

The influence of associations of hydrocarbon oxidizing microorganisms to the microbial cenosis and oil destruction in soil

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ABSTRACT

Mesocosms experiments were used to study the influence of associations of hydrocarbon oxidizing microorganisms on the microbial cenosis and oil destruction in soil. The introduction of active oil oxidizing microorganisms associations into the soil purify of soil from oil to 59.8% - 63.8% after 2 months with 5% oil content and with 10% to 49.2% - 55.6%. As a result of oil destruction there was enlarging of the number of all investigated groups of microorganisms.

Keywords: Oil Oxidizing Microorganisms; Bioremediation; Associations of Hydrocarbon Oxidizing Microorganisms; Oil Polluted Soils

1. INTRODUCTION

The soil pollution owing to oil and oil products which increases by high industrial activities becomes one of the main factors, which endanger the environment. Oil products are kept long time because of high adsorptions abilities of soil, changing microbiological and biochemical processes, make structural changes of biocenosis, low activity of soil-forming processes [1-3]. There is a complex pollution of soils: except of oil products, several heavy metals arrive into the soil, it occurs salinization soils by salted reservoir waters. They disturb water, nitric and phosphoric regimes of soil. The part of not hydrolyzed residue in soil humus content increases [4].

The area of the oil polluted soils in the Western Kazakhstan makes 194 thousand hectares, and volume of the poured oil-more than 5 million tons. Improvement of environment in oil-winning regions of Kazakhstan becomes an urgent state problem. In this connection the most important problem becomes a problem to invent technologies for production and use of biological preparation and restoration and rehabilitation of soils in oil-producing regions of Republic of Kazakhstan.

In spite of the fact that modern practice of recultivation works possesses many varieties of methods for cleaning soil from the oil products, the full restoration of biocenoses is provided only with those technologies in which basis is put the biological method, or bioremediation. The technology of bioremediation is based on biodegradation of hydrocarbons of oil owing to acting of hydrocarbon oxidizing microorganisms. Complexity of biodegradation by oil microorganisms depends on multicomponent and heterogeneity of substances which build it, therefore the creation of the associations consisting of microorganisms, capable to destroy different fractions of oil, certainly, it is a perspective direction at bioremediation of polluted ecosystem [5-7].

In this connection it can be important for Kazakhstan to develop and introduce highly effective biotechnologies on the basis of using local strains of microorganisms- destructors of oil hydrocarbons.

The complexity of biodegradation of petroleum products is heterogeneous and multi-component materials that build up the oil, so it is important to purify oil-contaminated soil to form associations which have a different ability to dispose of some fractions of petroleum hydrocarbons.

The subject of this research was to study oil degradation in the soil by active association of hydrocarbon oxidizing microorganisms, as well as the study of quantitative changes in the composition of the soil microbial cenosis.

2. MATERIAL AND METHODS

For the estimation of influence of active associations of bacteria for destruction in soil and on changing microbial was performed modeling experiments with 5% and 10% oil polluted soil. The soil was selected from horizon 0 - 10 sm. In plastic containers placed on 100 g soils, then it is artificial polluted by oil. The associations consist of strains *Arthrobacter* sp. P1, 24, K3 and *Bacillus subtilis* 72.

Experiment consists of following variants:

- 1) Soil + oil 5 g/100 g soils (control);
- 2) Soil + oil 5 g/100 g soils + association 1 (strains P1 + 24);
- 3) Soil + oil 5 g/100 g soils + association 2 (strains P1 + 24 + K3 + 72);
- 4) Soil + oil 10 g/100 g soils (control);
- 5) Soil + oil 10 g/100 g soils + association 1 (strains P1 + 24);
- 6) Soil + oil 10 g/100 g soils + association 2 (strains P1 + 24 + K3 + 72).

Titer of the brought cells has made 10^9 cell/g. $(\text{NH}_4)_2\text{HPO}_4$ is used as a mineral nutrition. Soil aeration was periodically spent; humidity was supported at level of 60% total moisture capacity.

The degree of oil destruction is defined by gravimetric method [8].

Number of the basic groups of microorganisms in the oil polluted and pure soil is defined by the standard microbiological methods [9].

Heterotrophic bacteria were considered on meat-peptone agar, spore bacteria-on the same medium after pasteurization at 80°C within 10 minutes, Petri dishes were cultivated at 28°C - 30°C during 5 days.

Oligotrophic microorganisms were considered on a poor agar, actinomycetes-on starch-ammoniac agar, cultivation spent at 28°C - 30°C during 7 days.

Micromycetes were considered on the Chapek medium, Petri dishes cultivated at 28°C - 30°C during 10 days.

Number of hydrocarbon oxidizing microorganisms defined by the method of limiting cultivations on Voroshilova-Dianova's medium. The oil from deposit Kosshagyl in quantity 1 ml on 100 ml of medium is used as a carbon and energy source. Test flasks cultivated during 14 days at 28°C - 30°C.

All experiments were performed in triplicate. Statistical processing of the received data spent by means of program Excel.

3. RESULTS AND DISCUSSIONS

During modeling experiment was defined the degree of oil destruction in soil after 1 and 2 months after enter-

ing of bacterial associations.

It is established that in soil samples with 5% oil content by entering of associations of active oil oxidizing microorganisms its recycling after 1 month has made 40.9% - 41.7% whereas in the raw polluted soil the natural decrease of oil was 10.9% (**Table 1**). After 2 months the quantity of oil in experimental samples has made 36.2% - 40.2% from the initial content. Thus the greatest activity destruction oil is found out in association 1.

At 10% oil pollution after 1 month after entering of associations of oil oxidizing bacteria the quantity of the utilized oil was approximately the same as well as at 5% oil pollution. But after 2 months the activity of introduced microorganisms had a little decrease, and the oil decrease in soil was lower and made 49.2% - 55.6 %.

During modeling experiment number of the basic groups of microorganisms in the oil polluted soil has been defined.

After 7 days after pollution in control samples there was a change of number of all investigated groups of the microorganisms allocated on firm environments. There was reorganization of microbes community and without that rather poor soil towards reduction of a biomass microflora (**Table 2**).

The opposite picture was observed at the account hydrocarbon oxidizing microorganisms. If in not polluted soil their number corresponded to thousand cages in 1 g soils at 5% pollution it has increased to 10^4 cell/g and at 10% - 10^5 cell/g. Thus it is possible to tell that the part microbial was switched to consumption of the brought hydrocarbon substratum but for some microorganisms it has appeared toxic.

Investigation of these same groups of microorganisms in the control sample in a month after the introduction of 5% of the oil showed that there was an increase in the number of heterotrophic, oligotrophic and spore-forming bacteria and actinomycetes in relation to the original data (**Table 3**). The quantity micromycetes and hydrocarbon oxidizing microorganisms remain at former level. It is connected by that for this period of time the microflora most part already adapted for an oil substratum.

Table 1. Degree of recycling of oil in soil samples of modeling experiment.

Variants	Destruction of oil. %			
	5%		10%	
	1 month	2 months	1 month	2 months
Control	9.7	10.9	9.9	10.9
Association 1	40.9	63.8	41.9	55.6
Association 2	41.7	59.8	39.4	49.2

Table 2. Quantity of the basic groups of microorganisms in initial samples of soil of modelling experiment.

Variant	Heterotrophic bacteria	Spore-forming bacteria	Oligotrophic bacteria	Actinomycetes	Micromycetes	Hydrocarbonoxidizing microorganisms*
1) Unpolluted soil	$(4.3 \pm 0.92) \times 10^5$	$(2.9 \pm 0.24) \times 10^6$	$(7.3 \pm 1.2) \times 10^5$	$(2.3 \pm 0.67) \times 10^5$	$(2.0 \pm 0.63) \times 10^3$	10^3
2) Soil with 5% oil	$(4.7 \pm 0.6) \times 10^4$	$(1.3 \pm 0.16) \times 10^3$	$(2.0 \pm 0.2) \times 10^4$	$(3.5 \pm 0.84) \times 10^4$	$(1.1 \pm 0.46) \times 10^3$	10^4
3) Soil with 10% oil	$(6.4 \pm 1.1) \times 10^3$	$(1.3 \pm 0.16) \times 10^3$	$(1.5 \pm 0.56) \times 10^3$	$(9.0 \pm 1.34) \times 10^3$	$(0.95 \pm 0.4) \times 10^2$	10^5

*-the most probable number of cells in 1 g.

Table 3. Number of the basic groups of microorganisms in soil at 5% oil pollution with associations of oil oxidizing bacteria.

Variant	Heterotrophic bacteria	Spore-forming bacteria	Oligotrophic bacteria	Actinomycetes	Micromycetes	Hydrocarbon oxidizing microorganisms*
1 month						
oil-contaminated soil	$(3.1 \pm 0.24) \times 10^6$	$(1.3 \pm 0.27) \times 10^4$	$(2.3 \pm 0.2) \times 10^5$	$(4.7 \pm 0.14) \times 10^5$	$(1.0 \pm 0.4) \times 10^3$	10^4
oil-contaminated soil + A1	$(4.6 \pm 0.14) \times 10^7$	$(5.1 \pm 0.9) \times 10^5$	$(4.7 \pm 0.6) \times 10^6$	$(8.0 \pm 0.9) \times 10^6$	$(1.0 \pm 0.4) \times 10^3$	10^6
oil-contaminated soil + A2	$(2.6 \pm 0.20) \times 10^7$	$(4.3 \pm 0.7) \times 10^5$	$(5.3 \pm 0.6) \times 10^7$	$(1.7 \pm 0.18) \times 10^7$	$(1.0 \pm 0.4) \times 10^3$	10^6
2 months						
oil-contaminated soil	$(3.0 \pm 0.22) \times 10^5$	$(5.1 \pm 0.56) \times 10^4$	$(2.8 \pm 0.3) \times 10^5$	$(9.3 \pm 1.3) \times 10^4$	$(0.9 \pm 0.4) \times 10^4$	10^4
oil-contaminated soil + A1	$(7.4 \pm 0.9) \times 10^6$	$(5.3 \pm 0.89) \times 10^6$	$(7.1 \pm 0.42) \times 10^6$	$(6.1 \pm 0.9) \times 10^6$	$(2.5 \pm 0.5) \times 10^3$	10^6
oil-contaminated soil + A2	$(8.7 \pm 0.89) \times 10^6$	$(4.2 \pm 0.96) \times 10^6$	$(4.2 \pm 0.2) \times 10^6$	$(4.5 \pm 0.8) \times 10^6$	$(3.4 \pm 0.6) \times 10^3$	10^6

*-the most probable number of cells in 1 g.

The increase in number of all groups of microorganisms, except micromycetes is extra noted for the same period of time in soil samples with entering of studied bacterial associations. At 5% oil pollution number heterotrophic, oligotrophic bacteria and actinomycetes has raised on three and spore-forming bacteria on two order. Also substantial increase of their number was in relation to the control which incubated within a month.

After two months' research of soil samples, it has shown that the quantity of heterotrophic bacteria has decreased both in control and in experimental samples approximately for one order. The number of spore-forming bacteria in the control remains flush with one-monthly indicator and with associations has increased 10 times. The quantity of actinomycetes has essentially decreased in a control variant and in a variant with entering of association 2. At entering of association 1 their number practically has not changed. The maintenance micromycetes has increased 10 times only in the control in samples of soil with the brought associations their number remains at former level. Quantity of hydrocarbon oxidizing microorganisms was stable and has not changed in comparison during 1 month.

At entering of oil into soil in number of 10% in one month incubation dynamics of change of number of microflora was similar about 5% pollution except for the control sample where the quantity of heterotrophic made

$(1.5 \pm 0.17) \times 10^5$ cell/g soils, *i.e.* this was lower (**Table 4**). It was less considered oligotrophic bacteria and actinomycetes on 1 - 2 order. Hydrocarbon oxidizing microorganisms in a variant with entering of association 1 was above (10^7 cell/g) than at 5% pollution.

After two months incubation the number of heterotrophic bacteria both in the control and in variants with the brought associations on one and two order accordingly has essentially decreased. Actinomycetes also were much less. The quantity of spore-forming bacteria and oligotrophic bacteria has increased. Number of micromycetes has practically not changed. Number of hydrocarbon oxidizing microorganisms in the control sample also was without change. It remained a high quantity of this group of microorganisms in samples of soil with association 1 and in samples from associations 2 has decreased with 10^6 to 10^5 cell/g.

Our studies correspond with the data of I. Ibatullina, *et al.* Preparations consisting of strains of *Rhodococcus* sp. and consortium from *Rhodococcus erythropolis*, *Arthrobacter* sp., *Candida lipolytica*, *Candida guillirmondii*, *Pichia guillirmondii*, *Fusarium moniliforme* and *Gliocladium deliquescens* have been tested. The results showed that the consortium was more effective against oil degradation compared to the monocultural preparation [10].

The study of the variety of hydrocarbon oxidizing

Table 4. Number of the basic groups of microorganisms in soil at 10% oil pollution with associations of oil oxidizing bacteria.

Variant	Heterotrophic bacteria	Spore-forming bacteria	Oligotrophic bacteria	Actinomycetes	Micromycetes	Hydrocarbon oxidizing microorganisms*
1 month						
oil-contaminated soil	$(1.5 \pm 0.17) \times 10^5$	$(1.3 \pm 0.5) \times 10^4$	$(5.0 \pm 0.4) \times 10^4$	$(1.1 \pm 0.1) \times 10^5$	$(2.4 \pm 0.6) \times 10^3$	10^4
oil-contaminated soil+A1	$(4.4 \pm 0.6) \times 10^7$	$(3.9 \pm 0.74) \times 10^5$	$(1.7 \pm 0.6) \times 10^5$	$(1.0 \pm 0.45) \times 10^4$	$(0.6 \pm 0.1) \times 10^3$	10^7
oil-contaminated soil+A2	$(2.4 \pm 0.2) \times 10^7$	$(2.2 \pm 0.26) \times 10^5$	$(2.4 \pm 0.6) \times 10^5$	$(1.2 \pm 0.4) \times 10^5$	$(0.5 \pm 0.08) \times 10^3$	10^6
2 months						
oil-contaminated soil	$(4.5 \pm 0.8) \times 10^4$	$(6.1 \pm 0.32) \times 10^5$	$(3.3 \pm 0.2) \times 10^5$	$(2.8 \pm 0.7) \times 10^4$	$(5.0 \pm 0.9) \times 10^4$	10^4
oil-contaminated soil+A1	$(4.8 \pm 0.9) \times 10^5$	$(4.0 \pm 0.4) \times 10^6$	$(6.0 \pm 0.7) \times 10^5$	$(5.2 \pm 0.8) \times 10^4$	$(4.0 \pm 0.5) \times 10^3$	10^7
oil-contaminated soil+A2	$(1.8 \pm 0.4) \times 10^5$	$(2.6 \pm 0.35) \times 10^6$	$(3.1 \pm 0.6) \times 10^6$	$(5.5 \pm 0.9) \times 10^3$	$(2.0 \pm 0.6) \times 10^3$	10^5

*-the most probable number of cells in 1 g.

microorganisms has shown that bacterial strains which capable to utilize the oil present basically following genera: *Rhodococcus*, *Microbacterium*, *Acinetobacter*, *Pseudomonas*, *Azomonas*, *Enterobacter*, *Bacillus* and *Arthrobacter*.

Under inoculation soil sample polluted oils product on the Myunc medium observed the development of the quite a number colony of actinomycetes. The colonies were of the same type-small, discoloured, leathery, lacking an aerial mycelium. All studied strains belonged to genus *Streptomyces* and did not show the significant aspectual variety. *Streptomicetes* present section *Albus* (63.5%), *Cinereus* (24.5%), *Azureus* (12%). The most frequency of occurrence is noted for series: *Albus-albus* (54.5%) and *Cinereus-chromogenes* (15.2%); in smaller amount are presented serieses-*Azureus-coerulescens* (12.0%). *Albus-albocoloratus* (9.0%) and *Cinereus-aureus* (9.0%).

Micromycetes were presented basically four dominant genera *Aspergillus*, *Penicillium*, *Trichoderma*, *Mucor* (50% and more), four rare genera *Fusarium*, *Alternaria*, *Ulocladium*, *Gliocladium* (25%) and very rare—*Absidia*, *Aureobasidium*.

Biodiversity of oil oxidizing bacteria in soils of Vietnam has been studied by A. Ivanova, et al. The next genera of oil oxidizing bacteria *Acinetobacter*, *Bacillus*, *Chromobacterium*, *Cupriavidus*, *Gordonia*, *Microbacterium*, *Mycobacterium* and *Rhodococcus* have been found with the density of population in uncontaminated soil to 10^2 - 10^3 cells/g, and oiled up to 10^6 - 10^7 cells/g [11].

There is a considerable identity in structure of oil oxidizing bacteria genera in soils from Kazakhstan and Vietnam.

4. CONCLUSIONS

At research of oil destruction in soil with various degree of pollution, it is revealed:

Throughout all experiment there was a decrease in quantity of oil under the influence of active associations of oil oxidizing microorganisms;

The association 1 consisting of two strains *Arthrobacter* sp has shown the greatest activity P1 and 24;

Entering of studied bacterial associations in oil pollution made active soil biocenosis therefore there was an increase in number of all investigated groups of microorganisms.

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