




Review

Recent Advances in Creating Biopreparations to Fight Oil Spills in Soil Ecosystems in Sharply Continental Climate of Republic of Kazakhstan

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Abstract: The problem of eliminating petroleum pollution and its consequences is currently very relevant for Kazakhstan, which is among the ten largest oil-producing countries. The specifics of natural conditions—the sharply continental arid climate—necessitate the development and application of adequate technologies for the restoration of oil-contaminated territories and the Caspian seashore. The key factors (temperature, moisture, alkalinity, salinity, low mineral and organic matter content) affect the self-purification processes and microbiological status of oil-contaminated soils of Kazakhstan. The assessment of taxonomic diversity and characteristics of oil-degrading microorganisms isolated from samples of soils and reservoirs contaminated with hydrocarbons are given. The review of biopreparations and biotechnologies developed and used in Kazakhstan for cleaning environments from oil pollution is made, and their effectiveness is shown. The analysis of the current state of research in the field of biodegradation of hazardous pollutants and bioremediation of oil-contaminated areas allows us to identify promising areas of further work and approaches to the development and improvement of technologies for environmental protection.

Keywords: oil spills; bioremediation; arid climate; Kazakhstan; desert soils; the Caspian Sea region; microbial biopreparations



Citation: Akhmetov, L.I.; Puntus, I.F.; Narmanova, R.A.; Appazov, N.O.; Funtikova, T.V.; Regepova, A.A.; Filonov, A.E. Recent Advances in Creating Biopreparations to Fight Oil Spills in Soil Ecosystems in Sharply Continental Climate of Republic of Kazakhstan. *Processes* **2022**, *10*, 549. <https://doi.org/10.3390/pr10030549>

Academic Editor: Inna P. Solyanikova

Received: 7 February 2022

Accepted: 9 March 2022

Published: 11 March 2022

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1. Natural Conditions, Soils, and Hydrocarbon Production in Kazakhstan

The regions where oil fields are located and oil is produced meet the main risk of oil and oil products pollution. About 60% of the world's oil reserves are located in the territories of countries with hot climates, where the specifics of environmental factors, especially temperature, force a more careful approach to the choice of the method of polluted lands remediation [1].

Kazakhstan is a land-locked country between Russia and China, and bordering Turkmenistan, Uzbekistan, and Kyrgyzstan. Kazakhstan currently ranks in the top 10 countries in oil and gas reserves. Its oil reserves are comparable to Nigeria and Libya, and no other country in Europe or Eurasia, except Russia, has more gas reserves. Kazakhstan's oil deposits are located primarily in the Western part of the country, near and under the Caspian Sea [2]. The Caspian Sea is the world's largest enclosed body of water. The sea area is 392,600 km². The biological diversity of the Caspian Sea is relatively small, but it is characterized by high endemism [3]. The environmental problems of the Caspian Sea deserve consideration in an additional review.

Interestingly, there are few places in the world comparable to the sharply continental climate of Kazakhstan; these are the desert of Uzbekistan adjacent to the territory of Kazakhstan, as well as the Patagonian desert in Argentina, and Gobi in Mongolia: isotherm $10\text{ }^{\circ}\text{C}$, arid climate—hot summer, winter from cool to cold, average annual rainfall across most of the territory is low—100–500 mm (aridity).

We failed to find information concerning oil spills and tests of oil-degrading biopreparations/single strains in the deserts of Mongolia and Patagonia. Nevertheless, Argentina ranks third in Latin America in terms of oil and gas reserves. Five oil and gas basins have been identified on the territory, four of which are located within the depressions of the pre-Andean deformation. There are 243 known oil and 52 gas fields in the country, concentrated in the basins in the Neuquen-Limay interfluvium (82 oil and 24 gas) and the San Jorge Lagoon (93 oil and 10 gas) [4].

Kazakhstan is the leader in oil production among the CIS countries, whose territories are located in regions with a hot climate. The largest oil fields on the territory of this state are Tengiz, Karachaganak, Uzen, Zhanazhol, and Kumkol (Figure 1). The density of the extracted oil mainly ranges from 0.79 to 0.82 g/cm^3 , the resin content ranges from 1.14% (Tengiz) to 8.2% (Kumkol), and sulfur 0.5–1.1%. The oil of the Uzen field differs in characteristics: it has a high density (0.84 – 0.87 g/cm^3), the resin content is up to 20%, and sulfur up to 2%.

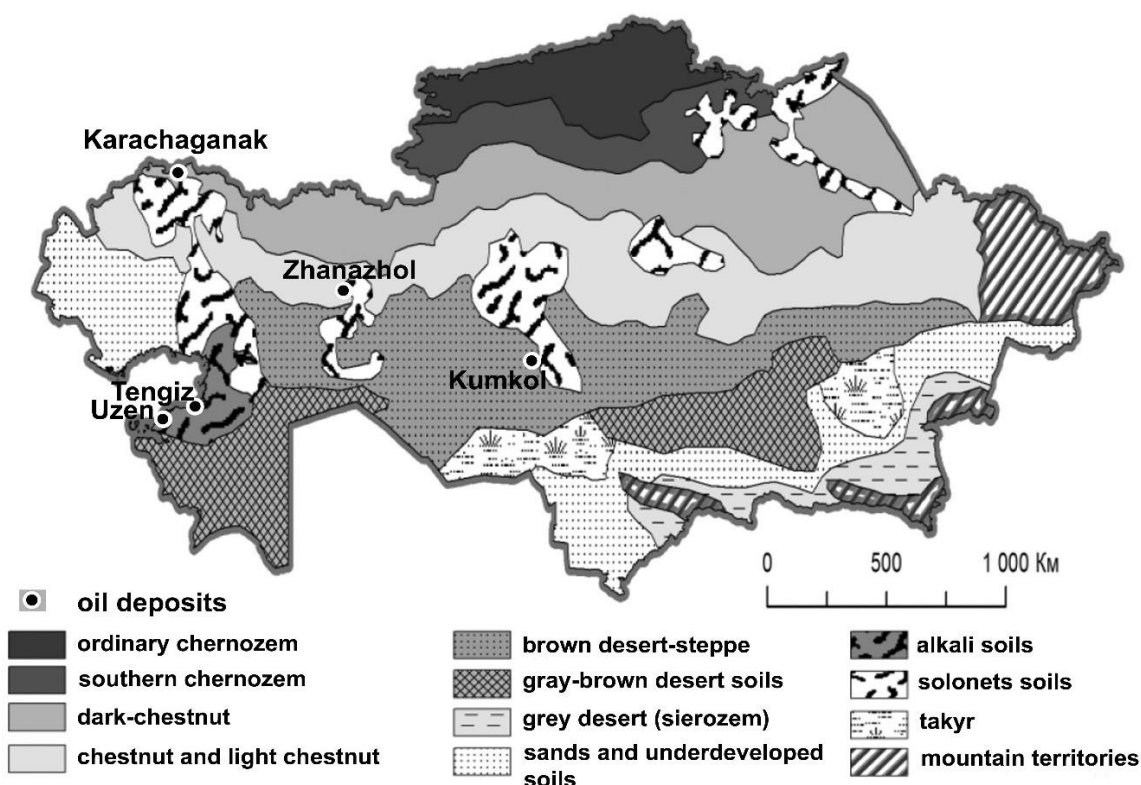


Figure 1. Soils of Kazakhstan and the largest oil deposits ([5], with modifications).

In most of the soils, the process of soil carbonization is under way to some extent; pH has alkaline values. Due to the aridity of the climate, the carbonates of the solution precipitate at a certain depth both in the form of separate new formations, for example, cranes in ordinary chernozems, and in the form of whole carbonate horizons in chestnut and many desert soils. In the most severe desert conditions, secondary gypsum accumulates, and gypsum salts are formed [5].

The best soils of the republic are chernozems. They are common in the north, under the steppe vegetation. Zones of chernozems and dark chestnut soils are agricultural, where mainly spring wheat is cultivated. Dark chestnut soils are most widespread in

the Irtysh area within the Pavlodar region. Light chestnut soils are common in the south of West Kazakhstan, Aktobe, Akmola, Central Kazakhstan, East Kazakhstan, and in the southernmost part of the Pavlodar region.

To the south of latitude 48, where a real desert begins, the summer is rainless and the winter is cold; brown and gray-brown soils are formed under very rare vegetation, which occupies 44% of the republic's land (117.3 million hectares). Brown soils are typical for the south of the semi-desert zone and the north of the desert zone. To the south, where it is even drier, gray-brown soils develop. They are almost always salted and carbonate from the surface. There are many alkali soils (salt marshes), and takyrs (gypsisols)—a kind of soil formations with a flat surface, completely devoid of any vegetation. Solonets soils are the result of the evolution of salt marshes, which occurs as a result of a decrease in the level of groundwater and some moisture from above.

In the semi-desert and desert areas in the south and west of Kazakhstan, primitive sandy soils (arenosols) are widespread on sands fastened by vegetation. There is very little humus in them—up to 0.5%; they are carbonate, almost not saline, and they easily pass moisture and retain it.

The Caspian region is characterized by a certain set of environmental problems: it is soil-plant (primarily man-made) desertification; depletion of water resources and their pollution with oil, petroleum products, and other toxic substances; the potential danger of oil-containing substances entering the water basin of the Caspian Sea and the likelihood of irreversible poisoning of its waters, and deterioration of public health as a result of environmental pollution. The ecological status of the territory of Western Kazakhstan is characterized as pre-crisis. The soil becomes an accumulator and repository of toxic chemicals that cause severe forms of hepatitis and diseases in the population's respiratory organs, tuberculosis, malignant tumors, etc. Rehabilitation of disturbed lands and improvement of the environment is becoming the most important state task and requires a speedy solution [6].

Man-made pollution in the form of oil spills of tens of thousands of tons was allowed on an area of more than 1.3 million hectares in the Atyrau region. The soil contamination in some oil fields reaches a thickness of up to 10 m. In the Caspian region of Kazakhstan alone, 0.6 million hectares of oil-contaminated lands have been identified [7].

It is worth mentioning the disaster at the exploration well No. 37 of the Tengiz field in Kazakhstan, when on 23 June 1985, a fountain of oil and gas with a high content of hydrogen sulfide occurred unexpectedly from a four-kilometer depth, which ignited after the collapse of the drilling rig. The gas pressure at the depth reached 750–800 bar, and 200–300 bar at the surface. The accident elimination took more than six months [8]. Similar accidents were repeated in 1991 during the Persian Gulf War, when Iraqi troops set fire to oil wells.

Mechanical and most physical methods reduce the oil content in soils and waters to 10–12%. However, such a residual concentration of oil is too high and requires further removal. As a result, bioremediation, the process of degradation or pollutants transformation into non-toxic compounds carried out by microorganisms, has become one of the promising methods of secondary purification of oil-polluted ecosystems [9].

2. Up-to-Date Studies Focusing on Hydrocarbon-Degrading Microorganisms in Kazakhstan

The diversity and metabolic potential of destructive bacteria are known to decrease with the shift of environmental conditions to a stressful area [10,11]. However, indigenous microorganisms adapted to specific climatic conditions are able to utilize oil hydrocarbons effectively even under conditions of seasonal temperature changes, low soil humidity and hypersalinity of seawater [12]. With a temperature increase, the solubility of hydrophobic pollutants enhances, the viscosity decreases, and the rate of transfer of, e.g., long-chain alkanes [13] from solid phase to liquid increases, which contributes to their more efficient removal from polluted biotopes in hot climates, including due to biodegradation.

Studies of bacterial degradation of oil have shown that the process accelerates at elevated temperatures, possibly due to the stimulation of individual enzymes involved in it [14]. The metabolites formed during the degradation of polycyclic aromatic hydrocarbons in thermophilic and mesophilic conditions may be different due to the influence of temperature on enzyme activity [15,16].

Obuekwe et al. [17] reported the isolation of oil-degrading mold (*Fusarium lateritium*) and fungi (*Drechslera* sp. and *Papulaspora* sp.) from a desert salt marsh in Kuwait. The mold strain and *Drechslera* sp. grew in the presence of 10% salt, the second in the presence of 5% salt.

In Kazakhstan, several closely interacting groups from the Institute of Microbiology and Virology in the city of Almaty, (and its branch “Applied Microbiology” in Kyzylorda), Al-Farabi Kazakh National University (Almaty), M. Auezov South Kazakhstan State University (Shymkent), and the Korkyt Ata Kyzylorda State University (Kyzylorda) investigate oil-utilizing microorganisms and develop methods of bioremediation of oil-contaminated soils.

Using four standard biotest systems, Ibragimova et al. [18] investigated the ecotoxicity of oil-contaminated soils of oil fields in Kazakhstan (Zhanatalap, Kumkol, and Aktas). Radish seed (*Raphanus sativa*) and a protozoan *Paramecium caudatum* were compared with cladoceran crustaceans *Ceriodaphnia affinis* and luminescent bacteria (“Ecolume” test system) and were less sensitive to old contamination with petroleum products.

Various oil-degrading microorganisms have been isolated from the soils ([19] and others), as well as the waters of the Caspian Sea [20,21], and there has been further study of bacterial strains (identification of strains, and biochemical and physiological properties, using methods of classical microbiology [20,22] and molecular biology [19]). In [19], Sadanov et al. studied the phytotoxicity of forty-five degrader strains in relation to the seeds of *Raphanus sativus*.

The ability to exhibit emulsifying properties in four hundred cultures of oil-utilizing microorganisms isolated from the coastal waters of the Caspian Sea has been revealed [21]. The four best cultures for the following indicators: cell wall hydrophobicity, emulsifying activity, production of extracellular biosurfactants, and emulsification index, were identified as *Sphingobacterium kitahiroshimense* wkar54, *Stenotrophomonas chelatiphaga* wka149, и wka151, and *Achromobacter* sp. wkar55.

Safary et al. [23] isolated an oil-degrading strain CpA1 with high emulsification activity from Iran seawaters. Later, also in Iran, *Pseudomonas balearica* S1-4-1 was also identified as the best emulsifying degrader marine strain of eighteen strains (among five pseudomonads, *Achromobacter xylooxidans*, two acinetobacteria, *Citrobacter freundii*, and some others) [24].

Oil-degrading microorganisms isolated in Kazakhstan belong to various taxonomic groups: actinobacteria, including the previously known deep-sea microorganism *Dietzia maris* (isolated from the soil of Kazakhstan), gamma-proteobacteria—pseudomonads and a representative of the genus *Enterobacter*, as well as eukaryotes—yeast and mold fungi (Table 1). It has been shown that the isolated sulfur-oxidizing chemoautotroph *Advenella mimigardefordensis* can utilize hydrocarbons [22]. Interestingly, the strain *Dietzia maris* 32d is able, in addition to soil and seawater, to survive in the reservoir water of an oil field [25].

Table 1. Taxonomic affiliation and source of isolation of petroleum-degrading microorganisms, Republic of Kazakhstan.

Microbial Group	Genus, Species, Strain	Source	Reference
<i>Prokaryotes (Bacteria)</i>			
Phylum Bacteroidetes			
	<i>Sphingobacterium kitahiroshimense</i> wkar54	Caspian Sea	[21]
Phylum Firmicutes, Class Bacilli			

Table 1. Cont.

Microbial Group	Genus, Species, Strain	Source	Reference
	<i>Bacillus amyloliquefaciens</i> I-15	soil/sewage	[26]
	<i>B. cereus</i> SBUG2056	Caspian Sea	[21]
	<i>B. aerius</i> KB-36		
	<i>B. cereus</i> IP-40-4		
	<i>B. cereus</i> P1-40-8		[27]
	<i>B. megaterium</i> P1-35-2		
	<i>B. subtilis</i> 72		
	<i>B. subtilis</i> 109KC	soil	[28,29]
	<i>Brevibacillus borstelensis</i> P2-50-2	soil	[30]
	<i>B. borstelensis</i> P2-50-5		[27]
Phylum Actinobacteria			
	<i>Arthrobacter luteus</i> 43-A	soil	[31]
	<i>Arthrobacter</i> sp. 12T		[32]
	<i>Arthrobacter</i> sp. 15T		[33]
	<i>Dietzia maris</i> 12K	soil	[19]
	<i>D. maris</i> 22K	soil	[34]
	<i>D. maris</i> 84T		[35]
	<i>D. schimae</i> 22K	soil	[19]
	<i>Gordonia alkanivorans</i> 25K	soil	[19]
	<i>G. amicalis</i> P1-35-14	soil	[30]
	<i>G. lacunae</i> 15K	soil	[19]
	<i>Microbacterium foliorum</i> 29K	soil	[19]
	<i>M. lacticum</i> 41-3	soil	[36]
	<i>Micrococcus roseus</i> 34	soil	[37]
	<i>M. roseus</i> 40	soil	[37]
	<i>Mycobacterium thermoresistibile</i> sp. 119-3GM		[38]
	<i>Rhodococcus equi</i> 51K		[38]
	<i>R. erythreus</i> AT7	soil	[34]
	<i>R. erythropolis</i> 7A	soil	[37]
	<i>R. erythropolis</i> 14K	soil	[19]
	<i>R. erythropolis</i> B12	soil	[39]
	<i>R. erythropolis</i> DH-1	soil	[26]
	<i>R. erythropolis</i> 119GM	soil	[29]
	<i>Rhodococcus erythropolis</i> KZ1	soil	[40]
	<i>R. erythropolis</i> KZ2	soil	[40]
	<i>R. erythropolis</i> SBUG2052	Caspian Sea	[21]
	<i>R. fascians</i> K3 (similarity 99%)	soil	[30]
	<i>R. globerulus</i> 51KC	soil	[29]
	<i>R. jialingiae</i> 4/5		[27]
	<i>R. jialingiae</i> 22PK		
	<i>R. maris</i> 65	soil	[41]
Phylum Proteobacteriae			
α -proteobacteriae	<i>Roseomonas</i> sp. wka124	Caspian Sea	[21]
	<i>Ochrobactrum</i> sp. wka148	Caspian Sea	[21]
β -proteobacteriae	<i>Achromobacter xylosoxidans</i> P2-35-9		[27]
	<i>A. pestifer</i> 25SH		
	<i>Achromobacter</i> sp. wkar55	Caspian Sea	[21]
	<i>Tetrathiobacter mimigardefordensis</i> (now <i>Advenella mimigardefordensis</i>) strains 24SH, 25SH, 26SH	Caspian Sea	[22]

Table 1. Cont.

Microbial Group	Genus, Species, Strain	Source	Reference
γ -proteobacteriae	<i>Acinetobacter calcoaceticum</i> 2A	soil	[36]
	<i>Azotobacter chroococcum</i>		[41]
	<i>Enterobacter</i> sp. 23SH	Caspian Sea	[42]
	<i>Pseudomonas</i> sp. 16-SH	Caspian Sea	[41]
	<i>Pseudomonas aeruginosa</i> 122AC	soil	[28,29]
	<i>P. azotifigens</i> 20K	soil	[19]
	<i>P. putida</i> KZ3	soil	[40]
	<i>P. xanthomarina</i> 17K	soil	[19]
	<i>P. stutzeri</i> A1		
	<i>Pseudomonas</i> sp. N2	artificial pond	[43]
	<i>P. alcaligenes</i> A5		
	<i>Serratia marcescens</i> N3K	soil/sewage	[26]
<i>Stenotrophomonas chelatiphaga</i> wkal49, <i>S. chelatiphaga</i> wkal51	Caspian Sea	[21]	
<i>Eukaryotes</i>			
yeasts	<i>Trichosporonoides</i> sp. V1		
	<i>Trichosporon cutaneum</i> R20CO2	soil	[28,29]
	<i>T. jirovecii</i> V2		
mold fungi	<i>Penicillium</i> sp.		
	<i>Mucor</i> sp.		
	<i>Endogone</i> sp.		[44]
	<i>Alternaria</i> sp.		
<i>Fusarium</i> sp.			
Order <i>Dothideales</i>	<i>Aureobasidium pullulans</i> P7	soil	[29]

Aitkeldieva et al. [30] studied in detail the spectrum of substrates consumed by isolated bacteria. The soil strains *Rhodococcus fascians* K-3, *Gordonia amicalis* P1-35-14, and *Brevibacillus borstelensis* P2-50-2 actively consumed *m*-xylene, naphthalene, and alkanes 1.7-methyltridecane (C₁₄H₃₀), dodecane, 2,6,10-trimethyl-pharnesan (C₁₅H₃₂), *n*-heptadecane (C₁₇H₃₆), pentadecane, 2,6,10,14-tetramethyl-norphitan (C₁₉H₄₀), Hexadecane, 2,6,10,14-tetramethyl-phitan (C₂₀H₄₂), and *n*-heneicosane (C₂₁H₄₄).

Pseudomonads *P. stutzeri* A1, *Pseudomonas* sp. N2, and *P. alcaligenes* A5, in addition to oil and diesel fuel, grew on toluene [43]. On the basis of active strains of hydrocarbon-oxidizing bacteria isolated from the soil of Western Kazakhstan, consortia have been created that effectively degrade a mixture of three isomers of xylene [42]. The consortium of *Bacillus megaterium* 1/1 an, *Bacillus thuringiensis* 2/4 fl, *Bacillus tropicus* 2/6 fl, and *B. megaterium* P1-fl1-5 strains utilized 89.8–95.5% of the studied substrates per day.

Kebekbayeva et al. [22] identified the most acceptable ways of storing oil-oxidizing microorganisms to prevent their loss of viability and cultural properties: under a layer of mineral oil and in a 10% layer of glycerin at low temperature. The authors of [39] suggest storing the *R. erythropolis* B12 strain under hypersalinity (NaCl 10%).

During the experiments, the concentration of oil is determined by gravimetry (weight method) [45–48], as well as using gas [19] and gas–liquid [41] chromatography. Mukhamedova et al. [45] used chromatography-mass spectrometry to determine the kinetics of the content of petroleum hydrocarbons of groups of alkanes, arenes and naphthenes during their degradation in the soil.

3. Bioremediation Approaches

In their research, Kazakhstan scientists used the well-known bioaugmentation method: after isolating the strains, their biomass was accumulated, followed by use in laboratory experiments on oil biodegradation in soil microcosms [41,45], and further scaling of the bioremediation process in the field [46].

The kinetics of the total number of soil microorganisms and their groups were studied [41,47]. It is shown that the number of many groups of soil microorganisms (spore-forming bacteria, mycelial fungi, actinomycetes) increased after the introduction of a complex organomineral fertilizer (manure, bird droppings, nitroammophos, ammonium nitrate) into the oil-contaminated soil both in laboratory soil microcosms [48] and in field conditions [47] of Akshabulak deposits (Kyzylorda region). As a result of experiments, the optimal amount of fertilizer applied to the soil was selected.

The microbiological and biochemical activity of soils under oil pollution in different doses was studied to assess the potential of soil self-purification. In that case the number of microorganisms and the intensity of respiration and lipase activity increased, which indicates the intensification of the processes of biodegradation of oil components of the soil microflora kurman [49].

Biotechnologies based on the use of microbial biopreparations made of the active biomass of hydrocarbon-oxidizing microorganisms are used to solve these environmental problems. For such microorganisms, hydrocarbons are a natural source of nutrition, therefore, in the process of vital activity, they multiply, consuming pollutants until that are completely exhausted.

It has been shown that the introduction of active cultures of microorganisms capable of oxidizing aliphatic, aromatic, and other hydrocarbons into the polluted soil of the Kazakhstan desert leads, as a rule, to an acceleration of soil clean-up and allows for the stability of the biological decay process at a relatively low cost of remediation [50–52].

The effect of the use of crushed zeolite aluminosilicate in bioremediation in laboratory [53] and microfield [41] conditions was studied, its positive effect on the change in the structure of microbiocenosis and the degree of soil clean-up from oil was revealed, the dose of application and the size of zeolite fractions were determined. At the K-Kurylys landfill of the Kumkol deposit in the Kyzylorda region, “Bakoil-KZ” biopreparation, with the addition of nitroammophos, biovermicompost, and zeolite, was tested [54].

Phytomeliorative methods are used at a low level of soil pollution as the final stage of purification. They are based on the sowing of appropriate varieties of herbs, during the development of which, due to the activation of microflora, the mineralization of petroleum hydrocarbons occurs. This method is auxiliary in nature and is used at the completion of work on the recultivation of oil-contaminated soils [52,55]. Cleaning of soil with a high degree of contamination is usually carried out with the extraction of contaminated soils.

The effectiveness of *in vitro* composting [56] the soil from the area of the Kumkol deposit, contaminated with oil up to 10%, in vessels with the addition of wheat straw and mineral salts was demonstrated, optimal mineral additives and their concentrations were selected, and the most appropriate dose of fluffier (wheat straw) was identified. The authors of [57], during composting in burst (Kyzylorda region), have shown that the selected cultivation conditions stimulate the growth of soil microflora and enhance the effectiveness of soil self-purification processes.

4. Enzymatic Activity of Oil-Degrading Microbes

The activity of dehydrogenase (DHA) and catalase (CA) characterizes the intensity of microbiological processes in the soil [58]. In conditions of soil pollution with oil products, catalase and dehydrogenase are involved in the decomposition of hydrocarbons. Catalase accelerates the oxidation of hydrocarbons, destroying the hydrogen peroxide formed as a result of the vital activity of microorganisms to the oxygen they need [59], and dehydrogenase catalyzes the dehydrogenation reaction—the elimination of hydrogen from hydrocarbons and their decomposition products [60].

CA increase, as well as its decrease in oil pollution, is associated with the dose of pollution, the type of polluting oil, and the buffer capacity of the soil [61]. Oil pollution can both inhibit DHA [61,62] and stimulate it [59,63,64].

According to [65,66], soil dehydrogenases, as the most sensitive enzymes to oil pollution, are inhibited to the greatest extent not by hydrocarbons themselves, but by intermediate products.

As a rule, in oil-contaminated soils, the activity of urease (UA), which hydrolyzes urea with the formation of ammonia and carbon dioxide, increases due to the content of organic C, the establishment of reducing conditions, and the presence of alkanes [59,67]. The change in its activity is in full accordance with the increase in the number of ammonifying microorganisms [59,68,69].

UA increase is noted during pollution against a background of medium and sometimes significant pollution, and a strong and very strong hydrocarbon content inhibits its activity [61]. A similar relationship was revealed in the case of oil pollution against the background of weak and strong salinization [70]. UA decrease can be attributed to the type of oil polluting, the dose, and the type of soil contaminated [61,69].

In the soils of three deposits in Kazakhstan [71], monitoring showed a threefold drop in the urease level, a 20% CA decrease and an increase in DHA levels from 30% to threefold, with an increase in the degree of chronic pollution from 0.5–0.9% and to the level of 2.8–3.4% (Table 2).

Table 2. Values of urease, catalase, and dehydrogenase activities in the oil-spilled soils from three Kazakhstan oil deposits [71].

Deposit	Enzyme	Clean Control	Medium Contamination 0.5–0.9%	Heavy Contamination 2.8–3.4%
Suhlтанат Балгимбаев	UA	4.4	3.1	1.1
Zhanatalap		5.5	3.3	0.8
Teren-Uzek		3.7	2.1	0
Suhlтанат Балгимбаев	DHA	0.4	0.7	1.2
Zhanatalap		0.4	0.5	0.6
Teren-Uzek		0.2	0.6	0.8
Suhlтанат Балгимбаев	CA	12.9	11.3	10.6
Zhanatalap		12.7	12.4	10.7
Teren-Uzek		10.6	11.0	7.8

Idrisova’s group investigated the change in dehydrogenase, catalase, and urease activity during oil biodegradation in laboratory and field conditions (Table 3).

In the soil from the Akshabulak field [46] with artificial 3% oil contamination, the initial UA was absent, and the dehydrogenase activity was suppressed. After four months of the microfield trial in the contaminated control, low UA (four times lower than the background) and increased DHA (two times higher than the background) were recorded. In the soil with oil and applied organic-mineral fertilizers, the UA value increased in comparison with the contaminated control and in some cases reached the level of pure soil, which could be associated with the stimulation of the activity of microorganisms.

Further, in the same place, the activity of the same enzymes was investigated at an artificially increased oil content of 5% and 7% [47]. Oil or its intermediates in three months suppressed UA (two to three times); however, with the introduction of fertilizers, UA increased (in the case of 5% oil, almost three times). Oil itself and fertilizers stimulated the DHA increase.

In laboratory and field conditions, the change in CA and DHA was studied when a combination of zeolite, vermicompost, nitroammophos and the biological product “Bakoil-KZ” was introduced into the oil-contaminated soil in the soil chronically contaminated with oil (10%), from the K-Kurylys landfill [54]. Interestingly, two months later, in a laboratory experiment, a threefold suppression of CA by oil and/or its intermediates was noted, and fertilizers contributed to the return of the CA level to a clean background. The DHA level seems to have returned to the background (clean) value. Intriguingly, under field

conditions, after the same time, the activity of both enzymes returned to the values of pure control, i.e., the process of degradation of available oil compounds was completed.

Table 3. Studies of research group headed by Prof. Idrisova focused on soil enzymes activities in oil-spilled soils of South Kazakhstan.

Experiments in the Soil at Akshabulak Oil Deposit							
Experiment Conditions	Enzyme	Before		After Four Months			
		Clean Control	Oil Control 3%	Oil Control	Oil Fertilizers		
Small field trials using organic and mineral fertilizers [46]	UA	0.66	0	0.155	0.2–0.9		
	DHA	0.131	0.085	0.214	0.6–0.8		
Experiments with the soil from K-Kurylys oil deposit							
	Enzyme	Before		After three months			
		Clean control	Oil control	Oil control		Oil fertilizers	
Small field trials using organic and mineral fertilizers [47]	UA	0.66	-	5%	7%	5%	7%
	DHA	0.131	-	5%	7%	5%	7%
Experiments with the soil from K-Kurylys oil deposit							
	Enzyme	Before		After two months			
		Clean control	Oil control 10%	Oil control 10%	Oil fertilizers		
Lab test At certain time intervals, loosening was carried out and the soil moisture was maintained at a level of 60% [54]	DHA	1.25		0.48	~1		
	CA	3.9		1.3	2.9–3.6		
Experiments with the soil from K-Kurylys oil deposit							
	Enzyme	Before		After two months			
		Clean control	Oil control 10%	Oil control 10%	Oil fertilizers		
Small field trials using zeolite, vermicompost, nitroammophos, and “Bakoil-KZ” [54]	DHA	1.25	-	1.38	~1		
	CA	3.9	-	4.7	3.1–4.2		

5. Microorganisms and Biopreparations to Remediate Oil-Spilled Soils in Deserts of Kazakhstan

Crude oil consists of some hundreds of different compounds, and in nature simultaneous consumption of petroleum occurs [72]. The representatives of the genera *Rhodococcus*, *Pseudomonas*, *Arthrobacter*, *Microbacterium* are the most common in oil-polluted sites [73]. Thus, multispecies biopreparations to fight oil spills seem to be promising.

There are several patents for single strains for combating soil and water pollution with oil products (four patents owned by a group headed by Prof. Sadanov [31–33,35] and a patent [39]); moreover, the efficiency of these destructors has been shown only under laboratory conditions and ranges from 55 to 90% (Table 4).

Table 4. Patented biopreparations/single strains for oil-spills bioremediation in Kazakhstan climate.

Biopreparation	Strain	Form	Application (if Mentioned)	Organization, Reference
Consortium	<i>A. calcoaceticum</i> 2A <i>M. lacticum</i> 41-3		to clean up soils from crude oil and petroleum products	Institute of Microbiology and virology [36,37]
“Bakoil-KZ”	<i>M. roseus</i> 40 <i>M. roseus</i> 34 <i>R. erythropolis</i> 7A bentonite as sorbent	paste	to clean up soils from crude oil and petroleum products	Institute of Microbiology and virology [37]
“Miko-Oil”	eight strains bacteria <i>B. subtilis</i> 109KC <i>P. aeruginosa</i> 122AC <i>R. erythropolis</i> 11GM <i>R. globerulus</i> 51KC yeasts <i>T. jirovecii</i> V2 <i>Trichosporonoides</i> sp. V1 <i>T. cutaneum</i> R20CO2 <i>A. pullulans</i> R7	paste	- high salinity (>4%) - a wide range of acidity (pH 4–10)	Al-Farabi Kazakh National University, KazEcoSolutions Inc. [28,29,74]
Consortium	<i>Penicillium</i> sp. <i>Mucor</i> sp. <i>Endogone</i> sp. <i>Alternaria</i> sp. <i>Fusarium</i> sp.	cell biomass, 10 ⁸ –10 ⁹ cells/ml	soil	M.Auezov South-Kazakhstan State University [44]
	<i>Arthrobacter</i> sp. 12T		to clean up soils and waters from crude oil and petroleum products 59.3–85.2%	Institute of Microbiology and virology [32]
	<i>Arthrobacter</i> sp. 15T		to clean up soils and waters from crude oil and petroleum products by 58.4–84.8%	Institute of Microbiology and virology [33]
	<i>D. maris</i> 84T		to clean up soils and waters from crude oil and petroleum products by 72.4–90.6%	Institute of Microbiology and virology [35]
	<i>A. luteus</i> 43-A		to clean up soils and waters from crude oil and petroleum products by 56.4–80.1%.	Institute of Microbiology and virology [31]
Consortium	host cyanobacterium <i>Phormidium</i> sp. K11 symbiotic degrader bacteria <i>P. stutzeri</i> A1 <i>Pseudomonas</i> sp. N2 <i>P. alcaligenes</i> A5		to clean up soils and waters from crude oil and petroleum products	Al-Farabi Kazakh National University [43]
“Enoil”	<i>R.erythropolis</i> DN-1 <i>B. amyloliquefaciens</i> I-15 <i>S. marcescens</i> N3K			Republic State Enterprise “National Center of Biotechnology” [26]
	<i>R. erythropolis</i> B12	biomass in water glycerol 1% and sodium chloride (10%) solution	to clean up soil and water ecosystems	Republic State Enterprise “National Center of Biotechnology” [39]

Table 4. Cont.

Biopreparation	Strain	Form	Application (if Mentioned)	Organization, Reference
“Mikot-rikh”	<i>M. thermoresistible</i> 119-ZGM <i>R. equi</i> 51KT <i>T. cutaneum</i> R20CO2	concentrated biomass		Al-Farabi Kazakh National University [38]
		concentrated paste	to clean up oil- and oil-product-spilled ecosystems and containers	Al-Farabi Kazakh National University [75]
Consortium	<i>R. erythropolis</i> KZ1 <i>R. erythropolis</i> KZ2 <i>P. putida</i> KZ3		soil	Korkyt Ata Kyzylorda State University [40]
“Peroil”	<i>R. erythropolis</i> DP 304 <i>M. luteus</i> B1Ag8G	lyophilizate		M.Auezov South Kazakhstan State University [76]
		lyophilizate and bentonite		[77]
“Kazbiorem”	<i>R. erythreus</i> AT7 <i>D. maris</i> 22K			Ecostandard.kz Ltd. [78]

For the patented biopreparations, only the results of laboratory tests (200 g of soil in vessels with 10% of crude oil) of “Kazbiorem” biopreparation in oil-contaminated soil from the Zhanatalap (5.3% pollution) and Kalamkas (2.4% pollution) deposits of Western Kazakhstan are known (Table 4) [34]. The degree of destruction in the soil from Zhanatalap reached 88.23% on the 7th day, and in the soil from Kalamkas 97.4% (in the control 9.3 and 6.4%, respectively). A consortium of strains *Acinetobacter calcoaceticum* 2A and *Microbacterium lacticum* 41-3 (Table 4) in model experiments reduced the oil concentration (initially 10%) by 64.4% in three months.

The consortium of strains *R. erythropolis* KZ1, *R. erythropolis* KZ2, and *P. putida* KZ3 (Table 5) has antagonistic activity against phytopathogenic fungi species *Fusarium graminearum* and *Gaeumannomyces graminis* var *tritici*, and the use of these strains in association on the lawn of micromycetes causes the appearance of zoning [40]. Now the results of field trials on the K-Kurylys landfill are processed.

As a rule, preparations are composed of two to three microbial strains; there are a couple of exclusions. “Miko-Oil” is a remarkable bioproduct and consists of eight degrade strains isolated from the soils of Western Kazakhstan: three strains of yeasts, an ascomycete, a bacilli strain, a pseudomonad, and two rhodococci strains. With its help, 20,000 tons of oil sludge and oily soils were processed on an area of about 4 hectares (Table 5). An association of five strains of molds is proposed to combat oil pollution (Table 4). A very interesting consortium of three degrader strains of pseudomonads, symbionts of the blue-green alga *Phormidium* has been developed [43]. To obtain a biological product, 100 mL of a suspension of the cyanobacterium *Phormidium* sp. K11 with a titer of 10^6 cells/mL was thoroughly shaken on a horizontal shaker and introduced into a 1 L plastic container, into which was placed a semi-liquid (with 0.5% agar) Zarruk medium with a volume of 600 mL [79]. After incubation for a month, a cyanobacterial paste of saturated dark green color with a homogeneous structure was obtained. 100 mL of *P. stutzeri* A1, *Pseudomonas* sp. N2 and *P. alcaligenes* A5 (titer 10^{12} cells/mL) mixed culture was added to the paste through the side of the container by piercing with the use of a disposable syringe. Six months after (from April to October 2011) the introduction of oil-oxidizing microorganisms in the soils, the concentration of C₁₄–C₂₇ and C₃₂–C₃₄ alkanes decreased by 3–10 times; the number of C₁₂–C₁₃ and C₂₈–C₃₁ decreased below the sensitivity threshold of the device (Table 5).

Table 5. Field trails of biopreparations in the soils of Republic of Kazakhstan.

Strain/Biopreparation	Test Place, Reference	Introduction Features	Pollutant, Concentration and Remediation Efficiency	
"Bakoil-KZ"	Kyzylorda Region [54] Small field trials on the landfill «K-Kurylys» plots 2 × 1 m (triple) periodic loosening and watering two months exposure	soil moisture 60% nitroammophos, biovermicompost, zeolite	Oil 10%	
			decrease by 68.3–82.2% (control 18.3%)	
"Bakoil-KZ"	Atyrau Region [37] landfill of "West Dala" LLC - landfill of 67.5 m ² - six plots 11 m ² each were - upper soil layer was isolated by strong plastic film - triple plowing down to 200 mm - using bentonite as sorbent	first inoculation—10 kg with 600 L water second inoculation—5 kg with 600 L water - introduction of organic (manure) and mineral (NH ₄ NO ₃) fertilizers		decrease by 80% (control 18%)
			Oil	
"Bakoil-KZ"	Atyrau Region [29] oil deposits one month exposure	one inoculation	"Bakoil-KZ": decrease by 73%	
"Miko-Oil"		double inoculation	"Miko-Oil": decrease by 99%	
"Miko-Oil"	Mangystau Region [74] test plot on "Uzen" oil deposit plowing, loosening	double inoculation application of organic and mineral fertilizers	oil	efficiency more than 93%
"Miko-Oil"	Atyrau Region [74] Oil Producing Companies "DossorMunaiGas", "Zhaikmunaigas", "ZhylyoiMunaiGas», JSC "EmbaMunaiGas" territories (four hectares) and oil sludge		Oil sludges (20,000 tons) and fuel oily soils	
Consortium of five molds	the city of Shymkent [44] trials on a territory of Research Institute of Problems in Biological Safety Bishimbaev ten plots of 1 m ² each, artificial black oil pollution. One month	ammophos 1%	Oil 10%	Fuel oil 10%
			Decrease by 75.1%	Decrease by 62.3%
	the city of Shymkent [44] Oil chronically spilled soils on a territory of "PetroKazasktan Oil Products" Ltd. Total area of spills was 325 m ² . Two weeks Plowing was made	ammophos 1%	~Oil 5.4%	Decrease by 69.8%

Table 5. Cont.

Strain/Biopreparation	Test Place, Reference	Introduction Features	Pollutant, Concentration and Remediation Efficiency				
Bacterial-cyanobacterial consortium	Aktau Region [43] field trials on a storage landfill of “KhimPromService—Aktobe” - plots 1 m ² each - periodic watering in six months	- the paste was diluted by water till 10 ⁶ cells/mL - consortium was added at the rate of 1 L/kg	fuel oil decrease by 80%				
“Enoil”	Zhambyl Region [26] a base of Eastern branch of “Fuel and Energy Complex—Kazakhstan”, the city of Tharaz two plots with chronic and emergency petroleum products spills; soil was loosened to a depth of 10–15 cm Soil was watered and mixed before and after biopreparation inoculation into the soil Four months	Concentrated suspension 10 ¹⁰ cells/mL was added into the soil until the final titer 10 ⁶ cells/g soil biopreparation was started in vessels of 200 L each with tap water, mineral medium, and diesel fuel. Nitroammophos was added	Oil 0.2%		Oil 0.3%		
			Decrease by 76.2%		Decrease by 82%		
“Mikotrikh”	Site unknown [38] three plots of 6 m ² each and 0.5 m in depth with high content of oil products (diesel fuel and black oil), regular plowing three times every month	Bacterial and yeasts cultures were mixed in the ratio 1:1. Concentrated biomass was diluted in water and added together with nitroammophos and sawdust	Diesel fuel 86% control by 17%		Fuel oil 37%		
“Mikotrikh”	the city of Almaty [75] small scale field trials on chronically fuel oiled soils (spill depth 0.5 m) of locomotive and carriage depots, temperature 28–35 °C, no rains	Concentrated biomass of single strains was mixed in the ratio 1:1:1 before introduction	Fuel oil (10%) Decrease by 89.5% (control by 13.2)				
“Peroil”	Site unknown [51] plowing every month and regular watering six sites with regular and single pollutions: Total area of territories under recultivation was three hectares, period—1.5 months	soil moisture 50–60% 1% ammophos	Oil 0.5%	Diesel fuel 1.0%	Fuel oil 2.5%	Sludge-like waste 2.3% >10% 9.9%	
			Content after bioremediation 0.05% 0.51% 0.27% 0.33% 0.99% 0.98%				
“Peroil”	the city of Shymkent [77] Territory of JSC “PetroKazakhstan Oil Products”. Total area was 6 hectares, the soil volume was 25 tons bentonite as an immobilizer with water suspension of lyophilized form		Oil 5.4%		Fuel oil 4.7%		
			content decreased by 66.2% in 15 days		content decreased by 73.1% in 20 days		

When being applied in situ, most of the biopreparations require additional organic and mineral fertilizers. Idrisova et al. [54] carried out small-plot field trials at the K-Kurylys test site of the Kumkol deposit in the Kyzylorda region using various combinations of nitroammophos, biovermicompost, zeolite, and the biopreparation “Bakoil-KZ”. Almost all biopreparations are offered in suspension form, only the authors of “Peroil” developed the lyophilized biopreparation—as free, and together with bentonite, a mineral sorbent. It should be noted that bentonite was used in a field experiment when studying the efficiency of “Bakoil-KZ” on “West Dala” LLC landfill [37]. The natural process of self-purification of the soil from oil under favorable conditions in a month led to the degradation of oil by 18%. After the introduction of “Bakoil-KZ”, the remediation process increased by 80% minus control.

In the soils of Atyrau oil deposits, “Bakoil-KZ” and “Myco-Oil” were tested [29]. After the double application of the biological product “Miko-Oil”, the degradation of crude oil in the soil was 99% as compared to the preparation “Bakoil-KZ” (73% utilization). We assume that the exposure took a month or more.

6. Conclusions

It is known that 60% of the world’s oil is located in areas with a hot climate [80], and it requires the development and application of adequate technologies for the remediation of oil-contaminated areas. Kazakhstani researchers are actively working on solving the problem of bioremediation of oil-contaminated desert soils as judged by the list of references in the review.

Analyzing the results testifies that researchers often overlook the influence of environmental factors (temperature, humidity) on the degradation process of oil and oil products, with insufficient data to assess the dynamics of the process (often data are collected only at the beginning and end of the experiment). Studies of the succession of microbial communities are rare, and methods of genomics (denaturing gradient gel electrophoresis of species-specific genes amplicons, fluorescence in situ hybridization, etc.), metagenomics, and transcriptomics, seemingly, have almost not been used for microorganisms in Kazakhstan ecosystems. Interestingly, still open for study are the issues of the presence and expression of heat and cold shock proteins in oil-degrading bacteria inhabiting the soil of the Kazakhstan deserts, that are capable of enduring from +60 °C on soil in summer to −50 °C in winter.

For the degradation of crude oil and its products in hot climates, the use of “thermotolerant” microorganisms, for which the conditions of a hot arid climate are not excessively stressful, could prove to be hopeful [81]. Such bacteria have a growth optimum that is shifted in comparison with mesophilic microorganisms. The development of biological products based on consortia of thermotolerant bacteria and their use for cleaning soil and water can be a promising solution.

When carrying out bioremediation measures, it is necessary to take into account not only the physicochemical characteristics of oil pollution, but also the weather and landscape conditions of the site. In countries with arid climates, such as Kazakhstan, it seems to be not fruitful to carry out remediation procedures in the midsummer, as the soil dries up, and microbiological activity in the soil decreases. Issaeva et al. [82] suggest starting bioremediation procedures for the autumn period to significantly reduce water consumption.

Therefore, the development of a technology for the remediation of oil-contaminated soils in an arid climate should include the search for the optimal time and conditions for the life of hydrocarbon-oxidizing microflora in the soil. Depending on the features of oil pollution and climatic conditions, bioremediation technologies should vary in a wide range of biotechnological possibilities, including, sequential stages of microbial recultivation, phytoremediation and vermicultivation.

Analysis of the current state of research in the field of biodegradation of hazardous pollutants and bioremediation of oil-contaminated areas in the Republic of Kazakhstan

allows us to determine promising areas for further work and approaches to the development and improvement of technologies for cleaning and protecting the environment.

Author Contributions: L.I.A.—discussion, writing, preparation of the manuscript; I.F.P.—writing, preparation of the manuscript, editing; R.A.N.—data analysis, discussion of the material, search for articles; N.O.A.—data analysis, discussion of the material, editing; T.V.F. and A.A.R.—summarizing data, search for articles, web information; A.E.F.—analysis of data, discussion of the material, coordination. All authors have read and agreed to the published version of the manuscript.

Funding: The reported study was funded by RFBR * and BRFFR **, project number 20-54-00002 “Peculiarities of the synthesis of surface-active compounds by bacteria that efficiently utilize oil at low and high temperatures”. * Russian Foundation for Basic Research; ** Belorussian Foundation for Basic Research.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available from public libraries/open sources according to references.

Conflicts of Interest: The authors declare no conflict of interest.

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