ANNOTATION

Thesis for the degree of Doctor of Philosophy (PhD) in the specialty
6D070100 - Biotechnology

Spankulova Gulzhan Abikulkyzy

"Development of a method for bioremediation of soils contaminated with oil and oil products, Kyzylorda region"

General description of work. The thesis is devoted to the development of a method for bioremediation of soils contaminated with oil and oil products in the Kyzylorda region.

Relevance of the research topic. The oil is one of the main and demanded sources of energy in the modern world. At present, the sustainable development of the economy of any country in the world, in particular Kazakhstan, depends on the resource and, above all, the oil potential. Increasing the pace of oil production, discovering new fields, transporting oil and becoming one of the leading exporters of hydrocarbons make research on the development of effective ways to clean up the environment in oil production sites a priority. Among the zones of ecological stress in Kazakhstan, a special place is occupied by a part of Kyzylorda region, where oil is extracted and processed. As a result of the activities of oil and gas complexes operating in the region, a rather difficult environmental situation has developed, soil pollution with oil and oil products.

It is not possible to completely eliminate its negative impact on the environment at the current level of development of the oil and gas complex. The problem of cleaning the environment from oil pollution is becoming increasingly acute due to the limited possibilities of mechanical and physio-chemical cleaning methods. Microorganisms play a decisive role in the processes of self-purification of soils from oil. Most known biotechnological purification methods are based on the use of pure and mixed cultures of microorganisms in combination with various substances or methods that stimulate their activity.

The aim of the work is to development of a bioremediation method for oil-contaminated soils in the Kyzylorda region based on the use of effective hydrocarbon-oxidizing microorganisms.

The following research tasks were solved to achieve this goal:

1. Isolation and selection of active destructor microorganisms from oil-contaminated soils of the Kyzylorda region;
2. Identification and study, phenotypic and genotypic characteristics of selected active cultures of microorganisms in order to establish their taxonomic position;
3. Study of the hydrocarbon-oxidizing ability of selected cultures of microorganisms;
4. Creation of an effective consortium of oil-oxidizing microorganisms;
5. Carrying out model experiments on the utilization of oil and oil products by a consortium of oil-oxidizing microorganisms.

The object of study. Oil-contaminated soil from the Kumkol deposit was used to isolate hydrocarbon-oxidizing microorganisms. We used a strain of bacteria isolated from oil-contaminated soils of the Kumkol deposit, Kyzylorda region

Research methods. In the course of the work, traditional microbiological (Koch method, microscopy method, perpendicular stroke method, etc.), biochemical, molecular genetic (16S RNA gene fragment sequencing) and physicochemical methods (gravimetric, colorimetric, spectrophotometric, gas chromatographic) were used.

Scientific novelty of the research.

The active strains of oil-oxidizing microorganisms as *Tessaracoccus sp.* and *Alcanovorax sp.* were distinguished in Kumkol in Kyzylorda region from the oil-contaminated soil of the field for the first time.

A high spasm of *Rhodococcus sp.* 1D/1; *Gordonia sp.* 12/5; *Rhodococcus erythropolis* 14/1 to the degradation of oil, fuel oil, diesel fuel and motor oils, as well as to the decomposition of mono- and polycyclic aromatic hydrocarbons (phenol, ortho-, para-, meta-cresol, naphthalene, phenanthrene, anthropene) are shown for the first time.

The associations of strains of oil-oxidizing microorganisms were created on the basis of active strains-destructors-bacteria. It was established that Association I (*Rhodococcus sp.* 1D/1, *Tessaracoccus sp.* 13/8, *Dietzia sp.* 13/4) and Association II (*Gordonia sp.* 12/5, *Dietzia sp.* 12/7, *Rhodococcus erythropolis* 14/1, *Arthrobacter sp.* 15/3) decompose n-alkanes, n-alkyls, branched chains, aromatic and polycyclic aromatic hydrocarbons of oil and petroleum products (fuel oil, diesel fuel).

A method for bioremediation of soils in the Kyzylorda region was developed which is based on the use of strains of bacteria-destructors of hydrocarbons of oil and oil products isolated from oil-contaminated soils.

Theoretical significance and practical value of the study. A collection of oil-oxidizing microorganisms has been created that can effectively degrade oil, fuel oil, diesel fuel, as well as mono- and polycyclic aromatic hydrocarbons (phenol, ortho-, para-, meta-cresol, naphthalene, phenanthrene, anthracene).

A chain of 16S rRNA nucleotides of active hydrocarbon-oxidizing bacterial strains isolated from oil-contaminated soil of the Kumkol field in Kyzylorda oblast is registered with GenBank. Access registration numbers: *Rhodococcus sp.* 1D/1 - MF188988.1; Gordonia *sp.* 12/5 - MF188989.1; *Dietzia sp.* 12/7 - MF188990.1; *Dietzia sp.* 13/4 - MF188991.1; *Tessaracoccus sp.* 13/8 - MF188992.1*; Rhodococcus erythropolis* 14/1 - MF188993.1; *Rhodococcus sp.* 14/3 - MF188994.1; *Arthrobacter sp.* 15/3 - MF188995.1; *Microbacterium arabinogalactanolyticum* 12/6 - MF188996.1; *Pseudomonas sp.* 14/2 - MF188997.1; *Microbacterium sp.* 16/1 - MF188998.1; *Alcanovorax sp.* 16/3 - MF188999.1.

The selected active strains and the associations created on their basis can serve as the basis for the creation of biological preparations used for bioremediation of soils contaminated with oil and oil products.

The developed method will improve the efficiency of the restoration of soils contaminated with oil and oil products, which will favorably affect the ecological situation in the region. The results of the work can be used for further biotechnological research and in the development of integrated technologies for the restoration of oil-contaminated soils in Kazakhstan.

Provisions for defense:

1. Staged screening among hydrocarbon-oxidizing microorganisms, isolated from oil-contaminated soil, made it possible to select strains growing on oil, oil products, mono- and polycyclic aromatic hydrocarbons.

2. Active strains of *Rhodococcus sp.* 1D/1; *Gordonia sp.* 12/5; *Rhodococcus erythropolis* 14/1 showed a high ability to degrade oil, fuel oil, diesel fuel and motor oils, as well as to decompose mono- and polycyclic aromatic hydrocarbons (phenol, ortho-, para-, meta-creslol, naphthalene, phenanthrene, anthracene).

3. The use of the association of microorganisms - oil destructors together with organ - mineral fertilizers, accelerates the process of remediation of contaminated soils in the Kyzylorda region by 1.5-2 times.

The main results of the study and conclusions:

The results obtained in the work allow us to draw the following conclusions:

1. The 182 cultures of hydrocarbon-oxidizing microorganisms were isolated from oil-contaminated soils of the Kumkol deposit of Kyzylorda region. To select the most effective oil decomposers, screening was carried out, as a result of which 12 crops were selected that most actively utilize crude oil and oil products (fuel oil, diesel fuel) in a liquid mineral medium - 43.9-88.8% for 14 days of cultivation.

2. New promising for biotechnology strains of hydrocarbon-oxidizing bacteria - *Rhodococcus sp.* 1D/1; *Gordonia sp.* 12/5; *Dietzia sp.* 12/7; *Dietzia sp.* 13/4; *Tessaracoccus sp.* 13/8; *Rhodococcus sp.* 14/1; *Rhodococcus sp.* 14/3; *Arthrobacter sp.* 15/3; *Microbacterium arabinogalactanolyticum* 12/6; *Pseudomonas sp.* 14/2; *Microbacterium sp.* 16/1; *Alcanovorax sp.* 16/3. 16S pRNA nucleotide sequences of the strains were registered in GenBank under registration numbers: *№ MF188988.1; № MF188989.1; № MF188990.1; № MF188991.1; № MF188992.1; № MF188993.1; № MF188994.1; № MF188995.1; № MF188996.1; № MF188997.1; № MF188998.1; № MF188999.1*.

3. The study of the hydrocarbon-oxidizing activity of selected strains of oil-oxidizing microorganisms showed their high destructive ability. The degree of oil degradation for 14 days of cultivation was 46.5-82.3% and 45.1-81.0%, fuel oil - 48.3-86.4% and 44.8-81.2%, diesel fuel - 40 .5-72.4% and 35.8-68.2%, respectively, at 3% and 5% content. The most active strains were 12/5, 12/7, 13/8, 14/1, 15/3, 12/6 and 1D/1.

4. Of all the selected active strains of oil-oxidizing microorganisms, three strains of Gordonia sp. 12/5, Rhodococcus erythropolis 14/1 and Rhodococcus sp. 1D/1 had a

high degradation potential and were able to fully assimilate aromatic hydrocarbons (naphthalene, phenanthrene, anthracene, phenol, xylene, ortho-, para-, meta-cresol).

5. On the basis of selected active microorganisms-destructors, associations have been compiled that are able to actively utilize the oil of the Kumkol field, fuel oil and diesel fuel. The most effective were two associations consisting of the bacteria *Rhodococcus sp.* 1D/1, *Tessaracoccus sp.* 13/8, *Dietzia sp.* 13/4 (Association I) and *Gordonia sp.* 12/5, *Rhodococcus erythropolis* 14/1, *Arthrobacter sp.* 15/3, *Dietzia sp.*12/7 (Association II). During 14 days of cultivation, the destruction of oil at 7% content was 77.5% and 78%, fuel oil - 74.5% and 73.7%, diesel fuel – 67.1% and 61.8%, respectively.

6. Laboratory model experiments have shown that the joint application of associations of oil-oxidizing microorganisms and organomineral fertilizers contributed to more effective soil purification from oil and petroleum products. For three months, the amount of oil and petroleum products in the soil decreased by 62.4-85.7% and 60.1-78.4% with 5% and 10% pollution, respectively. At the same time, the association II showed the greatest activity.

7. To develop a method of bioremediation of soils of the Kyzylorda region contaminated with oil, small-scale field studies were conducted. Experimental batches of biopreparations based on active associations of oil-oxidizing microorganisms (Association I and Association II) have been developed at the pilot plant of LLP SPC Microbiology and Virology. It was found that the most effective soil purification was observed with the joint application of association II and organomineral fertilizers. The oil content in the soil decreased by 73.4% and 70.7% after 2 months, with a pollution level of 5.7% and 7.2%, respectively.

Personal contribution of the author. The analysis of literature data concerning the problem under study, the conduct of experimental studies, the analysis of the results obtained and statistical processing, the presentation of the dissertation work were carried out personally by the author.

Approbation of work.

- Fifth International Conference "Ecological Engineering and Environmental Protection", 2017 Plovdiv, Bulgaria.
- VII International Conference on Ecological Industrial and Applied Microbiology. October 2017 Madrid, Spain.
- International scientific and practical conference "Actual problems of science", 2018. Almaty, Kazakhstan.
- XV International scientific-practical conference "Actual problems of modern science". June 07 -15, 2019. Przemysl, Poland.

Publications. The 10 scientific publications based on the research materials were published in the open press. Of these, 2 article is included in the Thomson Reuters database; 3 articles in republican scientific journals recommended by the Committee

for Control in Education and Science of the Republic of Kazakhstan; 2 articles and 1 abstract in conference materials, 1 article in the journal Microbiology and Virology. A patent is also received for the invention (№ 34047, 2019).

The structure of thesis. The dissertation is presented on 125 pages of computer text and consists of designations and abbreviations, introduction, review of literature, materials and methods, results and discussion, conclusion, list of references from 235 titles, contains 16 tables, 25 figures and three appendices.