Study of the Dynamics of Oil Destruction with the Use of Bacterial Biological Preparations in the Conditions of the Far North

M. Yu. Menshakova[1,*], *K. K.* Moskvin[1], and *S. A.* Khodjaliev[2]

[1]Murmansk Arctic State University, Egorova str., 15, 183038, Murmansk, Russia
[2]Kadyrov Chechen State University, Sheripova Street, 32, 364024, Grozny, Russia

Abstract. A study of the dynamics of crude oil decomposition was carried out using the bio-preparations "Glaukoil" and "Lenoil" and various methods (aeration, application of a sorbent and a biological product at different times, the use of a polymer shelter, the addition of a moisture-retaining component and the distribution of the dose of mineral fertilizers) in order to optimize the oil destruction technology at emergency oil spills. It is shown that the most effective methods are the use of a polyethylene film to maintain a higher temperature during oil decomposition, additional aeration, and the use of a water-retaining component. Other methods did not show a significant acceleration of oil degradation.

1 Introduction

Petroleum hydrocarbons were formed in the earth's crust in a natural way, and in nature there are many microorganisms capable of assimilating these compounds. But in reality, rapid biodegradation to eliminate oil spills is difficult for a number of reasons:

- a specific type (strain) of bacteria can break down only individual components of oil, which is a complex mixture of hydrocarbons. The qualitative and quantitative composition of which is individual for each deposit [1].

- individual components of oil are not only difficult to break down by bacteria, but also have a bactericidal and bacteriostatic effect, which makes it difficult to break down even the most easily assimilated hydrocarbons

- in case of spills of oil and oil products on soils, part of the hydrocarbons can be firmly bound to soil components, which reduces their availability for oil-destructing microbes [2].

To accelerate the decomposition of oil that has entered natural ecosystems, it is necessary to provide certain conditions, namely, the temperature regime, the availability of minerals, the optimal level of humidity, oxygen access, the use of highly efficient crops of natural and artificial origin.

Crucial in the process of oil biodegradation is played by abiotic environmental factors, such as temperature, humidity, availability of oxygen and minerals.

Most strains are active in the range from 10 to 40 0C. The turning point is a temperature of +50C, below which biodegradation slows down sharply. At temperatures below 0°C,

*Corresponding author: dendrobium@yandex.ru

bacterial reproduction and biodegradation stop completely, and after defrosting, all bacteria used resume their activity [3].

To maintain the activity of bacteria at a sufficient level, it is necessary to maintain a soil moisture content of at least 40%. In the southern regions, where the soil is sufficiently water-intensive or the moisture capacity of the soil is provided by the remnants of the grass sod, the required water content is maintained by periodic sprinkling and reservoir turnover. For coastal gravelly, sandy or rocky soils, maintaining the required level of moisture can be very difficult.

The bacteria used are aerobes, so providing air access becomes one of the decisive factors. As a rule, plowing is enough for this, but in the conditions of the coasts of the North Seas, the use of such a technique seems to be very difficult.

The composition of nutrient media for the cultivation of oil-degrading microorganisms, as a rule, includes nitrogen, potassium, phosphorus, sulfur, magnesium, sodium, and chlorine [3].

Bacteria-biodestructors of oil and oil products were first isolated from canal water, and later from soil. Some preparations use bacteria that live in the intestines of farm animals. In particular, one of the biological preparations contains bacteria isolated from pig farm waste (Bacillus pumilus, Bacillus sphaericus, Micrococcus hylae, Arthrobacter viscosus, Bacillus licheniformis) [4].

In conditions of fertile soils with rich microflora, to eliminate small spills, when the degree of pollution with oil products does not exceed 10%, it is possible to do without the use of biological products and the introduction of mineral growth factors. In the conditions of the North, where soils are poor in hydrocarbon-decomposing bacteria and nutrients, it is necessary to apply both biopreparations and fertilizers even with slight pollution [3].

Bacillus cereus, Artrobacter simplex, Artrobacter mysurens Brevibacterium fuscum, Flavobacterium solare, Flavobacteruim fucatum, Xanthomonas oriza, Nocardia minima, Bacillus megatherium [3-5].

Natural strains isolated from the soil are used in isolation or form artificial associations, for example, Acinetobacter, Bacillus and Pseudomonas 1:1:1, and it has been shown that during cultivation on a nutrient medium containing glucose, there is no change in the ratio of the number of bacteria of different species

In addition to natural strains, genetically modified microorganisms can be used in the biodegradation of oil and oil products. In particular, the introduction of certain plasmids into bacterial cells of the genus Pseudomonas makes it possible to split individual oil fractions: the OCT plasmid provides for the decomposition of octane and hexane, the XYL plasmid - xylene and toluene, the NAH plasmid - naphthalene, the CAM plasmid - camphor.

Isolated OCT and CAM plasmids cannot exist in the same cell, since they have homologous regions; therefore, if it is necessary to introduce two plasmids at once, they are prehybridized to form a larger hybrid CAM/ OCT plasmid [6]. As a result of sequential hybridization of plasmids and bacterial strains, pseudomonads were obtained containing all four types of plasmids listed above.

Genetic engineering solutions make it possible to outline the prospect of obtaining microorganisms capable of breaking down hydrocarbons at low temperatures. For example, the TOL plasmid (providing toluene assimilation) of a mesophilic strain of Pseudomonas putida was introduced into the cells of a psychrophilic (with a low temperature optimum) strain capable of cleaving salicylic acid at a temperature of about 00C. The results of this experiment, of course, only show the possibility of obtaining hybrid oil-degrading microorganisms capable of functioning at low temperatures, but so far such strains have not found practical application and there are doubts that oil biodegradation using transgenic bacteria will be the main technology in the near future [7]. The most promising drugs are considered to be a complex of several types of natural strains of bacteria with the addition of transgenic forms [8].

One of the approaches to soil restoration is phytoremediation, a technology in which the decisive role is assigned to higher plants, in the rhizosphere of which oil-destructing bacteria intensively spill. In this case, no significant acceleration of oil components is observed, but due to the fact that with this approach a closed plant community is quickly formed at the spill site, phytoremediation receives the support of environmental NGOs and the public.

The possibility of transformation of natural ecosystems as a result of the use of microbial biological preparations for remediation after accidental spills is not ruled out [7]. The possible consequences of the use of bacterial preparations for soil biota are assessed mainly by the antibiotic activity of the studied bacterial strains. At the same time, the authors note the resistance of the studied species to a number of antibiotics [3].

The vast majority of modern preparations for the remediation of oil-contaminated soils do not require additional measures to deactivate the microorganisms that make up their composition.

The purpose of the study is to modify the technology of using biotechnological oil destructors to the conditions of the Far North.

Tasks:

- study of the influence of environmental factors on the rate of oil degradation (temperature, aeration, the procedure for introducing mineral growth factors)
- study of the dynamics of oil degradation with the combined introduction of two preparations with different microbiological composition

2 Research Methodology

For the experiment, two domestic certified drugs were used: Lenoil-nord and Glaukoil. These drugs have a similar purpose, but differ significantly in technical regulations. Manufacturers of drugs declare the possibility of oil destruction in one season (provided that the degree of pollution does not exceed 10%)

The drug "Lenoil-nord" contains Pseudomonas sp. as oil-degrading bacteria. and does not contain a sorbent component and mineral additives. Before use, in accordance with the technical regulations, in most cases, preliminary activation is required when diluted with industrial water (5 kg of the drug per 1000 liters of industrial water), adding 0.5 kg of nitrogen-phosphorus-potassium mineral fertilizer and 1 liter of diesel fuel to ensure a non-difference nutrition of bacteria .

The preparation "Glaukoil" is much richer in the content of hydrocarbon-decomposing bacteria (Bacillus megaterium, Bacillus subtilis, Pseudomonas putida, Pseudomonas sp.), but the technical regulations of this preparation require the introduction of a significant amount of mineral fertilizers, in particular, for the disposal of 1 ton of oil, the introduction of 393 kg of nitrophoska is required.

The use of the drug "Econadin" in the experiment had to be abandoned due to the liquidation of the manufacturer.

Fucus algae were used as a moisture-retaining component. quantities of this material in materials amounts of materials and materials out of quantities, it can be in sufficient amounts of materials volunteers in the immediate vicinity of spills, while other materials need to be specially transported to the venue, collection on the coast can be difficult.

For the study, model experiments were carried out, during which portions of the soil were placed in plastic containers with a capacity of 2 l, 100 ml of crude oil, mineral fertilizers were added in accordance with the technological regulations of each drug and various additives in accordance with the experimental scheme (tables 1-6) . Optimization of the temperature regime was carried out using a polyethylene film. The work was carried out on the territory of the educational and scientific base of the Federal State Budgetary

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Table 1. Study of the influence of the order of application of mineral growth factors

Components and manipulations	Experience Options						
	1	2	3	4	5	6	7
A drug	Lenoil 2г	Lenoil 3г	Lenoil 2г	Lenoil 2г	Lenoil 2г	Lenoil 2г	Lenoil 2г
Nitroammophoska	1,8 г	1,8 г	1,8 г	1,8 г	1,8 г	1,8 г	1,8 г
Procedure for adding mineral factors	single	single	single	double by 0,9 г	double by 0,9 г	triple in 0,6 г	triple in 0,6 г
transparent lid	-	-	+	+		+	-

Table 2. Study of the effectiveness of biotechnological preparations together with a biodegradable sorbent (sphagnum and TSR)

Components and manipulations	Experience Options			
	8	9	10	11
A drug	Lenoil 2 g	Lenoil 2 g	Glaukoil 3 g	Glaukoil 3 g
Nitroammophoska	1,8 g	1,8 g	1,8 g	1,8 g
Procedure for adding mineral factors	single	single	single	single
transparent lid	+	+	+	+
sorbent	Sphagnum	Sphagnum	Silicon carbon sorbent TShR TU 2164-011-02698192-2006	Silicon carbon sorbent TShR TU 2164-011-02698192-2006
Sequence of application of sorbent and biopreparation	separate	simultaneous	separate	simultaneous

Table 3. Study of the effect of aeration on oil degradation

Components and manipulations	Experience Options	
	12	13
A drug	Lenoil 2 g	Lenoil 2 g
Nitroammophoska	1,8 g	1,8 g
Procedure for adding mineral factors	single	single
transparent lid	+	+
Mixing	single	double

Table 4. Study of the combined action of drugs

Components and manipulations	Experience Options
	14
A drug	Lenoil 2 g + Glaukoil 3 g
Nitroammophoska	1,8 г
Procedure for adding mineral factors	single
transparent lid	+

Table 5. Study of the effect of water-retaining additives (fucus thallus)

Components and manipulations	Experience Options
	15
A drug	Lenoil 2 g
Nitroammophoska	1,8 g
Procedure for adding mineral factors	single
transparent lid	+

Fucus thallus	300 g

Table 6. Study of the effect of shading on oil degradation

Components and manipulations	Experience Options
	16
A drug	Lenoil 2 g
Nitroammophoska	1,8 г
Procedure for adding mineral factors	single
Opaque lid	+

The total content of oil products was determined on the basis of the FBUZ "Center for Hygiene and Epidemiology in the Murmansk Region" by the fluorographic method during the extraction of oil with hexane. The initial concentration of oil in the experiment averaged 85550 mg/kg.

The sum of effective temperatures was determined based on the fact that in this case the temperature above +5 0C is considered effective. We used data on the average daily temperature in the village of Tuloma on the resource www.gismeteo.ru.

3 Results and Discussions

Data on the oil content in the samples during the period of the experiment are shown in Table 7.

Table 7. Dynamics of oil content in samples and the sum of effective temperatures

No. option experience	Oil content in samples (in mg per 1 kg of soil) by dates					
	12.07	20.07	02.08	16.08	09.10	03.11
1	85590±989	82789±1080	73650+54	70567±567	67910±195	64678±896
2	85590±989	84789±992	70400+42	63456±514	54677±367	52789±789
3	85590±989	85995±1015	70300+101	55678±562	44226±161	44376±674
4	85590±989	82678±997	64132+103	56748±782	43074±327	43005±567
5	85590±989	84568±892	69337+84	65782±739	62575±614	61678±564
6	85590±989	85989±982	62231+558	54825±602	44848±831	40678±562
7	85590±989	84562±999	66691+92	65783±1097	63624±166	60678±507
8	85590±989	83567±981	65452+136	52783±1015	49739±993	50078±561
9	85590±989	84678±876	65646±763	58682±592	43100±1578	42562±672
10	85590±989	83756±899	69801+175	54892±649	48623±965	46893±562
11	85590±989	82898±1002	68320+270	60673±992	44977±374	42367±902
12	85590±989	83789±989	49495±673	43678±782	37819±945	35783±992
13	85590±989	84567±768	56714+1478	37782±892	29269±156	29783±894
14	85590±989	83598±986	47820+120	39578±672	28416±1060	27783±674
15	85510±897	84988±876	50990+377	42789±569	34309±533	32876±563

16	85590±989	85100±922	73725+3431	60567±948	44002±475	40362±673
17	85510±897	86102±672	79783±675	75673±1023	72783±672	70782±672
18	85510±897	84783±765	80672±783	79782±672	75783±902	76201±562
Sum of effective temperatures	-	262	645	1026	1905	1939

It follows from Table 7 that the presence of a film cover is crucial for accelerating oil degradation, which allows to accumulate heat and create a sufficiently high temperature even with variable cloudiness. This pattern can be traced for all options for bookmarking experience. In the early stages of oil degradation, even at high air temperatures, the decomposition of oil occurs rather slowly, which is apparently associated with a long period of activation of microbial preparations.

Aeration is of great importance for accelerating oil degradation: double mixing of soil containing oil allows achieving good results. But the application of this technique in practice is associated with large expenditures of funds and labor of volunteers, especially if the spill occurred in a remote area of the coast.

The division of the dose of applied mineral fertilizers into two and three portions does not lead to an acceleration of oil degradation.

Increasing the dose of the drug by one and a half times "Lenoil-nord" without the use of a film cover does not give a significant activation of oil degradation, therefore, such a method at significant financial costs will not give good results.

The best results were achieved with a combination of two preparations, however, in practice, the combined use of two biodestructors is difficult due to the high cost of the Glaukoil preparation and the need to apply high doses of mineral fertilizers. In the conditions of the Far North, where soils are extremely poor in nitrogen and phosphorus, and the reproduction of hydrocarbon-decomposing bacteria is slow (hence, the assimilation of inorganic substances is slow), the application of fertilizers in such an amount will lead to new pollution, this time with minerals.

The use of TSHR sorbent and sphagnum together with microbiological preparations did not cause a significant acceleration of oil degradation, both with the sequential introduction of the sorbent and the biological product, and with simultaneous application.

More promising, in comparison with other methods, in our opinion, is the addition of thalli of fucus algae, which, firstly, play the role of a moisture-retaining component and allow stabilizing the water content in the soil, and secondly, gradually decomposing, the algae themselves become a source of minerals for the growth of bacteria, thirdly, the thalli of fucuses are quite dense and allow, when mixed, to create a looser structure of the sample, which improves aeration. Fucus algae grow in abundance in the littoral of the Barents Sea and it is quite possible to collect them in large quantities to clean up spills by volunteers already during the initial treatment of the oil-contaminated part of the coast. During the experiment, the appearance of mold fungi on the surface of the contents of the container was noted. It is obvious that the spores of these fungi were introduced into the containers on the fucus thalli. During the next growing season, an analysis will be carried out to determine the species of these fungi and their possible participation in the process of oil degradation.

Measurements of the content of oil products in the control variants of the experiment show that in the conditions of the Far North it is impossible to achieve any significant splitting of oil due to oil-degrading bacteria that it contains. With the sum of the effective temperatures accumulated during the experiment 1939, about 10% of the oil is split in the control variants.

Thus, such manipulations as the creation of a film shelter and the addition of thalli of fucus algae are of the greatest importance for accelerating oil degradation.

In general, in none of the variants of the experiment, the result declared by the manufacturers was achieved - complete oil destruction in a season, however, the implementation of certain techniques can significantly increase the rate of oil destruction.

4 Conclusions

The most effective methods for accelerating oil destruction in the conditions of the Far North are

- creation of a film shelter
- the introduction of fucus algae as a moisture-retaining component
- carrying out periodic loosening in order to aerate the oil layer

Compliance with these measures makes it possible to achieve a three-fold reduction in the oil content in the soil (with an initial degree of pollution of 10%).

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