Application of biological preparation for bioremediation of oil-contaminated soils of the tank farm

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Abstract. The paper presents the results of an experiment on the biological purification of permafrost soils from oil pollution with use biological preparations on the basis of hydrocarbon-oxidizing microorganisms. Biological preparations were used in two modifications - in liquid form as a suspension and in dry form as a suspension immobilized on organic granules. It has been established that dry form of a biological preparation is more effective for the purification of permafrost soils from oil than a liquid microbial suspension. For 1 month in dry conditions and a strongly alkaline soil environment, the use of a dry biological preparation allowed reducing the concentration of oil pollution by up to 60 %. The change of the chemical structure of extracts of soil samples was investigated with use the method of IR-Fourier spectroscopy. The increase of the relative absorption coefficients of oxygen-containing groups and bonds pointed (indicated) on the course of oxidative destruction.

1. Introduction

In present time the development of the oil and gas industry in Yakutia is rapidly gaining momentum. It is largely due to the fact that considerable natural hydrocarbon resources - oil, natural gas and gas condensate - are concentrated on the territory of the republic [1-2]. The functioning of oil and gas facilities is almost always accompanied by spills and various leaks. In extreme climatic conditions of Yakutia, the liquidation of the consequences of accidental spills is complicated by the specific feature of the natural ecosystems of the northern regions, namely the presence of a cryolithozone. The processes of biodegradation of petroleum hydrocarbons in permafrost soils proceed much slower than in regions with favourable climatic conditions. Destruction of oil and its derivatives in the conditions of the North can last more than 50 years [3-8]. As a result, the soil pollution is remained for years forming areas with a steady high level of pollution. It is necessary to solve problems related to monitoring of disturbed areas, scientifically-based selection of technologies for the liquidation and remediation of oil-contaminated soils.

Now a biotechnological approach is the most promising method for remediation disturbed areas, based on the development of biological preparation on the basis active strains of oil-degrading microorganisms [9-14]. Innovative biotechnologies allow not only to reduce the time of cleaning the lands from oil contaminations but also allow to accelerate processes of self-remediation of natural ecosystems.
2. Models and Methods

Experimental work on the purification of permafrost soils polluted by crude oil with use of the biological preparation on the basis of hydrocarbon-oxidizing microorganisms (HOM) was carried out in one of the tank farm territory, located in south-east of Central Yakutia, in an open ecosystem. The territory of the tank farm was polluted because of accidental crude oil spill in 2007. Soil samples collected from experimental sites in the summer season (July-August, 2018) before biological treatment (June) and after (August) served as materials for research. The microscopic Penicillium soil fungus strain isolated from soil samples from the Arctic territory (Samoylov Island, Lena river delta, 72.37704° N; 126.48022° E) was used as HOM. As a control, background samples of soils collected from a considerable distance from the tank farm were investigated. The scheme of the experiment is shown in Table 1.

For the preparation of a liquid form of a biological preparation the original Penicillium strain was used, which was washed with distilled water so that at least 250 ml of microbial suspension with a titer of at least 1 billion microbial cells per 1 ml of water was obtained. Then 10 ml of the obtained microbial suspension and 100 ml of a previously prepared sterile nutrient medium were introduced into a flask with a volume of 250 ml [15-17]. In this way required for remediation the microbial suspension was prepared and then cultivated for 3 days at a temperature of +20 °C. After cultivation, the accumulated liquid was poured into a container and used for the treatment of oil-contaminated soil in the amount of 1 liter of suspension per 1 m² of polluted area.

For the preparation of the dry form of the biological preparation, the obtained microbial suspension was mixed in equal ratios (1: 1) with a previously prepared sorbent carrier. The obtained mixture was activated for 3 days, then passed through a granulator. The granules were dried at room temperature for 3 days. The biological preparation was packaged in sterile bags. For the experiment 200 g of a dry biological preparation per 1 m² of contaminated area was applied to the soil.

The content of residual crude oil in soils was determined by method of cold chloroform extraction by the yield of the chloroform extract. The processes of transformation of oil pollution were studied with use the method of IR-Fourier spectroscopy. IR-spectra were recorded on the IR-Fourier spectrometer of Protege 460 of Nicolet in the range of 500-4000 cm⁻¹. The interpretation of the spectra was carried out according to the atlas of the IR-spectra and the wavenumber tables [18].

3. Results and Discussion

The content of hydrocarbon compounds in the background soil sample was 232 mg kg⁻¹ (Table 1). The initial oil content in the experimental sites 1 and 2 before the biological treatment in the samples was 44,705 mg kg⁻¹ and 1,051 mg kg⁻¹, respectively. The difference in the values of the initial oil content in the soil is due to the uneven distribution and age of pollution. According to the classification V M Goldberg [19], the level of contamination of the samples is characterized as high and moderate. In a month after the biological treatment, the residual oil content in all samples decreased. The degree of destruction at site 1 was 59.89%, and at site 2 - 13.22%.

| № experimental sites | Scheme of experiment | Yield of the chloroform extract, (mg kg⁻¹) Before treatment (Jule) | After treatment (August) | Destruction degree, (%) |
|----------------------|----------------------|---------------------------------------------------------------|-------------------------|------------------------|
| 1                    | Suspension of fungi immobilized on organic granules            | 44,705                                                          | 17,927                  | 59.89                  |
| 2                    | Water-base suspension of fungi                                | 1,051                                                          | 912                     | 13.22                  |
| Background           | Clear soil                                                     |                                                                | 232                     |                        |
Estimation of oil contamination of soils was carried out by the comparison of background samples. The reduction of the oil concentration was also accompanied by the change of the structural group composition of the soil extracts. IR-spectra of chloroform extracts of soil samples before biological treatment and spectrum of extract of the background sample are represented in Figure 1.

![Figure 1. IR-spectra of chloroform extracts of soil samples before bioremediation.](image)

According to IR-Fourier spectroscopy, in the chemical structure of the background sample extract oxygen-containing compounds prevail over hydrocarbon components. The absorption band in the region of 3300 cm⁻¹ indicates the presence of hydroxyl groups that are part of carboxylic acids, esters and ketones. The intensive absorption band in the region of 1700-1740 cm⁻¹ is associated with a high content of carbonyl groups. In the spectra of the background sample are found absorption bands ethereal bonds (1170 cm⁻¹). IR-spectra the initial oil-contaminated soils are the same. In the spectra the dominance of the absorption bands of hydrocarbon structures is observed. This is confirmed by the fact that the absorption bands of the methylene groups (1460 cm⁻¹) in the samples are most pronounced in comparison with the absorption band of oxygen-containing groups. Absorption in the region of 1600 cm⁻¹ is due to the content of aromatic compounds in the composition of chloroform extracts of contaminated samples.

The course of oxidation processes in the experimental sites after the biological treatment can be estimated by the change of the relative absorption coefficients of oxygen-containing groups in relation to the hydrocarbon groups.

The relative absorption coefficients of the structural groups were calculated by the following equation (1) [20]:

\[
D_v^f = \frac{D_v}{D_{1460}} ,
\]

where

- \(D_v\) – optical density of the absorption band;
- \(D_{1460}\) – optical density of the absorption band 1460 cm⁻¹.
An increase of the relative absorption coefficients of oxygen-containing groups and bonds after the introduction of biological preparation confirms the course of biological oxidation processes (Figure 2).

![Figure 2. Relative absorption coefficients of oxygen-containing functional groups and bonds before and after bioremediation.](image)

Accumulation process of heterotrophs using ready-made organic compounds for their nutrition was more active in the soil of the site with a higher concentration of oil pollution. Probably, the reason for the intensification of the development of microorganisms here was the introduction into the site of an organic sorbent carrier onto which were immobilized HOM. The introduction of an organic sorbent carrier into the soil also led to the activation of nitrogen fixation and the accumulation of HOM in the experimental site (Table 2).

### Table 2. Results of microbiological studies.

| Parameters                                | Experimental sites |          | Background sample |
|-------------------------------------------|--------------------|----------|-------------------|
|                                           | 1                  | 2        |                   |
| Sampling time                             | July               | August   | July              | August           |
| Total number of heterotrophic microorganisms in soils | 5.3·10⁸          | 1.0·10¹⁰| 9.5·10⁸          | 1.6·10⁸          | 1.8·10⁸          | 7.0·10⁸          |
| - bacteria                                | 3.5·10³           | 4.1·10⁸ | 2.2·10³           | 6.2·10⁶          | 2.5·10³           | 6.6·10⁴          |
| - fungi (cells g⁻¹ dry soil)              |                    |          |                   |                  |                   |                  |
| Activity of *Azotobacter*, (%)            | 4.0                | 25.0     | 8.0               | 8.0              | 25.0              | 40.0             |
| Total number of HOM, (cells g⁻¹ dry soil) | 2.1·10³           | 2.1·10⁷ | 0.4·10³           | 1.6·10⁴          | 0.2·10³           | 0.4·10³          |
| Characteristic of soil environment        |                    |          |                   |                  |                   |                  |
| Soil pH                                   | 8.81               | 8.93     | 9.15              | 9.12             | 8.97              | 8.93             |
| Absolute humidity (%)                     | 22.40              | 5.50     | 14.50             | 11.90            | 16.20             | 18.80            |
Soil treatment with a biological preparation based on organic granules allowed to reduce the phytotoxicity of the soil in the experimental site, in comparison with the experience where used the liquid form of the biological preparation. The seeds of common oats (lat. *Avéna satíva*) and oil radish (lat. *Raphanus oleifera*) were used as a test-plants. In the biotesting radish oil seeds showed greater sensitivity to oil pollution (Table 3).

**Table 3. Results of soil biotesting.**

| Parameters                      | Experimental sites | Background sample | Germination control |
|---------------------------------|--------------------|-------------------|--------------------|
|                                 | 1                  | 2                 | July               | August  | July | August  | July | August  | 92.0 | 92.0 | 96.0 |
| Germination of *Avéna satíva*, (%) | 16.0              | 24.0              | 24.0               | 32.0    | 88.0 | 92.0    | 96.0 |
| Germination of *Raphanus oleifera*, (%) | 12.0              | 16.0              | 24.0               | 92.0    | 92.0 | 96.0    |      |

The reason for the insufficient activity of the liquid form of a biological preparation was a strong alkaline reaction of the soil environment and insufficient soil humidity. It is known that fungal forms of microorganisms prefer wet (at least 60%) and acidified (pH 5.0–5.5) substrates (Table 2). In addition, for activation the process of microbial degradation, it is necessary to add mineral fertilizers into the soil and to aerate the polluted soil layer, since all currently known HOM are aerobes that require oxygen for their development.

4. Conclusion

Thus, results of the experiment on the cleanup of oil-contaminated soils of the tank farm showed the possibility of application biological method of purification. The maximum degree of destruction of oil pollution was established at the site 1, the soils of which were treated by the biological preparation on the basis of the mycelial strain HOM of the genus Penicilium immobilized on organic granules. The results of the structure-group composition of chloroform extracts of soil samples, in the chemical structure of which the predominance of oxygen-containing groups and bonds over hydrocarbon structures is established, point to the course of oxidative destruction processes.

Introduction of biological preparation in oil-contaminated soil allowed to enhance nitrogen fixation by soil bacteria, increase microbiological activity of disturbed lands, significantly reduce phytotoxicity and concentration of oil products up to 60 % for 1 month in dry conditions and a strongly alkaline soil environment.

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