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Bioremediation of oil-polluted coastal soils of the Caspian Sea with active oil-oxidizing associations

AmankeldySadanov,Svetlana Aitkeldiyeva, Elmira Faizulina, Olga Auezova, Larissa Tatarkina, ArayNurmukhanbetova
Institute of Microbiology and Virology, Kazakhstan
E-mail: ecomicrolab@gmail.com

ABSTRACT
Model studies on the purification of oil-polluted coastal soil by bacterial and bacterial-yeast associations were carried out. It is established that when they are introduced, changes in the composition of the microbiocenosis occur. In this case, the number of heterotrophic bacteria and hydrocarbon-oxidizing microorganisms is significantly increased. During the entire experiment, the bacterial-yeast association showed the greatest activity. Under its impact, oil utilization amounted to 62.3-84.0%. In this case, the use of manure with ammonium nitrate was more effective than the introduction of urea. Experimental data showed that more intensive destruction of oil occurs with the introduction of preparations in an amount of 1-2 g per 300 g of contaminated soil.
***Keywords*:**bioremediation, oil-polluted soil, association of oil-oxidizing microorganisms, oil destruction, organic-mineral fertilizers

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I. INTRODUCTION

At the current level of the oil industry development, it is not possible to completely exclude its negative impact on the environment. Purification of natural ecosystems from oil pollutants is an important task of environmental biotechnology. Successful, ecologically safe remediation of such pollutions is possible only due to a microbiological method using active cultures of oil-oxidizing microorganisms [1-3]. Therefore, the main efforts are aimed at the search for microorganisms-destructors capable of degrading pollutants that are resistant to unfavorable environmental conditions and stably retaining their biodegradable potential, the introduction of which leads, as a rule, to acceleration of soil purification from oil [4, 5].

The multistage nature of the biochemical processes forhydrocarbondecomposition by different groups of microorganisms, complicated by the diversity in the chemical composition of oil and petroleum products, makes difficult the regulation of the stable process for their decomposition. Therefore, the participation of consortia of microorganisms-destructors belonging to different taxonomic groups is required to degrade all components of crude oil and completelydecompose them [6-8].

The purpose of this study is to examine the destructive ability of associations of oil-oxidizing microorganisms isolated from coastal soils of the Caspian Sea in a model experiment.

II. MATERIALS AND METHODS

To set up a laboratory model experiment on the purificationof oil-polluted coastal soilwith active associations of microorganisms, 300 g of preliminary grinded and sifted soil, artificially polluted with oil from the Kashagan field, were introduced into plastic vessels in an amount of 5%.

Two associations of active oil-oxidizing microorganisms were introducedin the experimental variants. The first association included strains of *Pseudomonas xanthomarina* 17K, *Rhodococcuserythropolis* 28K, *Dietziamaris* 84T, the second one - *Acinetobacter* sp. 15PK, *Rhodococcus* sp. 22PK, *Yarrowialipolytica* 11d. The associations were introduced in the form of a pasty preparation made with the use ofbentonite in an amount of 0.5, 1.0, and 2.0 g. Mineral and organic fertilizers were added in the following amounts: ammonium nitrate - 178 mg/300 g, manure - 10 mg/ 300g, urea - 134 mg/300 g. The soil was periodically moistened and loosened. Oil-polluted soil, soil with the addition of fertilizers without the introduction of microorganisms servedas control. The number of major groups of microorganisms was determined by inoculating the appropriate selective nutrient media with soil suspension [9].

The residual oil content in the soil was determined

using the gravimetric method [10].

III. RESULTS AND DISCUSSION

A laboratory model experiment on the purification of oil-contaminated coastal soilswith active associations of microorganisms wasset up. As is known, bioremediation of oil-contaminated soils is more effective with the use not only of active microorganisms-destructors, but also with the addition of organomineral fertilizers [11, 12]. To determine which fertilizers are needed, chemical soil analysis was performed. It was found that there was an increased phosphorus(38-45 mg/kg) and very low nitrogen content (14-16.8 mg/kg) in the soil. Inthisconnection, onlynitrogenfertilizerswereintroduced.

Prior to the introduction of microbial associations, organic and mineral additives, the number of major groups of microorganisms in the control background soil was determined. Soil oil pollution led to a decrease in the number of heterotrophic bacteria by one order of magnitude, the number of mycelial fungi increased in the same way (Table 1). The number of actinomycetes did not change and was about 10^5 CFU/g. Since the soil contained a significant amount of mobile phosphorus, the number of phosphate-mobilizing bacteria was also high - 10^5 CFU/g. With oil pollution, their number decreased by an order of magnitude.

The results of the study showed that the introduction of associations of oil-oxidizing microorganisms together with organomineral fertilizers in 1 month resulted in an increase in the number of heterotrophic bacteria as compared to the control. When manure was applied with ammonium nitrate, their number increased by two orders of magnitude, and was even higher by an order of magnitude than in pure, unpolluted soil. Under the effect of associations 1 and 2 together with urea, an increase in the number of heterotrophic bacteria by one and two ordersof magnitude, respectively, was observed.

1 month later, there was a significant decrease in the number of phosphate-mobilizing microorganismsin the control variants of the experiment. When the associations were introduced into the soil, their titer was within 10^3-10^4 CFU/g.

The number of actinomycetes in all variants of the experiment was practically on the same level and amounted to 10^3-10^4 CFU/g, but it was one order of magnitude less than in the initial soil. In almost all cases, the number of mycelial fungi reached 10^3CFU/g. Yeast microorganisms were not identifiedin the initial soil samples and control variants, whereas the introduction of the examined associations together with the fertilizers promoted their development.

The number of hydrocarbon oxidizing microorganisms in pure, unpolluted soil was 10^3 MPN cells/g. 7 days after the pollution, their number remained at the same level, because for such a period of time this group of microorganisms had not yet developed. 1 month after the experiment was set, their content in the soil control samples increased by one order of magnitude. The introduction of associations of oil-oxidizing microorganisms contributed to an increase in their number up to 10^5-10^6 MPN cell/g.

In 2 months, no significant changes in the number of heterotrophic bacteria were observed in the control variant with the addition of ammonium nitrate and manure. In control variants without fertilizers and with urea, their number increased by an order of magnitude. In experimental variants with the introduction of association 1 together with urea there was also an increase in the number of this group of microorganisms by 1 order of magnitude. No significant changes in their numbers were recordedin the remaining experimental variants.

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Table 1.The number of major groups of microorganisms in soil with 5% oil pollution in 1 month

Experiment variants	Heterotrophs, cell/g	Phosphate-mobilizing bacteria, cell/g	Actinomycetes, cell/g	Yeast, cell/g	Fungi, cell/g	HOM, MPNcell/g*
Uncontaminated original soil	$(3.0\pm0.3)\times10^{7}$	$(1.3\pm0.3)\times10^{5}$	$(3.9\pm0.4)\times10^{5}$	-	$(1.2\pm0.3)\times10^{4}$	10^{3}
Soil 7 days after 5% oil contamination	$(3.2\pm0.4)\times10^{6}$	$(3.0\pm0.9)\times10^{4}$	$(3.1\pm0.4)\times10^{5}$	-	$(1.2\pm0.2)\times10^{5}$	10^{3}
Control 1.Soil + oil	$(1.1\pm0.07)\times10^{6}$	-	$(8.5\pm0.9)\times10^{3}$	-	$(2.4\pm0.3)\times10^{3}$	10^{4}
Control 2. Soil + oil + urea	$(4.0\pm0.9)\times10^{7}$	units	$(1.2\pm0.2)\times10^{4}$	-	$(4.5\pm0.9)\times10^{3}$	10^{4}
Control3.Soil + oil + manure + ammonium nitrate	$(4.6\pm0.5)\times10^{8}$	units	$(1.2\pm0.2)\times10^{4}$	-	$(3.2\pm0.4)\times10^{3}$	10^{4}
Soil + oil + manure + ammonium nitrate + ass.1 (0.5 g)	$(4.0\pm0.1)\times10^{8}$	$(8.0\pm0.9)\times10^{3}$	$(1.4\pm0.3)\times10^{5}$	$(1.43\pm0.3)\times10^{3}$	$(1.3\pm0.3)\times10^{3}$	10^{5}
Soil + oil + manure + ammonium nitrate + ass.1 (1.0 g)	$(3.8\pm0.1)\times10^{8}$	$(1.5\pm0.3)\times10^{4}$	$(1.2\pm0.2)\times10^{4}$	units	$(1.02\pm0.2)\times10^{3}$	10^{5}
Soil + oil + manure + ammonium nitrate + ass.1 (2.0 g)	$(9.0\pm0.1)\times10^{8}$	$(4.0\pm0.9)\times10^{3}$	$(1.5\pm0.3)\times10^{4}$	$(1.6\pm0.3)\times10^{4}$	$(1.2\pm0.2)\times10^{3}$	10^{6}
Soil + oil + urea + ass. 1 (0.5 g)	$(1.4\pm0.08)\times10^{7}$	$(1.0\pm0.2)\times10^{4}$	$(6.0\pm0.9)\times10^{3}$	units	$(1.9\pm0.1)\times10^{3}$	10^{4}
Soil + oil + urea + ass. 1 (1.0 g)	$(5.8\pm0.5)\times10^{7}$	$(8.0\pm0.2)\times10^{3}$	$(9.0\pm0.8)\times10^{3}$	$(4.5\pm0.3)\times10^{3}$	$(1.4\pm0.3)\times10^{3}$	10^{5}
Soil + oil + urea + ass. 1 (2,0 g)	$(4.1\pm0.5)\times10^{7}$	$(9.0\pm0.2)\times10^{3}$	$(1.0\pm0.2)\times10^{4}$	units	$(2.2\pm0.3)\times10^{3}$	10^{5}
Soil + oil + manure + ammonium nitrate + ass.2 (0.5 g)	$(2.0\pm0.1)\times10^{8}$	$(4.0\pm0.8)\times10^{3}$	$(1.1\pm0.2)\times10^{4}$	-	$(1.8\pm0.3)\times10^{3}$	10^{5}
Soil + oil + manure + ammonium nitrate + ass.2 (1.0 g)	$(1.6\pm0.09)\times10^{8}$	units	$(6.0\pm0.9)\times10^{4}$	-	$(1.4\pm0.3)\times10^{3}$	10^{5}
Soil + oil + manure + ammonium nitrate + ass.2 (2.0 g)	$(1.4\pm0.08)\times10^{8}$	units	$(1.3\pm0.3)\times10^{4}$	-	$(1.6\pm0.3)\times10^{3}$	10^{6}
Soil + oil + urea + ass. 2 (0.5 g)	$(10.4\pm0.7)\times10^{7}$	$(6.0\pm0.8)\times10^{4}$	$(1.6\pm0.3)\times10^{4}$	units	$(1.0\pm0.2)\times10^{3}$	10^{5}
Soil + oil + urea + ass. 2 (1.0 g)	$(1.1\pm0.07)\times10^{8}$	$(9.0\pm0.8)\times10^{3}$	$(9.0\pm0.8)\times10^{3}$	$(7.0\pm0.9)\times10^{2}$	$(1.1\pm0.2)\times10^{3}$	10^{5}
Soil + oil + urea + ass. 2 (2.0 g)	$(1.4\pm0.08)\times10^{8}$	$(7.0\pm0.9)\times10^{3}$	$(1.3\pm0.3)\times10^{4}$	$(1.4\pm0.3)\times10^{3}$	$(1.5\pm0.3)\times10^{3}$	10^{5}

*HOM – hydrocarbon-oxidizing microorganisms, MPN cell/g – the most probable number of cells in 1 g

In 2 months, no significant changes in the number of heterotrophic bacteria were observed in the control variant with the addition of ammonium nitrate and manure. In control variants without fertilizers and with urea, their number increased by an order of magnitude. In experimental variants with the introduction of association 1 together with urea there was also an increase in the number of this group of microorganisms by 1 order of magnitude. No significant changes in their numbers were recordedin the remaining experimental variants.

The number of phosphate-mobilizing microorganisms decreased sharplyafter 2 months. This is probably due to the fact that mobile phosphorus was intensively used by the entire complex of hydrocarbon oxidizing microorganisms during the destruction of oil hydrocarbons.

The number of actinomycetes practically did not change, butthat of yeasts in most variants decreased. The biomass of mycelial fungi was one orderof magnitudelarger in the variants with the addition of manure and ammonium nitrate, both in the control and experimental samples, as compared with the 1 month incubation of the examined associations.

The number of hydrocarbon oxidizing microorganisms remained at the same level, except for the control variants with oil polluted soil and soil with urea, where it decreased by 1 orderof magnitude.

The residual oil content in all variants of the experiment was determined after 1 and 2 months (Table 2, 3). The results of the study showed that when the association 1 was introduced into the soil with 5% contamination, the degree of oil destruction in a month was 38.8-59.5%. Association 2 was more active, and under its impact oil utilization was 49.7-65.2%. The natural loss of oil was 7.2%, while with the addition of urea or manure with ammonium nitrate to the polluted soil it reached 10.5% and 14.2%, respectively.

Table2. Oil destruction in soil with 5% oil pollution in 1 month

Research Associations	Dosage injections, g/300 g ofsoil	Experiment variants	
		Soil + oil + manure + ammonium nitrate	Soil + oil + urea
		Degree of destruction, %	
Association 1	0.5	47.1	38.8
	1.0	59.5	53.3
	2.0	57.9	45.0
Association 2	0.5	62.4	54.1
	1.0	62.2	49.7
	2.0	65.2	57.0
Control	-	14.2	10.5
Note: In the control (soil + oil), the degree of oil destruction is7.2%			

2 months later, the amount of utilized oil increased both in the control and experimental versions (Table 3). Association 2 was the most active, when it was introduced together with urea, the oil loss was over 60%, and in combination with manure and ammonium nitrate - over 80%. It was found that the most optimal fertilizers are manure and ammonium nitrate. Their introduction together with the examined associationsincreased the oil destruction of by 8.2% -19.4% compared with urea. The number of hydrocarbon oxidizing microorganisms in pure, unpolluted soil was 10^3 MPN cells/g. It was shown that the most effective oil destruction in the soil under the effect of association 1 occurred at a dose of 1 and 2 g/300 g soil. At the same time, the degree of oil utilization by association 2 at all the doses studied was approximately the same level.

Table3. Oil destruction in soil with 5% oil pollution in 2 month

Research Associations	Dosage injections, g/300 g ofsoil	Experiment variants	
		Soil + oil + manure + ammonium nitrate	
		Degree of destruction, %	
Association 1	0.5	63.3	44.5
	1.0	69.5	61.3
	2.0	73.4	57.2
Association 2	0.5	80.2	62.3
	1.0	82.1	66.5
	2.0	84.0	64.6
Control	-	29.6	28.1
Note: In the control (soil + oil), the degree of oil destruction is 13,9%			

IV. CONCLUSION

Model studies were carried out on the purification of oil-polluted coastal soil with bacterial and bacterial-yeast associations: 1) *Pseudomonas xanthomarina* 17K, *Rhodococcuserythropolis* 28K, *Dietziamaris* 84T; 2) *Acinetobacter* sp. 15PK, *Rhodococcus* sp. 22PK, *Yarrowialipolytica* 11d. It was established that when they are introduced, changes in the microbiocenosis composition occur. At that, the number of heterotrophic bacteria and hydrocarbon-oxidizing microorganisms significantly increases.

During the entire experiment, the bacterial-yeast association exhibited the greatest activity. Under its effect oil utilization amounted to 62.3-84.0%. At the same time, the use of manure with ammonium nitrate was more effective than the introduction of urea.

Experimental data showed that oil destruction occurs more intensively with the introduction of preparations in an amount of 1-2 g per 300 g of polluted soil. Since there was no significant difference in the degree of oil utilization at these doses, a dose of 3 g/kg of oil can be considered economically justified.

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