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Research Article

Bioremediation of Oil-Contaminated Soils of the Zhanazhol Deposit from West Kazakhstan by *Pseudomonas mendocina* H-3

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The culture of *Pseudomonas mendocina* H-3 was selected as the microorganism for oil destruction, and its effect on oil-contaminated soil from the Zhanazhol deposit in West Kazakhstan was studied. After conducting model laboratory experiments, field experiments were carried out. Six and twelve months after the treatment of the oil-contaminated field with microorganisms, the amount of oil fractions in the soil decreased noticeably, while the content of asphaltenes remained constant. Analyses show that the composition of the oil fraction changes—the concentration of paraffin-naphthenic—polycycloaromatic components decreases, whereas the relative amount of mono- and bicycloaromatic hydrocarbons increases. The results of the efficiency assessment showed that the use of *Pseudomonas mendocina* H-3 cell suspension in natural conditions leads to a decrease in the content of hydrocarbons in the soil from 55 to 70%. The lower efficiency of bioremediation with cell cultures in field experiments (on average, 61%) compared with laboratory model studies (reduction of oil content to 79%) is apparently associated with climatic conditions.

1. Introduction

Petroleum product refining processes are accompanied by various losses associated with production, storage, and transportation. These problems are associated with imperfect production technologies, defects in containers for storing petroleum products and vehicle tanks, and damage and leaks in pumping systems. All these losses lead to the entry of petroleum products into the soil and their contamination.

Soil contamination with petroleum products has a strong negative effect on the environment. Thus, only 2 g of petroleum products per 1 kg of soil renders plants and microflora unsuitable for life. Water permeability sharply decreases, and the ratio between carbon and nitrogen increases (due to oil carbon), which leads to a deterioration in the nitrogen regime and disruption of plant root nutrition [1, 2].

Bioremediation is a set of methods for cleaning soils from oil pollution based on the use of the biochemical potential of microorganisms (bacteria, fungi), algae, and higher plants. The most important advantage of these technologies is their safety for the environment; they are based on the self-purification processes of living nature. With bioremediation, there is no secondary waste generated during physical and chemical remediation methods: combustion, the use of surfactants, bioventilation, electrochemical treatment, and the use of ultrasound. A special role in the bioremediation process belongs to the aerobic hydrocarbon-oxidizing group of microorganisms [3].

The mechanism of chemical oxidation of saturated hydrocarbons with the formation of low-molecular-oxygen derivatives is disclosed by Engler's theory [4]. Oxidative processes take place with the participation of microbial enzymes such as dehydrogenase, urease, catalase, polyphenol oxidase, and peroxidase resulting in the formation of intermediate metabolic products—alcohols, aldehydes, ketones, fatty and carboxylic acids, and phenols, which are eventually oxidized to CO_2 [5–7].

Various hydrocarbon compounds of oil are available for the action of bacteria, but their oxidation does not occur in the same way. First of all, n-alkanes degrade [8]. Deep degradation of aromatic compounds in oil is carried out in contact with microflora obtained by the selective method [9].

Saturated hydrocarbons are the only oil components that are completely degraded to form biopolymers, biological surfactants, organic acids, alcohols, gaseous products, and other metabolites [10]. The rate of destruction of oil depends on the elements of mineral nutrition and the number and activity of hydrocarbon-oxidizing microflora. In the soil contaminated with oil, the number of cells is determined to be up to 35 million/g [11].

Biooxidation of the resinous-asphaltene part was arranged in the following order of decreasing propensity to biooxidation: normal alkanes > isoalkanes > isoprenoid alkanes > cyclanes > arenes > asphaltenes > resins [12]. However, during the degradation of crude oils by marine and freshwater microbial populations, large losses of naphthalene and alkyl aromatic hydrocarbons were noted.

The works of the past years have proved the fundamental possibility of the biological oxidation of oils both under aerobic and anaerobic conditions. The studies [13, 14] carried out showed that the processes of microbiological transformation of oils are very intensive. Depending on the chemical type of the initial oil and the duration of the experiment (from 3 to 39 months), microorganisms destroyed from 13 to 31% wt. (by weight) of the oil taken in the experiment. At the same time, the content of high-boiling fractions increased due to the residual accumulation of resins, as well as the formation of various new oxygen-containing compounds [15].

In many laboratories, research is being carried out to isolate, select, and study hydrocarbon-oxidizing microorganisms from contaminated environmental sites (Table 1), but domestic biological products for remediation of oil-contaminated territories are still not produced.

It is well known that oil from different fields differs in its physical and chemical characteristics. Therefore, *Pseudomonas mendocina* H-3 was selected as the microorganism for oil destruction culture, and its effect on oil-contaminated soil from the Zhanazhol deposit in West Kazakhstan was studied.

2. Materials and Methods

2.1. Description of Zhanazhol Deposit Site Location. Samples of soilswere collected at the territory of LLP «Khimpromservice-Aktobe», located on the territory of the Zhanazhol deposit (Mugalzhar district of the Aktobe region) which is 15 kilometers south of the Shengelshiy village and 70 kilometers west of the republican highway A-26 (Aralsk-Aktobe). The coordinates of the object in Google Maps are 48.39318042331913 and 57.43037065031205. The climate of the territory is sharply continental; the annual temperature fluctuation ranges from +27.5°C in summer to −14.0°C in winter. The average annual precipitation range is presented in Table 2.

Climatic data on air temperature and humidity, as well as soil temperature and moisture regim were obtained from the Database for open use of observation results at the meteorological station Mugaldzhar, Kz-AKT of the RSE "Kazhydromet" [24].

2.2. Zhanazhol Deposit Site Soil Properties. The processes of carbonation, salinization, and solonchakization are actively occurring on the soil of the Zhanazhol deposit area, and solonezes occupy 40% of the area. The main characteristics of the soil are presented in Table 3.

The upper horizons of light chestnut soil had no salinity; in the B and Bc horizon, there is an increase of salinity, i.e., sulfates up to 9.74 mmol/100 g of soil. An almost neutral medium is observed throughout the profile (pH 7.2–7.6).

Table 4 presents data on soil moisture and soil temperature regimes in the Mugalzhar region. On average, the soil surface warms up in the third ten days of April to 9–15°C; in the first ten days of May, above 15°C; in the third ten days of May, it reaches 20°C; and in June, it exceeds 23°C.

Moisture reserves are measured from the moment of thawing and drying of the soil before the start of harvesting; after harvesting, the measurement is resumed and continues until the date of stable transition of daily air temperature through 5°C in the fall.

Thus, according to Table 4 in the Mugaldzhar district of the Aktobe region, soil moisture according to RPM data is characterized as satisfactory in the first half of the agricultures, growing season and as unsatisfactory in the second.

2.3. Bacterial Cultures Isolation. Bacterial cultures of *Pseudomonas mendocina* H-3 were isolated from oil-polluted soil and used at a concentration 10^7 CFU/g dry soil [19, 20]. Synthetic broth medium E8 was used for the growth of the microorganisms. The composition of this medium is presented in Table 5. The pH value of the medium should be 6.8.

To prepare the inoculum, a cell suspension with a titer of $10^{7\text{-}8}$ CFU is added to a bioreactor with an E8 mineral medium containing 7% diesel fuel as a hydrocarbon source. Next, this suspension is grown with constant stirring at a speed of 250 rpm for 2 days until the nutrient medium becomes completely turbid and the cell titer reaches 10^{10} CFU. Cultivation conditions are aerobic, at 28–30°C, pH 6.8.

For the target product (cell mass), the supernatant liquid was first drained from the fermenter, and the thick biomass was poured into sterile 1-liter jars. The biomass jars were kept for 24 hours to thicken. The forming liquid above the cellular biomass was sterilely drained. The resulting thick biomass was left in jars for long-term preservation of the

Table 1: Literature examples on the use of microbial cultures for soil bioremediation.

No	Cultures	Observed effect	Reference
1	*Pseudomonas stutzeri, Pseudomonas putida, Bacillus cereus*	Alkanes C_{21}–C_{29} were subjected to deep oxidation up to 80%	[16, 17]
2	*Pseudomonas* sp. NCIB 9816	Naphthalene dioxygenase catalyzes the oxidation of more than 50 aromatic compounds (including anthracene and phenanthrene)	[18]
3	Pseudomonas fluorescens strain 26 K	75% transforms phenanthrene with the formation of phenanthrenone, 7,8-benzocoumarin and the cleavage products of one of the aromatic rings - 1-carboxy-2-naphthylbutane, 1-carboxy-2-naphthylpropionic, 1-carboxy-2-naphthoic acids	[19, 20]
4	*Enterobacteriaceae, Stenotrophomonas, Pseudomonas, Acinetobacter, and Achromobacter*	Total petroleum hydrocarbon (TPH) removal was 80.05%	[21]
5	*Geobacillus kaustophilus*, *Geobacillus jurassicus*, *Geobacillus thermocatenulatus*, *Parageobacillus caldoxylosilyticus*, *Anoxybacillus geothermalis*, *Geobacillus stearothermophilus*	Completely degrade C_{37}–C_{40} and increase the ratio of C_{14}–C_{18}	[22]
6	*Rhodococcus, Sphingomonas, Variovorax*	The byproducts of oxidizing of aromatic hydrocarbons dihydroxylated cleave by intradiol or extradiol ring cleaving dioxygenases through ortho- or meta-cleavage pathway result in intermediates such as protocatechuate and catechols	[23]

Table 2: Average monthly air temperature and monthly precipitation range.

Month	Average monthly air temperature (°C)	Monthly precipitation (mm)
1	−13.4	23
2	−13.2	16
3	−5.1	17
4	7.8	20
5	15.6	29
6	23.0	21
7	27.5	17
8	26.6	15
9	24.6	10
10	8.0	25
11	−3.0	23
12	−14.0	22

drug in the refrigerator at 8°C. Transportation of cell cultures to the field for testing was carried out using portable refrigerators with constant temperature control.

2.4. Soil Sampling and Soil Preparation for Bioremediation in Laboratory and Field Experiments. The interstate standard GOST 17.4.3.01-2017 "Nature protection. Soils. General requirement for sampling" was used for soil sampling [25]. Soil sampling is carried out on test plots (2 m × 3 m) laid out in such a way as to exclude distortion of test results under the influence of the environment. The composite samples were mixed to obtain a representative sample of the plots determined by setting predefined sampling points. Samples are taken along a profile of soil horizons or layers in such a way that, in each case, the sample represents a part of the soil typical of the genetic horizons or layers of a given soil type.

To conduct experiments in laboratory conditions, three soil samples were taken from the Zhanazhol deposit with different organic content. In sample No. 1, the content of the organic part is 13.0 wt. %, in sample No. 2–20.0 wt. %, and in sample No. 3–23.0 wt. %. Synthetic medium E-8, containing cell cultures of *Pseudomonas mendocina* H-3, in an amount of 5, 10, and 15 ml (concentration 10^6 CFU/ml), was added to oil-contaminated soils with a mass of 30 g, and the change in the content of the organic part in the soil under the influence of the strain was determined for 15 to 80 days.

To conduct field tests of bioremediation by cell cultures, the quadrant sampling technique was used. 5 small areas measuring 2 × 3 m were fenced off from humans and animals with barbed wire. The surface layer of soil in the oil-contaminated area was loosened. Cell suspensions of oil-oxidizing microorganisms in concentrations of 10^7 CFU/g (10^{10} CFU/l) were introduced using a submersible pump at a rate of 5 liters per 1 square meter of plot. The air temperature at the time of introducing cell cultures (mid-March 2022) was +15°C. Twice a month, the top layer of soil (0–20 cm) of the site was loosened and watered to maintain soil moisture at ~15%. After 6 and 12 months, soil samples were taken and submitted for analysis to determine the content of oil.

2.5. GC Analysis of Oil Composition. Chemical analysis of oil content was performed using an Agilent 6890N gas chromatograph equipped with an Agilent 5973N mass detector (Agilent, Germany). Separation of diesel hydrocarbons was carried out on a DB-XLB GC-column (30 m × 0.25 mm, 0.50 μm particle size) using the following temperature program and conditions: 40°C –10 min., with 2°C min^{-1} to 250°C – 20 min; ion range – 10–300 Da, injection volume –0.2 μl with flow separation 1 : 50; carrier gas – helium at a flow rate of 1 ml·min^{-1}.

In this work, the quantitative gas chromatographic method of simulated distillation (SimDis) was used to determine the fractional composition in accordance with standards ASTM D2887, D7213, and D7398 [26]. The described technique makes it possible to relatively quickly determine the fractional composition for small quantities of samples and also gives results on the content of asphalt-resin compounds in the studied oil. The chromatography results were processed using special software such as AC SimDis and DHA Plus.

The program calculates the mass fractions (semi-quantitatively) of the following 11 classes of hydrocarbons:

(i) Paraffins;
(ii) Noncondensed cycloparaffins;
(iii) Condensed cycloparaffins with 2 rings;
(iv) Condensed cycloparaffins + all cycloparaffins with 3 rings;
(v) Benzenes;
(vi) Naphthenobenzenes;
(vii) Dinaphthenobenzenes;
(viii) Mothballs;
(ix) Acenaphthenes;
(x) Fluorenes;
(xi) Phenanthrene.

The boiling diagram of the samples is plotted in the range of 40–250°C. To construct a boiling diagram, calibration against normal alkanes in the required boiling point range is necessary, which can be done using a standard sample of

Table 3: Soil physiochemical properties.

Soil taxonomy	Horizontal	Depth (cm)	Dense residue (%)	pH	Humus content (%)	Ion content (mmol/100 g)					
						K^+, Na^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	HCO_3^-
Subtype: light chestnut; genus: light chestnut carbonate, dark chestnut solonetzic	Ah	0–21	0.08	7.2	4.75	0.02	1.36	0.22	0.13	0.37	0.24
	B	22–40	0.78	7.6	3.62	6.47	12.55	0.35	1.24	9.74	0.25
	Bc	41–102	0.89	7.6	0.51	6.01	11.29	0.48	2.52	7.66	0.43
	C	102-	0.73	7.3	—	0.12	0.33	—	0.16	—	0.15

Table 4: Soil moisture and soil temperature regimes of Mugalzhar region.

Temperature regime of soil	Month	4	5	6	7	8	9
	t_{min}	4	15	23	25	23	20
	t_{max}	13	20	28	30	28	22
	t_{ave}	9	18	26	28	26	21
Reserves of productive moisture (RPM), mm	0–20 cm depth	29	23	16	11	8	16
	20–100 cm depth	102	99	89	77	63	15

Table 5: The composition of medium E8.

No	E8 medium components	Quantity
1	Potassium dihydrogen phosphate KH_2PO_4	0.7 g
2	Ammonium hydrogen phosphate $(NH_4)_2HPO_4$	1.5 g
3	Sodium chloride NaCl	0.5 g
4	Magnesium sulfate $MgSO_4$	0.8 g
5	Agar-agar	20.0 g
6	Distilled water	1.0 l

alkanes or a sample of oil with a high content of normal alkanes. Determination of the component composition is possible without calibration.

2.6. IR Spectroscopy. Changes in the composition of storage-pit oil by infrared (IR) spectroscopy were estimated by comparing the spectra determined at the beginning of the experiment and after 7 as well as 14 days of bacterial growth. IR spectra were recorded using a two-beam automatic spectrometer UR-20 in the range 400–4000 cm^{-1}. Analyses were carried out with plates of potassium bromide (KBr) in which the oil was incorporated under pressure. The thickness of the absorbing layer was 0.01 mm. Oil samples were analyzed as thin films by squeezing a drop of liquid solution between potassium bromide salt plates. To obtain an IR spectrum, a small amount of sample (20–40 μg) is required. An aliquot was taken with a microsyringe with a filter attachment at the end. (Finntip).

The structural and group composition of oils and components is determined by the intensity of the characteristic absorption bands in the IR spectra using a common baseline with fixed points of 1850 and 650 cm^{-1}. For an average molecule, the content of methylene groups (CH_2) is estimated from the absorption band of 720 cm^{-1}, methyl groups (CH_3) from the absorption band of 1380 cm^{-1}, carbonyl groups (CO) in the region of 1720–1700 cm^{-1} relative to aromatic C=C bonds along the absorption band 1600 cm^{-1}.

2.7. Assessment of the Effectiveness of Soil Bioremediation. The effectiveness of soil bioremediation was assessed by reducing the concentration of crude oil in soil samples using the petroleum products analyzer Concentometer KN-2 m. The operation of the device is based on a photometric method for determining petroleum products, fats, and nonionic surfactants in carbon tetrachloride in the infrared region of the spectrum at a wavelength of 3.42 micrometers.

Soil samples from the Zhanazhol field (at least 10 samples) for analysis were taken at the beginning and end of the experiments—after 6 and 12 months of treatment with *Pseudomonas mendocina* H-3 cells.

A sample of dry soil was scattered on paper, or tracing paper and large lumps were crushed with a pestle. Then inclusions were selected—plant roots, insects, stones, glass, coal, and animal bones. The soil was ground with a mortar and pestle and sifted through a sieve with a hole diameter of 1 mm. The required amount of soil was taken for analysis. A 250 ml flask was used to extract oil from the soil. A soil sample was poured into a flask, and 60% of the required volume of carbon tetrachloride was added there. The standard solution GSO 7822-2000 (PSI) with the composition isooctane 37.5%; hexadecane 37.5%; benzene - 25% was used for calibration of the device. The certified characteristics of the standard are the mass of petroleum products contained in 10 ml of GSO - 50.00 mg; the limit of absolute error of the certified value: ±0.25 mg, at $p = 0.95$.

Effectiveness of bioremediation was expressed as the following equation:

$$E = \left[100\% - \left(\frac{C_{12m}}{C_0}\right)x\,100\%\right] \pm \text{RSD}\%, \quad (1)$$

where C_0 is the concentration of oil before the treatment, mg/kg of soil; C_{12m} is the concentration of crude oil after 12 month of treatment by cell cultures, mg/kg of soil; and RSD is the relative standard deviation, %.

The results of chromatographic, group, and IR spectroscopic analysis of the organic part of the soil after biodegradation show the following scheme for the transformation of paraffinic hydrocarbons (Figure 1).

2.8. Statistical Analysis. Statistical differences among measurements were determined by analysis of variance (ANOVA) using Excel and Microsoft Office. Means were compared using the least significant difference (LSD) test at $p < 0.05$ and presented as the values of five varieties of restricted plots determined by the envelope technique.

3. Results and Discussion

The dependence of the content of organic matter in soils on the time of exposure to *Pseudomonas mendocina* H-3 cells is shown in Figures 1–4.

As can be seen from the figures, with the addition of a 5 ml medium in all soil samples, the content of the organic part decreases slowly, but with an increase in the number of cells, the content of the organic part decreases significantly.

Figure 2 shows the results of processing soil contaminated with oil in the amount of 13 wt. %. When 5 ml of the suspension is added, the amount of the organic part in the soil gradually decreases, and after 60 days, its amount is 9.5 wt. %, then sharply decreases to 5.52 wt. %. When the volume of the medium is between 10 and 15 ml, the amount of the organic part decreases uniformly, and after 80 days, it reaches 3.51–5.04 wt. %. In general, in soil with 13% pollution under the influence of biooxidative processes, the degree of biodegradation in 80 days was 73%.

When a microbial environment is introduced into the soil (with a contamination of 20%), the amount of the organic part decreases gradually, but, as in the first soil sample, the amount of the introduced microbial suspension has a noticeable effect on the oxidative processes (Figure 3). Due to the biodegradation of oil in the second soil sample after 80 days, the amount of the organic part decreased from 20 to 4.2 wt. %, i.e., the degree of soil purification was 79%, which indicates deep processes of oxidation of oil hydrocarbons.

Figure 4 shows the change in the mass fraction of the organic part in the soil, with its initial content of 23%, after its biodegradation with a different amount of medium with *Pseudomonas mendocina* H-3 cells.

The introduction of small volumes (5, 10 ml) of microbial suspension reduces the content of the organic part in 80 days only to 16–20 wt. %., while when adding 15 ml of microbial suspension, the consumption of hydrocarbons increases significantly (Figure 5). The content of the organic part in the soil during 80 days decreased from 23 to 4.5 wt. %, giving a degree of destruction of the pollution in the soil of 80%.

Thus, in all soil samples, with an increase in the volume of the microbial medium to 15 ml, a decrease in the content of the organic part to 3.5–4.5 wt. % could be achieved.

Field studies were also carried out at the sludge accumulator LLP «Khimprom service-Aktobe», located on the territory of the Zhanazhol deposit (Mugalzhar district of the Aktobe region).

In field experiments, soil samples for analysis were taken at the beginning and end of the experiments. For introduction, cell suspensions of oil-oxidizing microorganisms in concentrations of 10^6 CFU/g were used. The experiments were carried out in five repetitions on sites measuring $2 \times 3 \times 0.8$ m. It should be noted that the areas under study, after 6 months, already visually differed from the original in color and smell. They were characterized by the color of light chestnut soils, and there was no acidic organic smell. Figure 6 shows areas of soils contaminated with oil from the Zhanazhol field before and after exposure to microorganisms.

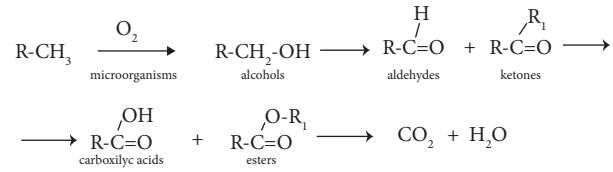

Figure 1: Scheme for the transformation of paraffinic hydrocarbons.

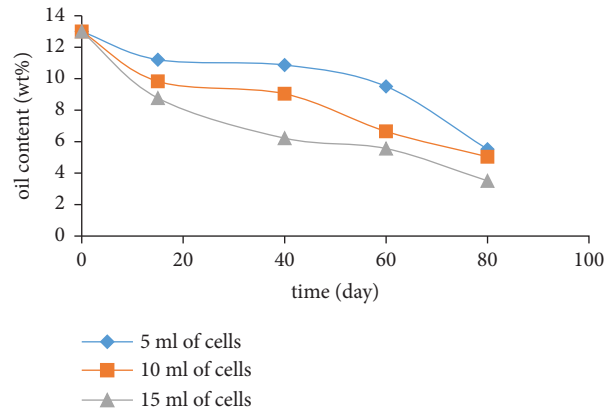

Figure 2: Results of bioremediation of soil contaminated with 13 wt. % oil, *Pseudomonas mendocina* H-3 cells.

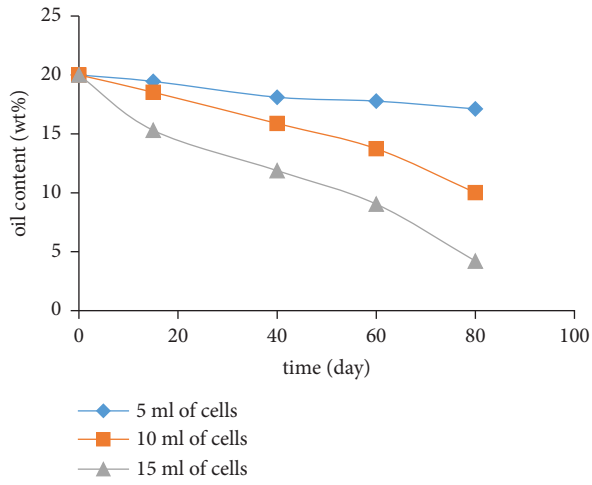

Figure 3: Results of bioremediation of soil contaminated with 20 wt. % oil, *Pseudomonas mendocina* H-3 cells.

The results of the chromatographic analysis of the component composition of the organic part of the soil showed that it is mainly represented by light paraffins (Table 6). The organic part of the original contaminated soil contains isoparaffins, cyclic, and aromatic hydrocarbons. The highest concentration is observed for 3-methylpentane, 1,5-dimethylnaphthalene, and C_{12} hydrocarbons. The chromatogram of the organic part of the soil, after 6 months of contact with microorganisms, showed significant changes in the amount of hydrocarbons. The content of low-molecular-weight hydrocarbons from C_{10} to C_{15} decreased. In

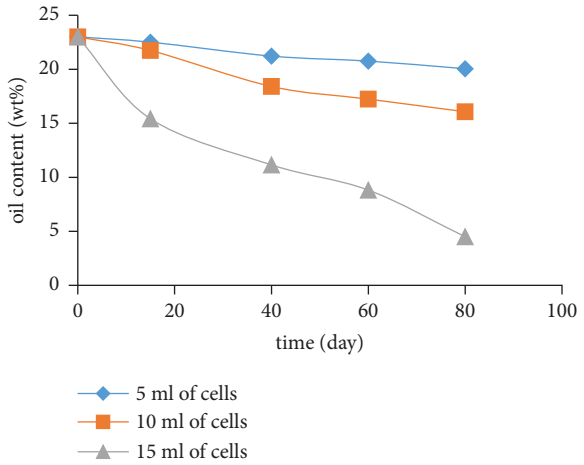

Figure 4: Results of bioremediation of soil contaminated with 23 wt. % oil, *Pseudomonas mendocina* cells.

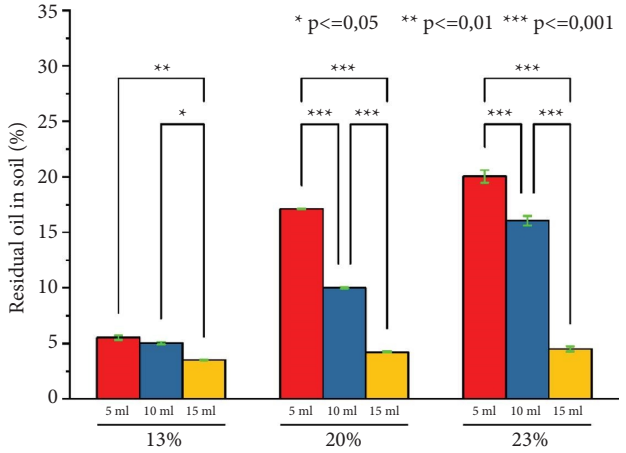

Figure 5: The content of the residual oil in soil after 80 days treatment by *Pseudomonas mendocina* H-3 cells. $^{*}p \leq 0.05$, statistical significance at the 5% level; **indicates a higher level of significance, $p \leq 0.01$; ***indicates a very high level of significance, $p \leq 0.001$.

(a) (b)

Figure 6: Oil-contaminated area of LLP "Khimprom service-Aktobe" before (a) and 6 months after exposure to microorganisms (b).

the composition of the organic part, the appearance of solid high-molecular hydrocarbons from C_{16} to C_{34} is observed. Biooxidative processes at 34% soil contamination for 6 months led to a decrease in the content of light paraffins in the soil and an increase in C_{16}–C_{34} components.

The method of chromatography was used to study the qualitative and quantitative changes in the chemical composition of the organic part of the soil contaminated with oil from the Zhanazhol field before and after bioremediation. Table 6 shows the results of chromatographic analysis of the original organic part of the soil 6 months after exposure to the microorganism *Pseudomonas mendocina* H-3.

An analysis of the group composition of the organic part of the soil from the sludge pond of "Khimprom service-Aktobe" LLP before and after cleaning with microorganisms based on the adsorption-chromatographic method was carried out, the results of which are shown in Table 7.

As can be seen from the tabular data, the composition of organic parts of the soil is dominated by oils (50.2 wt.%) and resins (41.3 wt.%). Among the oils, the highest content is in paraffin-naphthenic oils (32.9 wt.%), and among resins in benzene resins (33.1 wt.%). The amount of asphaltenes is 8.5 wt. %.

6 months after the introduction of the environment with microorganisms into this soil, the amount of oil fractions in the soil decreased markedly, while the content of asphaltenes increased. In the composition of oils, the concentration of paraffin-naphthenic and polycycloaromatic components decreased, while the amount of mono- and bicycloaromatic hydrocarbons increased. Although the total resin content did not change, the concentration of benzene resins decreased by more than 10 times and amounted to only 2.4%. The increase in the content of alcohol-benzene resins may be associated with the secondary oxidation of compounds. It should be noted that the content of asphaltenes in the organic part increased 2.5 times.

Analysis of the vibrational spectra of organic compounds in the infrared region is a reliable method for determining the nature of functional groups and their structural transformations during oxidation. The IR spectra of oil degradation products can be used to trace the rate of oil oxidation and, in comparison with the control, to evaluate the acceleration of the destruction processes.

To determine the change in the composition of the organic part of the soil, IR spectroscopic analysis was also carried out. Figure 7 shows the infrared spectrum of the organic part extracted from the original contaminated soil. The spectrum reveals intense absorption bands in the range of 2953, 2922, 2851, 1456, and 1377 cm^{-1}. The appearance of absorption bands at these wavenumbers indicates the presence of a significant amount of saturated hydrocarbons. An absorption band with high intensity at 728 cm^{-1} appears due to the stretching and bending vibrations of elongated normal hydrocarbons. This means that paraffinic hydrocarbons predominate in the oil of the Zhanazhol field. The presence of absorption bands at 1604 and 1044 cm^{-1} in the spectrum indicates the presence of aromatic structures associated with benzene rings in the organic part. The medium-intensity band at 1707 cm^{-1} indicates the presence

Table 6: Component composition of the organic part of the soil contaminated with oil from the Zhanazhol field before and after bioremediation.

Components	Original sample Content (wt. %)	After 6 months
2-methylpentane	3.3	—
3-methylpentane	16.6	—
n-hexane	9.5	—
2,2,3-trimethylbutane	1.5	—
1,1-dimethylcyclopentane	2.4	—
2,2,4-trimethylpentane	1.3	—
C_7	2.8	—
C_8	0.9	—
C_9	1.0	—
C_{10}	2.7	0.01
C_{11}	1.1	0.3
C_{12}	17.7	1.0
C_{13}	3.5	1.7
C_{14}	7.1	2.7
1,6-dimethyl naphthalene	4.7	—
1,5-dimethyl naphthalene	13.2	—
1,3-dimethyl naphthalene	3.0	—
C_{15}	7.7	2.6
C_{16}	—	2.4
C_{17}	—	2.1
C_{18}	—	2.0
C_{19}	—	2.1
C_{20}	—	2.5
C_{21}	—	3.0
C_{22}	—	4.4
C_{23}	—	4.6
C_{24}	—	4.6
C_{25}	—	6.7
C_{26}	—	7.8
C_{27}	—	9.7
C_{28}	—	9.8
C_{29}	—	8.1
C_{30}	—	6.6
C_{31}	—	6.4
C_{32}	—	4.8
C_{33}	—	3.0
C_{34}	—	0.8

Table 7: Group composition of the organic part of the soil contaminated with oil from the Zhanazhol field before and after the introduction of a cell suspension of *Pseudomonas mendocina* H-3.

Group composition	Organic part of oil-contaminated soil	
	Initial	After 6 months
Oils (wt. %):		
Paraffin-naphthenic	32.9	27.7
Monocycloaromatic	1.4	5.4
Bicycloaromatic	0.3	2.9
Polycycloaromatic	15.6	1.4
Total	50.2	37.4
Resins (wt. %):		
Benzene	33.1	2.4
Alcohol-benzene	8.2	39.0
Total	41.3	41.4
Asphaltenes	8.5	21.2

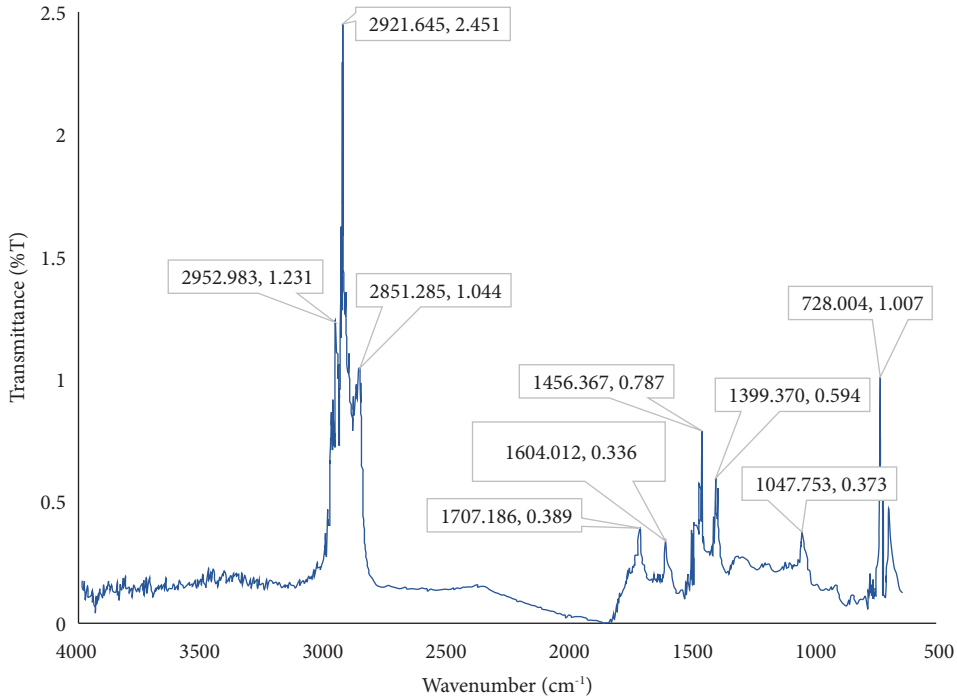

Figure 7: IR spectrum of the organic part of the soil contaminated with oil from the Zhanazhol field.

of carbonyl groups in organic oxygen-containing compounds. The appearance of this band is explained by the fact that when stored in the open air for a long time, sunlight stimulates the growth processes and oxygenase activity of the soil microflora. This leads to a gradual increase in the content of oxygen-containing components in the organic part of the soil.

As shown in Figure 8, exposure to microbial cells for 6 months led to a change in the composition of the organic part of the soil. The IR spectrum of the organic part of the biodegraded soil contains absorption bands of aliphatic hydrocarbons at 1377, 1462, 2851, 2922, and 2954 cm^{-1} (stretching and deformation vibrations of the $-CH_2$ and $-CH_3$ groups). The intensity of these characteristic absorption bands in comparison with the spectrum of the organic part of the original soil has significantly decreased. This is evidenced by the values of the signal intensities, which are shown next to the values of the wavenumbers.

The absorption band at 728 cm^{-1} has disappeared, and the absorption band of aromatic compounds at 1604 cm^{-1} has a weak intensity. New absorption bands with wavenumbers of 1073, 1122, and 1273 cm^{-1} appear in the spectra due to vibrations of the C-O and C-O-C functional groups of various oxygen-containing organic compounds: alcohols, ethers, and esters, which are intermediate products of metabolism during the microbial oxidation of n-paraffins. The absorption band at 1730 cm^{-1} can also be considered as a sign of the presence of oxygen compounds, while its intensity also increases after biodegradation.

The data of the IR spectroscopic analysis showed that because of bioremediation, the number of oxygen-containing components of oil increases and the amount of paraffins and aromatic compounds decreases. First of all, the selective oxidation of heterosubstituted unsaturated compounds occurs, then of normal alkanes and the replacement of bonds with low breaking energy by bonds with high breaking energy, which is confirmed by a change in the intensities of bands in the regions of 1707 and 1730 cm^{-1}, which characterize oxygen-containing fragments of the structure. By the end of the experiment, the products of oil transformation were characterized by the predominance of compounds with ether, carbonyl, and carboxyl groups.

In order to assess the effectiveness of soil bioremediation using a culture of microorganisms, we studied the reduction in the concentration of crude oil in soil samples from the Zhanazhol field (Mugalzhar district of the Aktobe region) using an oil product analyzer (concentometer KN-2 m). Soil samples were taken in the amount of 10 specimens from each area at the beginning of the experiment (without treatment with microorganisms) and after 6 and 12 months of treating the field with *Pseudomonas mendocina* H-3 cells to carry out the analysis. Results are presented in Table 8.

The results of the efficiency assessment showed that the use of *Pseudomonas mendocina* H-3 cell suspension in natural conditions leads to a decrease in the content of hydrocarbons in the soil from 55 to 70%. According to chromatographic and infrared spectral analysis, only heavy resinous hydrocarbons—asphaltenes and carboxylic compounds of cyclic components—remain in the soil. The lower efficiency of bioremediation with cell cultures in field experiments (on average 61%) compared with laboratory model studies (reduction of oil content to 79%) is apparently associated with climatic conditions, namely average temperature differences from −14.0 to +27.5°C throughout the year and other weather events (snow, rain, wind, etc.) that effect on the cell culture activity.

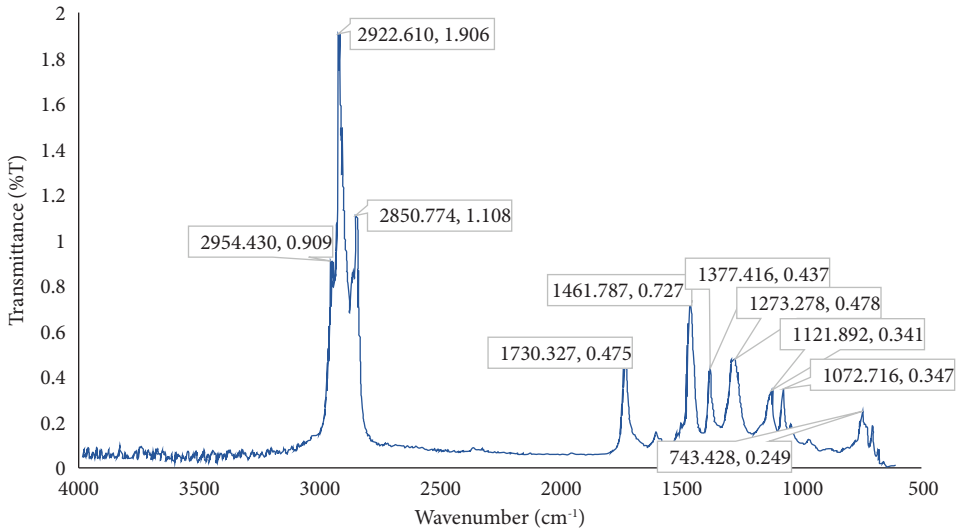

Figure 8: IR spectrum of the organic part of the soil contaminated with oil from the Zhanazhol field after exposure to microbial cells.

Table 8: Effectiveness Zhanazhol field soil bioremediation by cell suspension of *Pseudomonas mendocina* H-3 in concentration 10^7 cells/g dry soil.

No of area	C_0 of oil (mg/kg)	C_{6m} of oil (mg/kg)	C_{12m} of oil (mg/kg)	Effectiveness % ± RSD%
1	146.5	87.2	55.8	61.9 ± 1.5
2	112.5	78.3	44.7	60.3 ± 1.8
3	215.4	135.4	64.7	69.9 ± 1.7
4	97.4	66.1	41.3	57.6 ± 0.9
5	177.9	98.4	79.1	55.5 ± 1.3

Overall, *Pseudomonas mendocina* H-3 strain has shown excellent potential in the remediation of soil in laboratory and field conditions. The oil-degradation/oil-oxidation activity of various *Pseudomonas* strains in laboratory and field conditions were shown in several studies. Strain *P. aeruginosa* WatG was able to degrade 60% total diesel oil and kerosene added to soil, and strain *P. aeruginosa* HokM was able to degrade 50–60% kerosene, diesel, and lubricating oil within 2 weeks [27]. Prof. Park et al. found that *Pseudomonas* aeruginosa-encapsulated alginate/gellan gum microbeads (PAGMs) were able to decrease diesel contamination in groundwater to 71.2% in 30 days, whereas pure culture was able to reach 32% effectivity [28].

The study [29] used burnt soil, which contains a high concentration of polycyclic aromatic hydrocarbons, to study *Pseudomonas putida* bioremediation effectivity. After two months of treatment of soil with *Pseudomonas putida* strains, the concentration of naphthalene decreased by 63.6%. This level of oil destruction effectivity is comparable with data obtained from our field studies in 6 months. However, using *Pseudomonas aeruginosa* BB-BE3 together with *Azadirachta indica* plants showed 95.71% removal of hydrocarbons from soil [30], so it is perspective to use *Pseudomonas mendocina* H-3 together with Kazakhstan plants to get this level.

To increase the efficiency of bioremediation by *Pseudomonas mendocina* H-3 strain in the future, it is necessary to select the optimal consortium of microorganisms rather than a separate type of microbial cell that also uses heavy oil as a nutrient and also uses hydrophobic carriers in order to ensure maximum fixation of microorganisms in the pores of the carrier. The formation of microbiocenosis on the surface of the sorbent material causes an increase in the stability of cells and directly depends on the nature of the carrier and microorganisms, the degree of the hydrophobicity of the surface of the material and the living cell, the charge of the surface of the living cell and the carrier, the ratio of the size of the cells of the microorganisms, and the pores of the carrier, and many other factors.

4. Conclusions

Thus, from the obtained research results, it follows that the introduction of oil-oxidizing bacteria *Pseudomonas mendocina* H-3 into oil-contaminated soil from the Zhanazhol depozit creates conditions for the activation of the processes of destruction of oil hydrocarbons, which, in turn, leads to the improvement of soils. Based on the results of group, chromatographic, and IR spectroscopic analysis with the introduction of these types of microorganism-destructors

into oil-contaminated soil, their effectivity was revealed, and the possibility of their use for cleaning oil-contaminated soils was shown. *Pseudomonas mendocina* H-3 strain has shown excellent potential in the remediation of soil in laboratory and field conditions (up to 79% and 69.9%, respectively). The results obtained in the course of experiments can serve as a basis for creating a technology for bioremediation of oil-contaminated soils.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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