 strains were selected for further work after initial screening, as they proved to be the most active hydrocarbon-oxidizing microorganisms. B1Ag16G, B1Ag8G, B1Ag6G, N 313-S14 belong to the genus Micrococcus; NP 301-9, G 311/1 – to the genus Bacillus; M 311/1-A1 – to the genus Pseudomonas; D 304-A2, DP 304-B7 –to the genus Rhodococcus. The ability to use various sources of carbon (hexadecane, benzol, naphthalene, diesel fuel, oil, and fuel oil) was studied in all cultures. Thus, 14 isolates were made from five strains: B1Ag16G, B1Ag8G, B1Ag6G, N 313-S14 (Micrococcus genus), and DP 304-B7 (Rhodococcus genus), which can grow in the presence of a high oil concentration. Isolates from *Rhodococcus sp. sp., Pseudomona* are known as alkane-degrading genera (Hamamura et al. 2006).

The oxidizing ability of strains was additionally studied in laboratory conditions. The study found that the best indices of oil consumption and cell growth rate were those of strains B1Ag8G, N 313-S14, and DP 304-B7, belonging to the Micrococcus and Rhodococcus genera, respectively. In order to use the strains in combination, their activity was measured in laboratory conditions by the rate of oxidation of 1% oil, both separately and in such combinations as N 313-S14 + DP 304-B7 and B1Ag8G + DP 304-B7. The highest rate of oil oxidation (99.3%) was displayed by the consortium of strains R. erythropolis DP 304-B7 and M. luteus B1Ag8G, which were then used to study the decomposition of petroleum products.

Decomposition of Petroleum Products by the Consortium of Microorganism Strains in Laboratory Conditions

The promising strains of R. erythropolis DP 304-B7 and M. luteus B1Ag8G cultures were tested for the ability to decompose oil and various petroleum products (aromatic hydrocarbons of diesel fuel and fuel oil) in laboratory conditions. Soil polluted with respective petroleum products was used for that purpose.

The consortium of strains *Rhodococcus erythropolis* DP 304-B7 and *Micrococcus* variants N313-S14 was added, together with sawdust and amorphous, to the oil-polluted soil sampled on plot A, 2 kg of which were placed in a glass desiccator. There were three samples that undergone IR spectroscopic analysis: oil from the Kumkol field; oil products isolated from soil before microorganisms were added; and oil products isolated from soil after biotreatment. Analysis revealed that oil from the Kumkol field has low aromatization index, since the stretching region of C=C band (1610 cm-1) is much smaller than the stretching region of C-C band (725 cm-1) (**Fig. 1**).

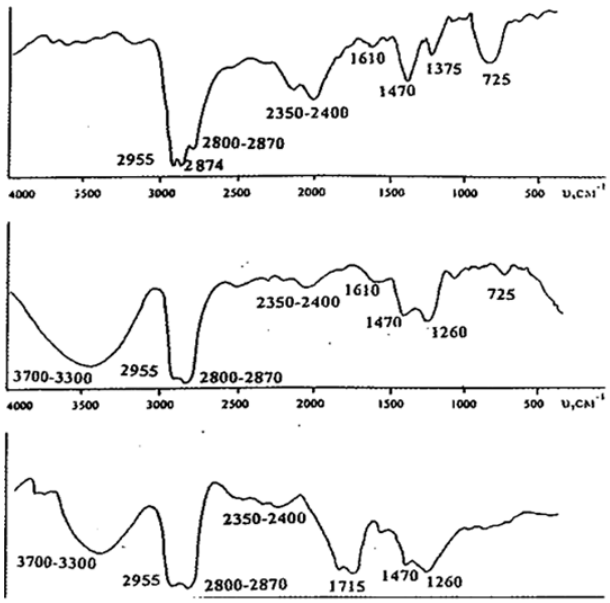

Fig. 1. IR spectra: a) oil from the Kumkol field, b) oil products isolated from soil before microorganisms were added; c) oil products isolated from soil after biotreatment

Oil products isolated from soil before microorganisms were added differs from the reference oil sample. For solids, O-H absorption band occurs in 3700-3300 cm-1 region; another band occurs at 1200 cm-1, which is a typical stretching frequency for C-OH bands; while the stretching region of band at 725 cm-1 narrowed. These changes indicate that hydrocarbons are going through the oxidation process by soil microorganisms. If we compare the absorption bands found for all three samples, it will become evident that n-alkane bands (725 cm-1) disappear. With microorganisms added, band that was at 1615 cm-1 (aromatic rings) disappeared, but another one appeared at 1715 cm-1, indicating a vibration of C=O of the COOH group (Adams et al. 2017). At that, we can assume that microbiological oxidation processes intensifies.

Bioremediation of Oil-polluted Soil using the Microorganism Consortium

A month's worth of exposure to air degraded the petroleum products by 15.2%, while maintaining the microorganism count in the soil at 10^6 cells/g; addition of ammophos increased their titer to 10^7 cells/g. However, a radical increase in petroleum product decomposition was achieved by adding the biological product to the soil. After the administration of the biological product (1:100 dilution), 60.6-65.5% of hydrocarbons degraded within one month, while the microorganism count was 10^9-10^{10} cells/g; with a 10-times smaller addition of bacteria (1:1000 dilution), the degradation percentage dropped to 44.1%, while the bacteria count dropped to 10^8 cells/g. Over the two months of reclamation using the biological product at 1:100 dilution, the percentage of hydrocarbon degradation reached 92.8-98.9%, while the bacterial titer was 10^{10} cells/g; at a 1:1000 dilution, the indices were 77.6% and 10^9 cells/g, respectively. A significant difference maintained in the degradation of fuel oil hydrocarbons with the addition of hydrocarbon-oxidizing bacteria to the soil with the biological product and the hydrocarbon-oxidizing microorganism biomass. With the addition of the hydrocarbon-oxidizing bacteria with the biological product, 60.6-65.6% degradation was reached; with biomass, this index was 28.3-36.7%. Over the two months of reclamation of soil polluted with fuel oil, the biomass provided for 65.4-68.5% of hydrocarbon degradation, which was 27.4 and 24.3% smaller than when using the biological product at 1:100 dilution.

The results of the experiment also showed that even a one-time addition of mineral salts of nitrogen and phosphorus intensified the biodegradation of oil; reduction of the amount of petroleum products occurs due to the sorption properties of sawdust. However, the highest rate of oil biodegradation (98.9%) was achieved by adding decomposer microorganisms to the soil and supplying them with additional nutrients and sawdust.

We also tested a biological product in the form of a paste based on bentonite, which was tested for bioremediation of soil polluted with fuel oil in the industrial zone of PKOP LLC, where the soil density exceeded 2.2 kg/m^3. Experiments found that using the biological product in the form of paste was inefficient due to the additional compaction of soil and disruption of the microorganism aeration regime.

It is necessary to improve the effectiveness of oil-polluted soil treatment in the conditions of the arid climate of Southern Kazakhstan, since the self-purification of such substrates is extremely slow and limited by a higher concentration of pollutants, biogenic element shortage, and low microbiological activity.

Most researchers focus on the Rhodococcus genus. Representatives of this genus have a high specific biodegrading activity and resilience to the effect of adverse factors. Biotechnological application of this group of bacteria is based on the peculiarities of their metabolism (ISO 11916-2:2013). Bioremediation by various bacterial strains of the genus Rhodococcus was succesful for the clean-up of polluted sites (Martínková

et al. 2009, Solyanikova and Golovleva 2011). The peripheral and central catabolic pathways in rhodococci are characterized for each type of hydrocarbons, phenols and other hydrocarbon compounds. Pathways involved in the hydrolysis of nitrile pollutants and the corresponding enzymes are described. The efficiency of the locally isolated Rhodococcus spp., according to (Norazah et al. 2017), optimum parameters have to be determined the effect of phenol concentration. Strain Rhodococcus sp. UCC0009 was selected with the ability to completely decompose 1.5 L-1 in twelve days. The performance of resting cells is no better than free cells in degrading phenol at high concentrations was established.

Known bacteria from the genus Rhodococcus metabolize a range of aromatic hydrocarbons and also produce a variety of value-added products, such as triacylglycerols and steroids (Pathak et al., 2013). Reports the draft genome sequence of Rhodococcus opacus strain M213 (9,193,504 bp with a G+C content of 66.99%), providing a comprehensive understanding of the repertoire of metabolic genes of this strain.

Strain Rhodococcus sp. 602 also showed the ability to synthesize triacylglycerols (Silva et al. 2010) during cultivation on naphthalene and naphthyl-1-dodecanoate. Triacylglycerols produced by resting cells in the presence of naphthyl-1-dodecanoate contained only short-chain length fatty acids (from C_8 to C_{12}), suggesting an initial attack of the substrate by an esterase releasing 1-naphthol and dodecanoic acid, which was subsequently degraded by β-oxidation. According to researchers, naphthalene seemed to be degraded by a mono-oxygenase yielding 1-naphthol, which was then transformed to 4-hydroxy-1-tetralone and to other possible metabolic intermediates.

A bacterial strain Rhodococcus imtechensis RKJ300 (= MTCC 7085(T) = JCM 13270(T)) was isolated from pesticide-contaminated soil is capable of utilizing 4-nitrophenol, 2-chloro-4-nitrophenol, and 2,4-dinitrophenol as sole sources of carbon and energy (Ghosh et al. 2010). The strain involved both oxidative and reductive catabolic mechanisms for initial transformation of these compounds. In the case of 2-chloro-4-nitrophenol, nitrite release was followed by stoichiometric elimination of chloride ions was established. Experiments using whole cells and cell-free extracts showed chlorohydroquinone and hydroquinone as the intermediates of 2-chloro-4-nitrophenol degradation.

Some studies were conducted using various species of the Micrococcus genus (Santhini et al. 2009, Wang et al. 2008). According to information (Ramadan et al. 2012) mixed cultures of Pseudomonas aureofaciens, Micrococcus varians, Lactobacillus coryniformis van degrade anthracene in soil, while Pseudomonas sp.strain with its ability to degradecrude oil can also degrade hexadecane in the presence of 5–10% NaCl. Dastgheibetal.

Our study used paraffin oil from the Kumkol field, which was added at 1% into the nutrient medium. As a result of the cultivation of the Micrococcus varians B1Ag8G strain, the amount of petroleum products dropped by 97.6% over the course of 10 days.

The lot of studies showed that using a consortium of two and more cultures increased the rate of oil hydrocarbon biodegradation, which was confirmed by our experiments. For example, the degradation percentage of solid phenanthrene at 200 mg/L in liquid medium after 6 days of incubation was higher than 95% under the condition of 37 °C and 120 r/min by microbial consortium. It was concluded that microbial consortium W4 might degrade phenanthrene via both salicylic acid and o-phthalic acid pathways (Janbandhu and Fulekar, 2011). Consortium including Sphingobacterium sp., Bacillus cereus and a novel bacterium Achromobacter insolitus MHF ENV IV has more effective phenanthrene-degrading ability than monoculture (Ichor et al. 2016). The biodegradation data of phenanthrene indicates about 100%, 56.9% and 25.8% degradation at the concentration of 100 mg/l, 250 mg/l and 500 mg/l respectively during 14 days. It was found that biodegradation of phenanthrene by a consortium of aerobic heterotrophic bacteria and cyanobacteria isolated from hydrocarbon polluted brackish water was monitored for 56 days. The initial quantity of phenanthrene in the treatment options monitored on day 0 was 2.8, 1.95, 3.17 and 2.76 mg L^{-1} for (A) Aerobic heterotrophic bacteria, (B) Cyanobacteria, (AB) aerobic heterotrophic bacteria+cyanobacteria and (C) Control, respectively on day 56.

It was found that the spread of hydrocarbon-oxidizing microorganisms across the horizons was nonhomogeneous and correlated to the molecular mass of petroleum pollutants in the oil-polluted plots of PKOP LLC. Thus, we isolated and created a collection of microorganisms from the soil environment at the PetroKazakhstan Oil Products LLC plant. It was found that these microorganisms were the most active

decomposers of oil. In order to isolate the microorganisms (from various oil-polluted sites), they were screened on media with different petroleum product fractions. Sixteen cultures of active oil-decomposing microorganisms were selected. Based on their morphological, physiological, and biochemical characteristics, these microorganisms were classified as belonging to the following genera: Micrococcus, Bacillus, Pseudomonas, Rhodococcus and Bacterium. The study of the oxidizing ability of strains found that the best indices of oil consumption and cell growth rate were displayed by strains DP 304-B7, B1Ag8G, and N 313-S14. The highest rate of oil oxidation (99.3%) was achieved by the consortium of strains R. erythropolis DP 304-B7 and M. luteus B1Ag8G.

Effective bioremediation on oil-polluted dense grey soils requires preliminary destructuring of soil via addition of sawdust for the purpose of reducing the soil density and the addition of biogenic elements for the purpose of activating the soil and added microflora. The results of the study can also be used in the bioremediation of oil-polluted soils at oil-extracting and oil-processing facilities.

CONCLUSIONS

The study of oil-polluted grey soil of the South Kazakhstan Region isolated oil-decomposing strains *Rhodococcus erythropolis* DP 304-B7 and *Micrococcus luteus* B1Ag8G, which were capable of growing on nutrient media with hexadecane, benzene, naphthalene, diesel fuel, oil, and fuel oil as the only source of carbon. These strains were isolated and selected from the natural microflora of oil-polluted grey soil at the oil refinery, at which bioremediation was conducted thereafter.

It was found that the isolated strains of hydrocarbon-oxidizing bacteria affected the qualitative composition of oil hydrocarbons and fully decomposed 1-butylbenzene, 1,2-ethylbenzene, tetramethyl benzene, naphthalene, phenanthrene, and acenaphtene. The oxidation rate of acenaphtene, tetramethyl benzene, and benzocarbazole was slower, which apparently is related to the strength of the covalent bonds of their chemical structure.

Microorganisms are adapted to a temperature range of 5-50°C, salt content of at least 1.0 g/l (since the area is characterized by a high salt content in the soil), and paraffin oil and petroleum products (since the oil from the Kumkol field is characterized by a high concentration of paraffin). Augmentation of biological products based on isolated strains of microorganisms does not produce antagonistic relations on the part of spontaneous soil microflora.

Thus, in order to improve the effectiveness of oil-polluted soil treatment in the conditions of the arid climate of Southern Kazakhstan by reducing water consumption, bioremediation should begin in autumn. During bioremediation, it is necessary to perform such agricultural actions as spudding and addition of biogenic elements. When adding the biological product, it is expedient to use sawdust, which serves as an immobilizer of microorganisms, destructurer of soil, and has a number of other advantages, such as cheapness, availability, sorption capacity, and biodegradability.

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