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Development and application of a dry form of a new biopreparation for remediation of oil-contaminated soils in extreme continental climate conditions

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Abstract: The article presents the results of research on the development of a dry form of the new biopreparation, the technology of production, and application for the reclamation of oil-contaminated soils in the extreme continental arid climate. A previously patented association of active crude oil degrader strains was used to develop the biopreparation (*Pseudomonas putida KZ3* and two strains *Rhodococcus* KZ1 and KZ2). Microorganisms were cultured on a semi-synthetic medium to achieve maximum abundance, and the genes for the biodegradation of petroleum hydrocarbons were induced by additives at the end of fermentations. The microbial abundance at the end of cultivation with the addition of salicylate reached 7×10^9, with the addition of glucose – 1×10^9 CFU/ml, and with the addition of diesel fuel – 3.2×10^9 CFU/ml. Lyophilization at 35°C and a pressure of 4 μm Hg yielded a dry preparation with a microbial abundance of 8.0×10^{10} CFU/g. After storage for 6 months at 4-8°C, there was no loss of viability of microorganisms. Field tests conducted at the Akshabulak oil waste landfill of Company-Daulet Asia LLP showed the effectiveness of the control sample of the biopreparation. The degree of soil purification from petroleum products in 4 weeks was 70%, and in twelve weeks – 89.67%.

Keywords: *Bacterial strains-destructors, Bioremediation, Petroleum, Development of a biopreparation, Hot arid climate.*

1. Introduction

Currently, environmental pollution with petroleum and petroleum products is a planetary hazard both in scale and toxicity. In terms of the degree of harmful impact on ecosystems, petroleum and petroleum products rank second after radioactive contamination. Oil losses during production, transportation, refining and storage of oil lead to significant losses, which reach 60-70 million tons per year, which is about 2% of the total global production. The problem of cleaning from oil contamination in regions with a hot arid climate has a number of features: high temperatures reduce the oil viscosity, accelerating its diffusion deep into the soil, intensive evaporation of light fractions of oil leads to air pollution with toxic products, while the remaining non-volatile components with high molecular weight form films that are poorly subject to biodegradation. In addition, drilling fluids that get into the soil during oil production contribute to the salinization of territories [1].

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In Russia, regions with a hot climate include the republics of the North Caucasus, the Krasnodar and Stavropol Territories, and the Rostov and Astrakhan Regions. The problem of environmental cleanup from oil contamination is also very relevant for Kazakhstan, where the largest oil fields are Tengiz, Karashyganak, Uzen, Zhanazhol and Kumkol. In the Pre-Caspian region of Kazakhstan alone, 0.6 million hectares of oil-contaminated land have been identified. According to the data, from 1.0 to 18.2% of petroleum and petroleum products are lost during production, preparation for processing and transportation [2]. A distinctive feature of oil contamination in the Aral Sea region of Kazakhstan is that these territories are located mainly in the zone of extreme continental arid climate with wide temperature fluctuations.

Physical, chemical and thermal methods of cleaning from oil contamination are not always effective and can cause additional damage to the environment. Therefore, the need to develop and apply new, effective, inexpensive and environmentally friendly cleaning methods is obvious. Bioremediation has great potential and competitive advantages, primarily due to environmental safety and low cost [3].

Below are presented the works of the authors, where bioremediation technology as an environmentally friendly and cost-effective solution is widely studied and considered.

The authors in the work [4] studied oil contamination, primarily caused by petroleum oil, poses a significant global issue causing bacteria to thrive in *oil-contaminated* environments. A study isolated fourteen culturable strains of oil-utilising bacteria from pumping stations in Khartoum, Sudan, using enrichment culture techniques. These isolates were isolated from the *soil* contaminated with crude oil for initial identification. The findings of this study showed that isolates from seven genera found in the contaminated sites had exceptional potential for use in biotechnological processes like hydrocarbon-polluted site *bioremediation.*

In the study by the authors [5] various bioremediation methods were implemented within 60 days to solve the problem of old oil pollution in hypersaline soil. It has been established that inorganic nutrients, used as biostimulation agents for the native microbial community in contaminated soil, were identified as potentially harmful.

The work demonstrates [6] developed an efficient microbial activator formula and conducted an in-depth study on its efficacy and mechanism in promoting the degradation of petroleum hydrocarbons in *oil-contaminated soil.* A 60-day microbial remediation experiment conducted on oily *soil* revealed that the microbial activators significantly boosted the activities of dehydrogenase and catalase, subsequently speeding up the degradation of petroleum hydrocarbons in the *soil.* The overall degradation rate reached as high as 71.23%, with the most significant degradation effect observed in asphaltenes, achieving a degradation rate of 93.98%. This was followed by aromatic hydrocarbons (90.45%), saturated hydrocarbons (84.39%), and asphaltenes (65%).

The study conducted by the authors Abdulhussein, et al. [7] was to screen for xylanase synthesis in Pseudomonas spp. and evaluate its efficiency as a bioremediator in removal of hydrocarbons from hydrocarbon-contaminated *soil.* It has been established that the purified xylanase led to removal of hydrocarbons from hydrocarbon-contaminated *soil* with time increasing manner until maximum removal after 15 days. Authors recommend using xylanase for cleaning up of *oil-contaminated* areas. Therefore, employing microorganisms as biological tools may be a more feasible way to handle one of the most serious issues in modern society which might be a more workable and affordable way to minimize waste and preserve natural resources.

The research results presented in the work [8] provide novel insights into the activation of native bacteria by the application of resuscitation agents, demonstrating a promising approach for the bioremediation of crude oil-contaminated soils.

This work demonstrates [9] the progress and application status of *bioremediation* technology for *oil-contaminated soil*, and analyzed the classification and principle of *bioremediation* technology. Through the comprehensive analysis of the actual cases at home and abroad, the actual effects and challenges of *bioremediation* technology are comprehensively evaluated. These cases not only show the remarkable effect of this technology in the treatment of *oil-contaminated soil*, but also reveal the problems existing in

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its practical application. On this basis, the future development direction of *bioremediation* technology is prospected.

The article Akhmetov, et al. [10] indicates that, given the characteristics of a hot arid climate, it is advisable to use halotolerant oil-degrading microorganisms with a wide temperature range for bioremediation, capable of degrading hydrocarbons in the absence of moisture.

Most of the known biopreparations for cleaning from oil contamination are used in liquid form, the disadvantage of which is a short shelf life and the cost of transporting large volumes of biomass to the place of contamination. The dry form of the biopreparation is convenient to use and has obvious advantages due to the long period of preservation of viability and degradative activity.

The purpose of this work was to develop a dry form of a new biopreparation, the technology for its production and application for the remediation of oil-contaminated soils in extreme continental arid climate conditions.

2. Materials and Research Methods

2.1. Association of Microorganisms

In this work, the association of crude oil degrader strains *Rhodococcus erythropolis* KZ1, *Rhodococcus erythrop*olis KZ2 and *Pseudomonas putida* KZ3, capable of utilizing both alkanes (aliphatic hydrocarbons) and polycyclic aromatic hydrocarbons of petroleum was used. Microorganisms were isolated from oil-contaminated soil of the Kumkol oil field, the Republic of Kazakhstan, studied and deposited in the Republican State Enterprise "Republican Collection of Microorganisms" of the Republic of Kazakhstan. The research conducted at the Research and Production Center of Microbiology and Virology of the Republic of Kazakhstan has shown that the microorganisms belong to the hazard class 4 and are not pathogenic [11].

The strain *Rhodococcus erythropolis* KZ1 (B-RKM-0800) is capable of using nonane, decane, undecane, hexadecane, diesel fuel, petroleum, phenol, benzene, ethylbenzene as the only carbon source in the temperature range of 4-45°C, in the presence of 8% sodium chloride in the medium and in the pH range of 4-8. It has emulsifying activity and produces biosurfactants.

The strain *Rhodococcus erythropolis* KZ2 (B-RKM-0798) is capable of using hexadecane, phenol, naphthalene, diesel fuel, petroleum as the only carbon source in the temperature range of 4 - 45°C, in the presence of 8% sodium chloride in the medium and in the pH range of 5-8. It has emulsifying activity and produces biosurfactants.

The bacterial strain *Pseudomonas putida* KZ3 (B-RKM-0799) is capable of using naphthalene, fluorene, phenanthrene, anthracene, salicylate, hexadecane, diesel fuel, petroleum as the only carbon source in the temperature range of 4-37°C, in the presence of 5% sodium chloride in the medium and in the pH range of 5-8. It has emulsifying activity and produces biosurfactants.

2.2. Obtaining the Biopreparation

The strains included in the biopreparation were cultured in a 10 L ANKUM-2M fermenter (Institute for Biological Instrumentation of Russian Academy of Sciences, Pushchino, Russia) with a filling factor of 0.6.

Semi-synthetic medium of the following composition was used for cultivation of *Pseudomonas putida* KZ3 strain in the ANKUM-2M fermenter: acid hydrolysate of casein – 10 g/L; yeast autolysate – 70 ml/L; $(NH_4)_2SO_4$ – 6 g/L; K_2HPO_4 – 2 g/L; glucose – 20 g/L; $MgSO_4$ – 0.3 g/L; $MnSO_4$ – 0.05 g/L; defoamer "SOFEXIL-1520" (20% water-oil emulsion of polymethyldisiloxane, Sofex-Silicon, Russia) - 1 ml/L; tap water up to 6 liters. When cultivating pseudomonads at the exponential growth stage, stimulating additives were additionally added: either sodium salicylate (content 0.2 g/L) or diesel fuel (0.45 ml/L) were used as additives.

The cultivation mode: temperature is 28°C; stirrer speed is 450 rpm; medium acidity pH 6.8±0.2 (maintained automatically by adding 12% ammonia solution to the medium); aeration with air 3 L/min from 0 to 4 hours of growth, then and until the end of the process – 6.0 L/min.

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The cultivation conditions for microorganisms of *Rhodococcus erythropolis* KZ1 and *Rhodococcus erythropolis* KZ2 strains were chosen similar to those for pseudomonads. The composition of the medium differed in the content of acid hydrolysate of casein – 5 g/L; yeast autolysate – 100 ml/L and the presence of peptone – 5 g/L. In the middle of the exponential growth phase, diesel fuel (1 ml/L) was added.

The cultivation mode was similar to that of pseudomonad culture, with the exception of the medium acidity (pH 7.0±0.2) and aeration (3 L/min from 0 to 8 hours of growth, then 6.0 L/min).

Concentrated suspension of microorganisms was obtained by centrifugation for 30 minutes on a K70 centrifuge (Janetzki, Poland) at a speed of 5000 rpm at 4°C.

2.3. Storage of Obtained Biomass of Microorganisms

The biomass of the preparation was stored by the following methods: at 2-4°C, the concentrated suspension – in liquid form without adding preservatives as a control, in diluted with 0.05 M sodium-potassium phosphate buffer pH = 7.0 form (1:1 by mass), as well as using various preservative solutions: with a 20% sucrose solution, 0.2% sodium benzoate solution and 0.2% sodium glutamate solution (biomass:solution ratio was 1:1 by mass). In frozen form, the biomass was stored at –20°C with the addition of a 20% sucrose solution as a cryoprotectant (1:1 by mass).

2.4. Lyophilization of Biomass of Microorganisms

Before lyophilization, the concentrated suspension of microorganisms mixed with protective medium was kept at a temperature of – 20°C for 24 hours. The protective media used were: for *Rhodococcus*, solution No. 1 (4% thiourea, 8% sucrose, 4% polyglucin), and for *Pseudomonas*, solution No. 2 (20% sucrose). The ratio of biomass: protective medium was 1:1 by mass. Lyophilization was carried out on a lyophilic drying unit KS30 (Frigera, Czech Republic), at a temperature of 35°C and a pressure of 4 μm Hg (0.5 Pa). The obtained dry preparations of strains KZ1, KZ2 and KZ3 were mixed to obtain a dry biopreparation with a microbial abundance of 8.0×10^{10} CFU/g.

2.5. Field Tests of Dry Biopreparation

The field experiment was conducted from August to November 2022 on a 3600 m² plot on the territory of the oil waste landfill of Company-Daulet Asia LLP (Kyzylorda region, Republic of Kazakhstan). The level of oil contamination was 6.97 g/kg of soil.

The experimental plot was plowed for natural aeration of soil. After that, cow manure (5 kg/m²) was applied to the soil as an organic fertilizer.

The biopreparation was applied to the plot after activation of the dry form together with mineral (nitroammophoska 10 g/m²) fertilizer. The final concentration of microorganisms of the biopreparation in the soil was 10^5 CFU/g.

To activate the biopreparation, 9 kg of dry biopreparation with a microbial abundance of 8.0×10^{10} CFU/g was dissolved in a container with 1000 L of fresh water, 1.25 kg of NaCl and 250 g of nitroammophoska, as well as 10 L of diesel fuel were added, and thoroughly stirred; aeration was provided (bubbling with a compressor, 20 L/min) for 14-18 hours. Then 8 kg of nitroammophoska were added to the solution. The solution was used within 24 hours.

The resulting solution (1000 L) was used to treat an area of 3600 m².

In order to determine the total microbial count and the oil content, averaged samples (10 g and 50 g, respectively) were taken from 5 different soil plots every 2 weeks.

After 4 weeks, the biopreparation was applied to the plot again without adding fertilizers.

2.6. Determination of Total Microbial Count in Soil Samples

Samples weighing 1 g were resuspended in 9 ml of phosphate buffer and stirred on a Paramix 2 mixer (Germany) for 1 minute at room temperature and, after appropriate standard dilutions, plated on

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dishes with rich medium and minimal Evans medium supplemented with diesel fuel. The dishes were incubated at 24°C for 3-7 days. The number of colony-forming units was calculated per 1 g of dry soil.

2.7. Determination of oil Content in Soil

The amount of oil in soil was determined by gravimetric method according to PNDF Federal Environmental Regulatory Document [12]. Soil samples were extracted with chloroform and separated from polar compounds by column chromatography after changing the solvent to hexane.

2.8. Determination of the Composition of Petroleum and Petroleum Products in Soil Samples by Capillary Gas-Liquid Chromatography

Hydrocarbons were extracted from air-dry soil samples using a Dionex ASE 200 Accelerated Solvent Extractor *[EPA Methods. Method 3545A, Pressurized fluid extraction (PFE). Revision 1. 2007]*. Extraction conditions: solvents ("standard" qualification): n-hexane, dichloromethane, acetone; temperature: 150°C; pressure: 1500 psi; number of cycles: 2.

The extract obtained after ASE 200 was dried with anhydrous sodium sulfate and the sample was purified from non-target components by passing an aliquot part of the extract through a 2 g aluminum oxide column.

Quantitative determination of the fractional composition of hydrocarbons in the sample was carried out on an Agilent 7890A/5975C gas chromatograph (Agilent Technologies, USA) with a flame ionization detector, a DBl-ms capillary column 30 m x 0.25 mm x 0.25 μm and a ChemStation data processing system.

A quantitative mixture of n-alkanes ($C_{11}H_{24}$-$C_{36}H_{74}$) containing pristane and phytane (Connecticut *n*-Hydrocarbon Mix) from Supelco was used as a standard according to the protocols [13, 14].

The quantitative composition of the compounds contained in oil was calculated by the method of internal normalization. The NIST-08 database was used as the mass spectra library.

3. Results

3.1. Effect of Stimulating Additives During Cultivation Process on the Microbial Abundance in the Fermenter

To develop a dry form of the biopreparation, a previously patented association of active crude oil degrader strains was used [11]. The strains included in the association (*Pseudomonas putida KZ3* and two strains *Rhodococcus* KZ1 and KZ2) were cultured in a 10 L ANKUM-2M fermenter with a filling factor of 0.6. The fermenter is equipped with a mechanical stirrer, HEPA filters, sensors for temperature, medium acidity, stirring speed and aeration air flow rate. Microorganisms were cultured on semi-synthetic medium to achieve maximum abundance, and the genes for the biodegradation of petroleum hydrocarbons were induced by additives at the end of fermentations. It is known that sodium salicylate is an inducer of enzyme systems for PAH biodegradation in pseudomonads [15] and the addition of diesel fuel (DF) at the end of fermentation leads to stimulation of the synthesis of alkane degradation enzymes and the production of biosurfactants. Therefore, when culturing pseudomonads at the exponential growth stage, stimulating additives were additionally added: sodium salicylate in one case, and diesel fuel in the other. When Rhodococci were cultured, DF was added.

When culturing *Pseudomonas putida* KZ3 strain in the fermenter (Fig. 1) on semi-synthetic medium, the lag phase was 5 hours, then the culture entered the exponential phase. At the same time, the values of the maximum specific growth rates were approximately the same for the two fermentations: 0.7 h^{-1}. However, in the first variant, after the addition of salicylate at the 11th hour of growth, the growth rate increased to 1.8 h^{-1}. In the second variant, after the addition of DF, the growth rate decreased. Glucose was added to maintain growth, after which the culture count reached 1×10^9 CFU/ml. The microbial abundance at the end of cultivation with the addition of salicylate reached 7×10^9, with the addition of glucose – 1×10^9 CFU/ml, respectively.

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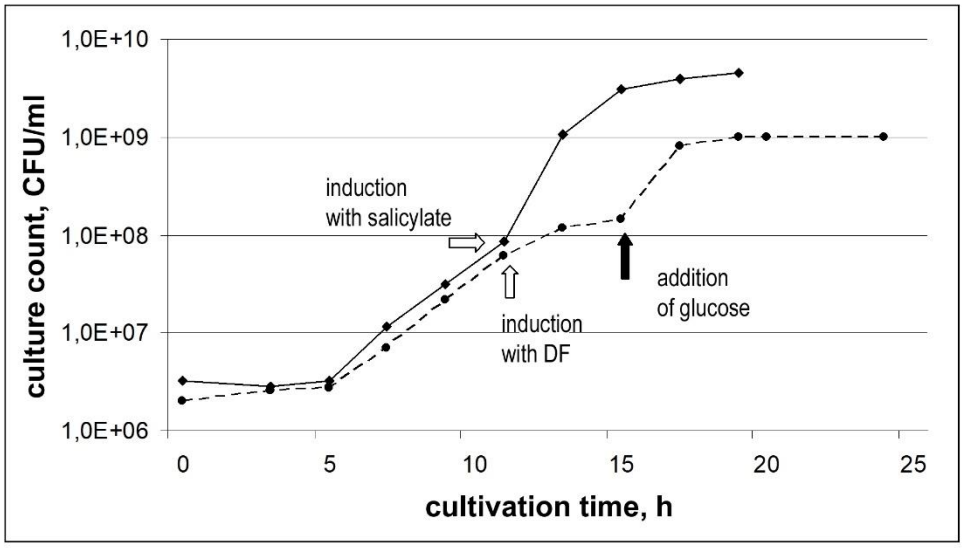

Figure 1.
Growth of *P. putida* KZ3 strain during cultivation in liquid semi-synthetic medium in the fermenter with various additives (Solid line – with the addition of salicylate, dash line – with successive additions of DF and glucose).

During fermentation of microorganisms *Rhodococcus* sp. *KZ1 and KZ2* (Fig. 2), the lag phase was slightly longer compared to *P. putida* KZ3. The maximum specific growth rate in the exponential phase was 1.0 h^{-1}. The microbial abundance in the culture liquid at the end of cultivation was 3.2×10^9 CFU/ml. After the microorganism culture entered the growth retardation phase, the cultivation process was stopped.

Thus, the media, conditions and modes of cultivation were selected to produce biomass with a high abundance of microorganisms included in the biopreparation.

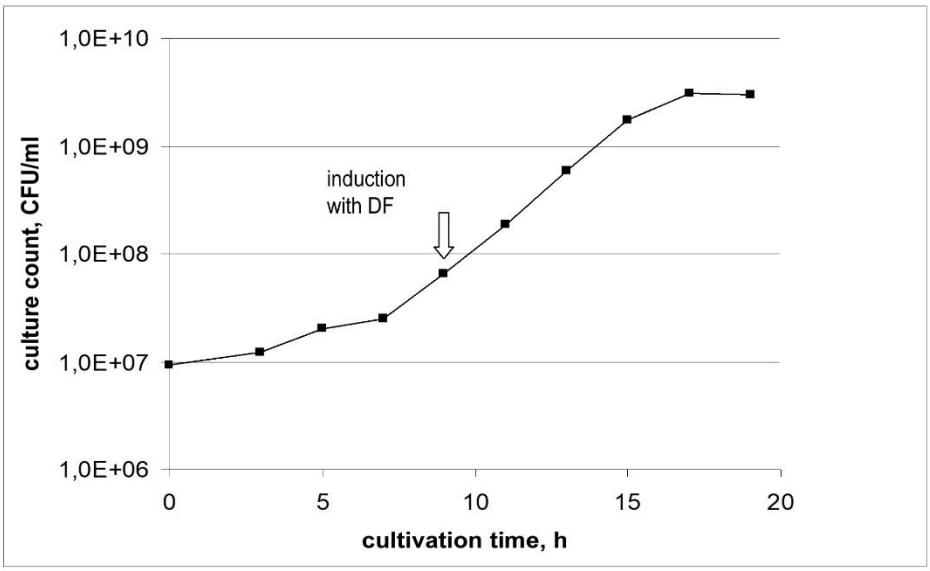

Figure 2.
Growth of *Rhodococcus* sp. KZ1 strain during cultivation in liquid semi-synthetic medium in the fermenter with the addition of diesel fuel.

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3.2. Storage of Biomass of Microorganisms

Storage of the obtained biomass was performed by the following methods: at 2-4°C, the concentrated suspension was stored in liquid form without the addition of preservatives as a control, in diluted with 0.05 M sodium-potassium phosphate buffer pH = 7.0 form (1:1 by mass), as well as using various preservative solutions: with a 20% sucrose solution, 0.2% sodium benzoate solution and 0.2% sodium glutamate solution (biomass: solution ratio was 1:1 by mass). In frozen form, the biomass was stored at –20°C with the addition of a 20% sucrose solution as a cryoprotectant (1:1 by mass).

Storing the biomass in frozen form allows preserving significantly more viable microorganisms than in the form of a concentrated suspension. After two months of frozen storage, the percentage of living cells was 2.9% for pseudomonads and 38.1% for Rhodococci. For raw biomass, these figures were 0.007% and 7.2%, respectively.

During lyophilization, a dry preparation was obtained with a microbial abundance of 8.0×10^{10} CFU/g, which was stored at a temperature of 4-8°C for 6 months. After storage, the preparation was tested for survival, the number of living cells was 7.8×10^{10} CFU/g, i.e., there was no loss of viability of microorganisms.

Thus, the storage conditions for biomass of microorganisms both liquid and lyophilized form have been selected. The choice of storage method of the produced microbial mass depends on the need for its quantity. In the case of small volumes, the biomass should be stored frozen with the use of cryoprotectants. If long-term storage and large volumes of the biopreparation are required, lyophilization is preferable. In addition, it is more convenient to transport the biopreparation in dry form to the place of remediation works, therefore, a dry form of the biopreparation was used in field tests.

3.3. Field Tests of the Biopreparation on the Territory of the Oil Waste Landfill of Company-Daulet Asia LLP

During the period of August to November 2022, field tests on the remediation of oil-contaminated soil (3600 m^2) using a dry form of biopreparation based on crude oil degrader strains (*Pseudomonas* sp. KZ3 and *Rhodococcus* strains KZ1 and KZ2) were conducted on the territory of the oil waste landfill of Company-Daulet Asia LLP (Kyzylorda region, Republic of Kazakhstan). The microbial abundance in the dry biopreparation was 8.0×10^{10} CFU/g of preparation. The rate of application of the biopreparation to the soil was calculated so that after its introduction into the soil of the plot, the number of crude oil degrader strains introduced was 1×10^{5} CFU/g of soil. Previously, a patent for invention of the Republic of Kazakhstan No. 33715 "Association of bacterial strains for the removal of oil and oil products from soils and waters in the extreme continental and hot arid climate" was received for this association of microorganisms [11]. According to the patent for invention, the association is applicable in the temperature range of 4-50°C, pH range of 4-9, salinity of the medium up to 8%, at low level of soil moisture - about 10% and level of oil contamination of the soil up to 10%.

Before testing the biopreparation, the qualitative and quantitative composition of oil in the contaminated area of the Akshabulak oil field was determined using the chromatography-mass spectrometry method. A large number of compounds were shown in the composition of oil from the contaminated soil sample, cleaned of asphaltenes and resins - 220 (Fig. 3).

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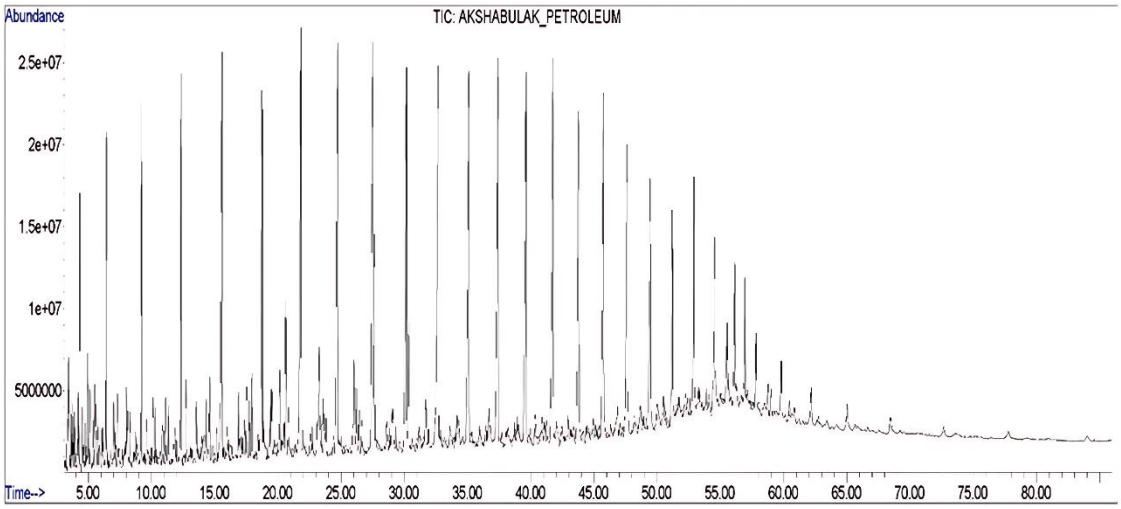

Figure 3.
Chromatogram of oil from the contaminated soil sample of the Akshabulak oil field.

The total content of hydrocarbons in the composition of oil from the contaminated soil sample of the Akshabulak oil field was 94.13%, the remaining 5.87% were sulfur-, oxygen-, nitrogen-containing, and halogen compounds. The results are shown in Table 1.

Table 1.
Composition of oil from the contaminated soil sample of the Akshabulak oil field.

Compounds	Mass fraction, %
Alkanes:	81.63
N-Alkanes	70.30
Branched Alkanes	11.33
Naphthenes	9.33
Aromatic compounds	3.17
Other compounds	5.87

According to the results of gravimetric analysis, the content of petroleum hydrocarbons at the experimental plot was 6.97 g/kg of soil.

Extreme continental arid climate of the Kyzylorda region is characterized by large fluctuations in seasonal and daily temperatures. Since the experimental plot was located in the region with prevailing high temperatures in summer, soil moisture was maintained by sprinkler method. Additionally, organic (manure) and mineral (nitroammophoska) fertilizers were applied to the plot (Fig. 4). The duration of the field test was 3 months (from August 13 to November 5, 2022). During the field experiment, changes in air temperature during the day and at night were monitored.

During the three months of the experiment, daytime air temperature gradually decreased from a maximum of 40°C to 5°C by October 5, 2022; nighttime temperature decreased from 24°C to 0°C.

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Figure 4.
Preparation of the experimental plot of the Akshabulak oil field for bioremediation work.

Figure 5.
Plot with contaminated soil before bioremediation (left) and after application of the biopreparation after 12 weeks (right).

To monitor the bioremediation process, averaged samples were taken from 5 different soil plots every 2 weeks. The total count of microorganisms and microorganisms-crude oil degraders and the oil content in soil were determined (Tables 2, 3). After 4 weeks, the biopreparation was re-applied.

During 12 weeks of field work, the oil loss on the plot with the biopreparation was 89.67% (Fig. 5). Thus, the effectiveness of the developed biopreparation in conditions of elevated temperature was demonstrated (in August and September 2022, the air temperature reached 40°C, and the soil warmed up to 50°C during the day).

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Table 2.
Residual oil content and its loss in the soil during field tests.

Work stages	Residual amount of oil, g/kg of soil	Oil loss, %	Date, duration of the experiment	Air temperature (day/night), °C
Application of fertilizers. Treatment with biopreparation	6.97	0.00	13.08.2022 0 point	37 /23 sunny
Sampling and watering.	4.60	34.00	27.08.2022 2 weeks	33/18 sunny
Sampling and re-treatment with biopreparation	4.04	42.04	10.09.2022 4 weeks	26/13 partly cloudy
Sampling and watering	3.20	54.09	24.09.2022 6 weeks	33/18 sunny
Sampling and watering	2.32	66.71	08.10.2022 8 weeks	19/6 sunny
Sampling and watering	1.46	79.05	22.10.2022 10 weeks	20/8 rain
Final sampling	0.72	89.67	05.11.2022 12 weeks	5/3 sunny

At the beginning of the field tests, the total microbial count in the soil was 7.3×10^6 CFU/g of soil, and the count of crude oil degraders was 1.2×10^5 CFU/g of soil, which was 1.6% of the total microbial count.

Table 3.
Change in the microbial count in the soil of the experimental plot during the bioremediation process.

Date	Total microbial count, CFU/g of soil	Count of crude oil degraders, CFU/g of soil, (% of total count)
13.08.2022 0 point	7.3×10^6	1.2×10^5 (1.6%)
10.09.2022 4 weeks	1.3×10^8	3.8×10^7 (29.2%)
08.10.2022 8 weeks	4.9×10^7	8.8×10^6 (17.8%)
05.11.2022 12 weeks	1.8×10^8	5.2×10^6 (2.9%)

Four weeks after the application of fertilizers and biopreparation, the total microbial count in the soil increased to 1.3×10^8 CFU/g of soil, and the count of crude oil degraders was already 29.2%. At the same time, the oil loss was 40%. At the end of the experiment, after 12 weeks, the oil loss reached 89.67% (residual amount of oil 0.72 g/kg of soil), and the count of crude oil degraders decreased to 2.9% of the total count.

4. Discussion

Analysis of literature data and a patent search on biopreparations shows that there are no universal microbial strains capable of equally efficient utilization of hydrocarbons of all oil fractions.

Scientists of the Russian Federation have developed biopreparations such as Microzyme Petrotrit (sanitary and epidemiological conclusion No. 77.99.02.515.D.001102.03.05 dated March 11, 2005), Biooil-Yugra [16] Devoroil (patent of the Russian Federation No. 2023686, C02F 3/34, 1994.11.30), and Destroil [17]. The disadvantage of the preparation "Microzyme Petrotrit" is the large number of strains, which causes difficulties in its production and its high cost. The common disadvantage of the preparations "Biooil-Yugra" and "Devoroil" is their low efficiency at elevated temperatures. The disadvantage of the preparation "Destroil" is the use of gram-negative strain as the main bioremediation agent, which limits the maximum temperature of use of the preparation.

The disadvantage of the known consortia of bacterial strains *Gordonia amicalis* VKM Ac-2720 D, *Rhodococcus erythropolis* VKM Ac-2722 D, R. pyridinivorans VKM Ac-2721 [18] is the lack of results of successful field tests of the consortium. (patent RU 2617941, priority dated 13.10.2015)

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Authors from Kazakhstan have developed a number of bacterial strains, *Arthrobacter* sp. 12T, *Arthrobacter* sp. 15T, *Dietzia maris* 84T, *Arthrobacter luteus* 43-A, which actively utilize oil from different oil fields of the Pre-Caspian region in mineral medium, sea water and in soil [18-21]. The common disadvantage of biopreparations containing one strain [22] is the lack of ecological plasticity, i.e., under unfavorable conditions, the biopreparation may lose its activity. In addition, there is no data on the ability of these bacterial strains to survive in a wide pH and temperature range.

The authors Sadanov, et al. [23]; Faizulina, et al. [24] and Idrisova, et al. [25] studied the destructive activity and phytotoxicity of strains of oil-oxidizing microorganisms isolated from various oil fields in the Aral Sea and Pre-Caspian regions.

However, in a number of cases, despite the presence of biopreparations with a working temperature limit of about 50°C, according to publicly available information, their use is mainly designed for moderate or even cold climates, and bioremediation measures need to be adapted to the conditions of extreme continental arid climate. The above suggests the use of microorganisms capable of utilizing hydrocarbons at low soil moisture and different pH and temperature values.

Our works Akhmetov, et al. [10]; Funtikova, et al. [26]; Narmanova, et al. [27] and Narmanova, et al. [28] present the current state of bioremediation of oil-contaminated territories and approaches to the development and improvement of environmental control technologies, as well as the results of research on the developed composition of the biopreparation [16] consisting of the bacteria strain *Rhodococcus erythropolis* KZ1, the bacteria strain *Rhodococcus erythropolis* KZ2 and the bacteria strain *Pseudomonas putida* KZ3, capable of consuming petroleum and petroleum products at 4-50°C. Since the soils of the Republic of Kazakhstan are saline and, as a rule, dry, the authors used microorganisms resistant to low humidity (below 10%) and high salt content (up to 8%) in the medium when creating an association of bacterial strains - destructors of petroleum and petroleum products.

The results of the field tests of the dry form of the new biopreparation showed its effectiveness in the remediation of oil-contaminated soils in the conditions of extreme continental arid climate.

Based on the results of the field tests of the dry form of the biopreparation, the conclusion was received from PetroKazakhstan Kumkol Resources JSC on the high efficiency of the developed biopreparation (degradation of 89.67% of petroleum in twelve weeks) for cleaning oil-contaminated soil in hot climate conditions.

5. Conclusion

The results of field tests on the territory of the Akshabulak oil waste landfill of Company-Daulet Asia LLP (Kyzylorda region) in the summer-autumn period of 2022 showed the effectiveness of the control sample of dry form of the biopreparation based on the strains *Rhodococcus erythropolis* KZ1, *Rhodococcus erythropolis* KZ2 and *Pseudomonas putida* KZ3. The degree of soil purification from petroleum products was 70% in four weeks, and 89.67% in twelve weeks. Thus, the use of the previously developed association of strains-destructors as part of the biopreparation is a promising and environmentally safe approach to solving the problem of cleaning oil-contaminated soils in the extreme continental climate of the Republic of Kazakhstan.

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Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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