ANNOTATION

theses by Sattarova Aigul Myrzahanovna on the topic:

«Development technology of bioremediation oil polluted South Kazakhstan soil by using the complex forms biopreparation"

for the PhD degree in specialty 6D070100 - "Biotechnology"

General description of the work: Dissertational work is connected with the research cycle on isolation and selection of new strains of hydrocarbon oxidizing microorganisms, studying the sorption parameters of sorbents for the development of complex forms of biologics, the development of a technological scheme and installation of a low-tonnage plant for the production of environmental biological products with the subsequent application of biologics for bioremediation of oil contaminated soils and concrete in the arid conditions of the south of Kazakhstan.

Relevance of the research topic

In addition to economic benefits, the intensive development of the oil and processing industry has negative sides as a negative impact on the environment. Oil pollution is affected by all links in the ecological system, manifested in inhibition of biological processes and violation of natural balance. There are a number of methods for the restoration of disturbed oil-polluted ecosystems, of which the most effective and appropriate are biotechnological methods based on the use of microbial life. Microbiological methods of bio-recultivation of oil contaminated soils are divided into two main directions: optimization of autochthonous micro-flora activity and augmentation of active strains of hydrocarbon oxidizing microorganisms into oil-contaminated soil.

To intensify the processes of bioremediation of oil contaminated soils, microbial biopreparations are developed, the market of which is supplemented each year with new types of products adapted to different types of soils and weather and climatic conditions of the terrain. Priority in the development of biologics of environmental action belongs to Russia and the United States. The scheme for the development of biopreparations consists of the stages of isolating the dominant groups of microorganisms, the selection of active strains, the study of oxidative capacity, the assessment of their effect on the environment, the efficiency of use in situ and ex situ conditions. However, despite the diversity of the biologics market, most of them are confined to the humid climate and humus-rich soils, the use of which in the arid climate of Southern Kazakhstan with high salinity in the soil may not always be appropriate.

Features of development and production of biological products of ecological effect for the south Kazakhstan are associated with moisture deficiency, high soil density, active insolation and increased salt content in water and soil. These moments become key obstacles when trying to use known foreign and domestic biologics for bio-recultivation of oil-contaminated soils in the south Kazakhstan.

Goal of the study: The goal of this work is to develop a technology for bioremediation of oil-contaminated soils in Southern Kazakhstan using complex forms of biological products.

Objectives of the study:

1. Isolation and selection of new strains of microorganisms with the study of sorption parameters of sorbents for the development of complex forms of biological products.
2. Development of the technological scheme and installation of a small-capacity installation for the production of environmental biological products.
3. Study of the possibility of using complex forms of biologics for bioremediation of oil-contaminated substrates.

Objects of the research: Various types of soils of the South Kazakhstan region (SKR), blocks of various types of concrete with a size of $10,0 \pm 0,9 \times 10,0 \pm 0,8$ cm, concrete surfaces of the sludge accumulator with an area of 420 m^2 on the territory of the treatment plant LLP PetroKazakhstan Oil Products (PKOP), mineral and organogenic sorbents.

Subject of the research: hydrocarbon oxidizing microorganisms; petroleum products extracted from oil-contaminated substrates; sorption characteristics of mineral and organogenic sorbents.

Methods of research

Microorganisms were grown on nutrient media: for thionic bacteria - Silveryan and Lundgren 9K, nitrifiers of the 1st phase - Vinogradsky I phase, micromycetes - Czapek, nitrogen fixers - Ashby. Nutrient media and microbiological dishes were sterilized according to conditions in a bacteriological autoclave (SPGA-100-I-HH). Cultivation of microorganisms was carried out in a thermostat with a programmable temperature (TC - 180 SDS). Distilled water was obtained on an aqua distiller (AE-10MO). Reagents weighed on an analytical scale (ScoutPro). Microscopy was carried out using light microscopes "Mikmed-5" with x40, x600, x1000 magnification (Russia), electron-raster microscope "Jeol JSM-6490 LV" (Japan).

For the taxonomic analysis of microorganisms, the "bacterial determinant" B. Berdzhi was used. Analysis of microorganisms for non-pathogenicity and non-allergenicity for humans and warm-blooded animals was carried out in the laboratory of Nutritest JSC (the founder of JSC Kazakh Academy of Nutrition).

To study the oxidizing ability of thiobacteria, pure cultures of microorganisms were used which, for 125 ml, were introduced into 250 ml volumetric flasks and placed on shakers (EKROS-6410M) at a fixed temperature of + 28 + 320 ° C. The dynamics of oxidation of ferrous iron was determined by titration with the use of the trilonometric method.

Sampling of soils for the experiment was carried out using the cutting ring method, while maintaining the structure of the native horizons of the soil profile. When determining the sorption capacity of the soil-absorbing complex (SAC) for Kumkol oil and the dynamics of its migration along soil type profiles, we conducted a laboratory experiment with native and averaged samples of the disturbed horizon of the studied soil types. The investigated samples, in order to exclude the influence on the parameters of the soil capacity of the general and non-capillary porosity (which can vary significantly in the native horizons due to various reasons and thus distort its true value), were previously passed through a

standard set of sieves. In the experiment, the combined fractions of mechanical elements measuring from 0.0001 to 3.0 mm were selected, which corresponds to a mixture from colloidal particles to gravel. 100g of the combined fractions of the studied soil types were placed in transparent plastic vessels 10, 0 cm in diameter and 12.0 cm in height, instead of the bottom in which metal sieves were installed. Oil was added to the soil until it was completely saturated, determined by the appearance of the first drop of oil through the lattice bottom of the vessel. To establish the value of the indicator of the effect of the temperature factor on the sorption capacity of soils, the experiment was repeated at 28, 20, 17, and 10°C, which corresponds to the temperature conditions of the summer and autumn-spring periods of the year. The dynamics of oil migration on the profile of different types of soils was studied at doses of oil contamination 2.0±0.2; 4,0±0,3; 5,0±0,5; 6.0±0.5; 8.0±0.5; 10.0±0.5; 15.0±0.5 and 20.0±0.5l /m^2.

Scientific novelty of dissertation work

From the oil-contaminated loamy gray soils of Southern Kazakhstan, four new strains of hydrocarbon oxidizing microorganisms were isolated: *Micrococcus varians Ag3U, Bacillus subtilis Ag1U, Aspergillus niger Ach1, Aspergillus flavipes* Ach2, which served as the basis for the composition Peroyl-Mius.

It is established that wood sawdust has the greatest indicators of sorption capacity from a number of oranic and mineral sorbents, while it is revealed that sawdust of the hardwoods sorbs oil and toluene better. It was established for the first time that the triple drying and saturation of sorbents with microorganisms increases their number in sorbents. Testing of the method of biorecultivation of oil-contaminated soils on the territory of LLP "SAC" during 2014-2016 with the use of microorganisms of the biological preparation "Peroil-Mius" immobilized on wood sawdust confirms the effectiveness of this form of biopreparations.

For the first time, a technological scheme was developed with further installation of a pilot plant for the preparation and packaging of complex forms of biological products from non-standard equipment. The device can produce liquid forms of biological preparations, pasty on bentonite and zeolite, friable - on wood sawdust, vermiculite, meal, husk, agloporite, expanded clay, etc.

For the first time it has been established that, in terms of its mechanical composition, the oil contamination of concrete at the plant is a mixture consisting of 21.4 ± 2.0% petroleum products, 72.6 ± 6.8% mechanical impurities, 6.0 ± 0.5% water. Spatial-structural arrangement of microflora shows that on the surface of oil contamination on concrete micromycetes of *Penicillium, Aspergillus* and *Micrococcus luteus* and *M.roseus, Pseudomonas, Bacillus*. A method for biological treatment of oil-contaminated concretes using a paste-like form of biopreparation "Biroks" based on the Thiobacillus ferrooxidans ThioA-1 strain has been developed.

The group composition of petroleum hydrocarbons extracted from bioremediated soils is presented, which is represented by polycyclonaphthenic, bicycloaromatic, monocycloaromatic compounds, toluene resins and asphaltenes. It was established that in the soils where microorganisms immobilized on wood sawdust were used, the processes of biodegradation of hydrocarbons are more intensive. It has been revealed that as a result of the vital activity of microorganisms, the oxidation of naphthenic oil is accompanied by an increase in its composition of aromatic fractions and a decrease in methane and naphthenic hydrocarbons. At the same time, according to the results of mass spectrometric analysis, the peaks of n-hexane, n-octane, decane, hexadecane, triacontane and tetra-trioctane were reduced.

The dynamics of oil transformation in soil depends both on the types of soils and on the seasonality of their conduct. Two peaks of the decrease in the rate of effectiveness of bioremediation measures in the winter were revealed, which is caused by a sharp drop in temperature and a summer period due to a decrease in soil moisture due to evaporation. The positive role of phyto-hydrates in activation of the processes of biodegradation of oil products in the soil is noted due to the creation of a favorable gas-air regime for microorganisms.

Theoretical and practical significance of the work

The results of the research contribute to the development of knowledge about the reaction of microflora of loamy gray soils to soil contamination with various petroleum products, qualitative changes in the composition of oil hydrocarbons under the influence of microbiological processes, the sorption characteristics of mineral and organic sorbents, and ways to increase their sorption capacity.

From a practical point of view, new strains of microorganisms that are involved in the process of biodegradation of petroleum hydrocarbons and are of interest for bioremediation activities are highlighted. Strains of microorganisms are non-pathogenic and non-allergenic for human and warm-blooded animals (Conclusion of Nutritest LLP No. 2-16 / 1633 of 11.10.2013). The strains are deposited in the RSE "Republican Collection of Microorganisms" (Conclusion № B-RKM 0696, 30.06.2016).

Sorption characteristics of various sorbents have been studied, some of which are agricultural wastes, the use of which is not only economically viable, but also solves the problem of their effective utilization. Three-fold drying and saturation of microorganisms with a biomass increases their sorption capacity, which contributes to an increase in the content of the active component in biological products.

A technological scheme has been developed and an installation for the low-tonnage production of complex forms of biologics with the capacity of 75.0 tons / year has been installed, which facilitates the commercialization of research results and the creation of additional jobs. Practical recommendations on the operation of the installation were made.

With the financial support and Terms of Reference of LLP SAK, a method has been developed for cleaning oil-contaminated concretes using a paste-like form of biopreparation "Biroks", where bentonite of the South Kazakhstan "Darbaza" deposit is used as a sorbent. A Scale of sorbent selection for complex forms of biological preparations and a method for bioremediation of oil-contaminated soils of Southern Kazakhstan with the use of the Peroil-Mius biopreparation are developed.

It has been established that for the arid climate of Southern Kazakhstan the most optimal period for carrying out biore-cultivation works is the autumn period of the year, and early spring for phytorectivation measures.

Basic provisions for the protection

Micrococcus varians *Ag3U, Bacillus subtilis Ag1U, Aspergillus niger Ach1, Aspergillus flavipes Ach2,* oxidizing n-alkanes, incl. n-hexane, n-octane, decane, hexadecane, triacontane and tetra-trioktane.

Based on the strains of *Micrococcus varians Ag3U, Bacillus subtilis Ag1U, Aspergillus niger Ach1, Aspergillus flavipes Ach2,* a composition "Peroyl-Mius" was developed, immobilization of which on wood sawdust leads to transformations of kumkol oil of naphthene type with an increase in its composition of aromatic proportions and a decrease in methane and naphthenic hydrocarbons. Three-fold drying and saturation of sorbents with microorganisms increases their number in sorbents.

A unit for the low-tonnage production of complex forms of biological preparations was developed and installed: liquid forms of biopreparations, paste-like on bentonite and zeolite, friable - on sawdust, vermiculite, shrot, husk, agloporite, expanded clay, etc.

is established that in terms of its mechanical composition, the petroleum contamination of the concrete at the refinery introduces a mixture consisting of 21.4% of petroleum products, 72.6% of mechanical impurities, 6.0% of water and populated with microflora. *Pseudomonas, Micrococcus, Bacillus, Penicillium, Aspergillus,* where a method was developed for the cleaning of concrete surfaces using a paste-like form of biopreparation "Biroks" based on thionic bacteria.

As a result of the life activity of the microorganisms of the biological preparation "Peroil-Mius", the oxidation of Kumkol oil is accompanied by an increase in its composition of aromatic fractions and a decrease in methane and naphthenic hydrocarbons, including: n-hexane, n-octane, decane, hexadecane, triacontane and tetra-trioctane .

The Scale of selection of sorbents for complex forms of biologics is developed.

Communication work with research programs

This work is related to the research plan of the Research Institute of Ecology and Biotechnology of the South Kazakhstan State University. M.Auezov on the topic: "Rational resource-saving technologies", with the grant of the Ministry of Education and Science of the Republic of Kazakhstan under the budget program 055 "Scientific and (or) scientific and technical activities", under the program "Targeted development of university science oriented to innovative results"

Production of biologics of a wide range of activities "(2011-2014), with a contract with LLP" PetroKazakhstan Oil Products "on the topic:" Development of a method for biological treatment of concretes polluted with oil and oil products "(2015).

Approbation of work

The results of the research and the main provisions of the thesis were reported and discussed at international and national scientific and practical conferences:

he use of micromycetes in the purification of oil-contaminated soils of the south Kazakhstan. // Tr. III European Conference on Biology and Medicine (Vienna, Austria 2014);

- On the issue of bioremediation of oil-contaminated soils of Southern Kazakhstan // Tr. 5th International Scientific and Practical Conference "European Conference on Innovations in Technical and Natural Sciences" (Vienna, Austria 2014);
- Study of the dynamics of vegetation cover restoration in oil-contaminated soils of Southern Kazakhstan // Mat. II All-Russian scientific-practical. Conf. with intern. uch. "Structural and functional organization and dynamics of vegetation cover" (Samara,Russia 2015);
- Study of the sorption qualities of mineral and technogenic materials // Sb. sciences. pub. «Велес» Int. Conf. "Actual problems of the development of world science"(Kiev,Ukraine 2016);
- Influence of phyto-inhibitors on the activation of microbiological purification of oil-contaminated soils of the South Kazakhstan region // Int. scientific-practical. Conf. "Contribution of microbiology and virology to the modern bioindustry", posv. 60th anniversary of the Institute of Microbiology and Virology – (Almaty, Kazakhstan 2016)
- On the issue of To the question of biological purification of oil-contaminated concretes // Mat. scientific-practical. Conf. "Modern problems of Biotechnology: from laboratory research to production", III Interd. Farabi readings(Almaty, Kazakhstan 2016)

Publications

The main provisions, results, conclusions and conclusion of the thesis are presented in 16 scientific papers, 3 of them in the journals included in the database of Thomson Reuters and Scopus, 3 articles in the republican scientific journals recommended by the Committee for Control in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan; 6 articles and 2 theses in the materials of international conferences of the far and near abroad; 1 thesis in the materials of the international conference of the Republic of Kazakhstan, 1 application was submitted for obtaining the patent of the Republic of Kazakhstan (No. 2016 / 0046.1 of January 20, 2013), 1 application for the patent of the Eurasian Patent Bureau (no. 02690587/26 dated May 10, 2016).

The structure and scope of the thesis: The thesis is presented in 136 pages and includes an introduction, a review of the literature, materials and methods of research, the results of the study and their discussion, conclusion. A list of used sources of 180 titles, the work contains 59 drawings, 14 tables and 6 applications.