Vermiculite of the Inagli Field as a Promising Material for Environmental Use at Reclamation Sites

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Abstract. This article discusses experimental studies of the use of local vermiculite from the Inagli field (Sakha Republic (Yakutia), Russia) as a recultivant on industrially disturbed permafrost-soil ecosystems of Yakutia after emergency oil spills. A brief description of the material composition, physical, chemical and technological properties of local vermiculite, which are close to the properties of vermiculite of the Kovdorskoe field (Murmansk oblast, Russia), is given. The sorption properties of Yakut vermiculite were tested on oil from the Talakan field, gasoline, diesel fuel, and toluene. Vermiculite of the Inagli field is a promising raw material for the production of oil sorbents, especially in connection with the increase in oil production, processing and transportation on the territory of Yakutia. The results of experimental application of hydrocarbon-oxidizing microorganisms (HOM) immobilized on vermiculite in oil-contaminated soils are presented. The technology of using vermiculite raw materials for oil pollution cleaning of various types of permafrost soils provides a significant environmental and economic effect, contributing to reducing the time of rehabilitation of disturbed lands and improving the environment of the disturbed territory.

1. Introduction

Despite intensive research on methods for restoring oil-contaminated soils, permafrost soils of Yakutia still remain poorly studied in this regard. Self-recovery of disturbed lands takes place here over the years. Many modern researchers believe that the most effective and environmentally safe for northern ecosystems polluted by oil and petroleum products are biological cleaning methods based on the use of bacterial preparations [1-7]. However, the use of industrial biologics used to restore soil cover are ineffective in cold climates. This is due to the fact that microorganisms do not compete with the local soil microflora, or lose their activity while they are at the technological stage of manufacturing a biological product. Since only about 1-30% of dried microorganisms remain viable after lyophilization, and even those can lose their oil-oxidizing ability.

These circumstances demand scientific research on obtaining new forms of biosorbents based on local natural materials capable of active destruction of oil pollution at low temperatures.

The purpose of this research is to develop an effective technology for cleaning soils from oil pollution in the environmental and climatic conditions of Yakutia using biosorbents based on local natural raw materials.
2. Materials and methods
Currently, more than two hundred different sorbents are used to eliminate oil spills, which are divided into inorganic, natural organic, organomineral, etc. The quality of sorbents is mainly determined by their capacity against oil, the degree of hydrophobicity, buoyancy after oil sorption, the possibility of desorption of oil, regeneration or utilization of the sorbent [8, 9].

Based on the world experience in the oil pollution control, new groups of products - biosorbents with both absorption and physical and chemical activity against petroleum products, and biological ones - were used as the basis for a new complex technology. These biosorbents are based on an absorption material obtained from natural aluminosilicates (perlite, vermiculite, zeolite, etc.) immobilized by natural bacteria that can destroy oil products collected by the preparation in a wide temperature range [8, 9, 10].

The materials for research were samples of soils and grounds selected at industrial and recovery facilities of the oil and gas complex (OGC) of Yakutia. The selection of samples for the extraction of hydrocarbon-oxidizing microorganisms (HOM) was determined by the presence of visually identifiable oil or petroleum product (PP) contamination at the sampling site. Background and conditionally pure samples were studied as a control.

HOM was isolated and cultured using classical methods in the mineral environment of Munz [11, 12]. Field experiments to assess the effectiveness of cleaning permafrost soils from oil pollution using the obtained biosorbents were carried out in experimental plots laid on the territories of the Yakutia’s OGC, after emergency oil and petroleum product spills, with different degrees and prescriptions of contamination, which excluded special contamination of clean soil areas. The concentration of petroleum products in soils was determined by the generally accepted method [13].

3. Experimental part
This paper presents the results of a study of the effectiveness of a biological petroleum sorbent for cleaning permafrost soils from oil pollution. The sorbent is represented by a solid carrier substrate and a biodestructor of oil pollution immobilized on its surface.

Thermally expanded vermiculite from Inagli field (Yakutia) is used as the substrate carrier; as the biodestructor of oil - a strain of bacteria Pseudomonas panipatensis VKPM B-10593 at a concentration of 1×10^9 cells/cm³ and cultured in a mineral medium with oil of the Talakan field, able to grow and degrade crude oil in soil at a wide range of temperatures (from +8 to +37 °C).

3.1. Characteristics of the strain
Pseudomonas panipatensis strain VKPM B-10593 was isolated from the water of lake Haly-Baly (Amginsky district, Central Yakutia), contaminated with Arctic diesel fuel. The strain was deposited in the all-Russian Collection of Industrial Microorganisms of FSUE Gosniigenetika (VKPM) (Moscow, 1st Dorozhny Ave., 1) under the registration number VKPM B-10593.

3.1.1. Cultural and morphological features
Gram-negative mobile rod-shaped cells with a size of 2.0-3.0×0.6-0.8 microns. Spores and capsules are not formed. In microscopic preparations the bacteria are arranged randomly, singly or in short chains (2-3). On nutrient agar (mass. %): hydrolyzate of fish meal - 1.2; peptone enzymatic - 1.2; sodium chloride - 0.6; agar - 1.0; distilled water - the rest; (pH 7.1-7.5) and on meat-peptone agar (mass. %): enzymatic peptone - 1.0; sodium chloride - 0.5; agar - 1.0; meat water - the rest, (pH 7.0-7.2), after 24 hours, the strain forms disc-shaped, moist, pasty consistency S-colonies of gray color, 1.5-5 mm in diameter, easily removed from the agar. At a temperature above +20°C, the strain forms a diffusing green pigment, which can be lost at a temperature of +4 °C and restored at a temperature of +20° C.
3.1.2. Physiological and biochemical features
The strain is aerobic. It grows at temperatures from + 8°C to + 41 °C. Optimum growth at +30...+37°C. It grows at pH 6.0-8.0 and in an environment with the addition of 0.1-2.0% NaCl. It has oxidase and catalase activity. As a source of carbon, it consumes acetate and citrate. Reduces nitrates. There is no denitrification activity. Does not hydrolyze gelatin. It has urease activity. Does not hydrolyze starch. Does not form lecithinase. Indole-negative. It oxidizes maltose and D-glucose. Does not ferment glycerin, Inositol, mannitol, rhamnose, sorbitol, raffinose, arabinose. Does not oxidize sucrose, mannitol, xylose, arabinose, fructose, galactose. Does not need additional growth factors.

The strain has a pronounced resistance to benzylpenicillin, furazolidone, metronidazole, amoxicillin, amoxiclav, kaotim, pefloxacin, amosin; weakly resistant to oxacillin, polymyxin, ampicillin, levomycetin; sensitive to moxiclav, kaotim, pefloxacin, amosin; weakly resistant to oxacillin, polymyxin, ampicillin, levomycetin; sensitive to moziklin, cefatoxim, ceftriaxone.

3.2. Characteristics of the carrier substrate
According to many authors, natural mineral materials are preferred for immobilization of HOM. In this case, the carrier sorbent used for cell immobilization must have the following main properties: permeability, oil capacity, non-slumping ability, porosity and flowability. Expanded vermiculite meets these requirements to the fullest extent possible (GOST 12865-67).

Vermiculite is a secondary mineral, resulting from the hydration of phlogopite and biotite and is related to complex ferrous-magnesium silicates of the group of hydromicas. Its chemical composition is variable and can be approximated by the formula \((\text{Mg}^{2+}, \text{Fe}^{2+}, \text{Fe}^{3+})_3[(\text{Si}, \text{Al})_2\text{O}_5]·\text{OH}_2·4\text{H}_2\text{O}\). Color bronze-yellow to brown, greenish. Leaves are soft and flexible. The structure is close to montmorillonite, the cleavage is perfect. Hardness 1-1.5; density 2.3 g / cm³; melting point about 1400 °C. [14, 15, 16].

In Russia the most well-known deposits of vermiculite ores are: Kovdorske (Murmansk oblast), Tatarske (Krasnoyarsk Krai), Koksharovsky (Primorsky krai), Buldymskoe and Potaninskoe (Chelyabinsk oblast), Uluntuysko and Syludyanskoe (Irkutsk oblast), Inaglinsky (Yakutia), etc. World production of vermiculite is about 500-600 thousand tons per year, including in Russia – about 35-40 thousand tons [17, 18].

Vermiculite has the ability to swell when heated and significantly (10-25 times) increase in volume with the formation of a loose granular material of a scaly structure, which, due to its low bulk mass, low thermal conductivity, high sound insulation properties, chemical inertia and fire resistance, is used in construction, agriculture, environmental protection, etc. [16, 17, 19, 20].

However, due to the lack of special equipment, the production of vermiculite and materials based on it in Russia is only beginning to develop and is still limited [20].

Vermiculite from the Inagli deposit (Yakutia), located 30 km to the west from the city of Aldan in the upper reaches of the Inagli stream, was used as an adsorbent and carrier for the immobilization of HOM. Inagli is the right tributary of the Aldan river, confined to the eponymous array of ultrabasic alkaline rocks. The deposit is associated with the weathering crust of chromdiopside-mica metasomatites formed from the dunes of the Inagli pluton. The field has estimated reserves of vermiculite of categories C1+ C2 in the amount of 618 thousand tons. The prognostic reserves of vermiculite in the Inagli massif (taking into account the explored ones) can be estimated at 1 million tons [14, 21].

The average chemical composition of vermiculite deposits, (%): \(\text{SiO}_2 - 48.30; \text{TiO}_2 - 0.99; \text{Al}_2\text{O}_3 - 11.10; \text{Fe}_2\text{O}_3 - 5.05; \text{FeO} - \text{sl.}; \text{MnO} - 0.07; \text{MgO} - 29.7; \text{CaO} - 3.83; \text{K}_2\text{O} - 0.66\).

The capacity of the expanded vermiculite of the Inagli field to oil and petroleum products is shown in Table 1 [22, 23].
Table 1. Sorption capacity of expanded vermiculite, kg/kg.

| Size fraction, mm | Oil  | Gasoline A-76 | Toluene | Diesel oil |
|-------------------|------|---------------|---------|------------|
| 1-2               | 5.4  | 5.1           | 5.0     | 5.7        |
| 2-3               | 4.7  | 3.7           | 3.2     | 3.6        |
| 3-5               | 2.8  | 2.7           | 2.3     | 2.7        |

Thus, expanded vermiculite is a promising carrier sorbent used for the immobilization of HOM cells, since it has the main properties listed above, namely: permeability, oil capacity, non-slumping ability, porosity and flowability; it combines the properties of a carrier for microorganisms and a sorbent for oil, which provides high efficiency and prolongation of reactions of destruction of petroleum hydrocarbons.

4. Results and discussion

Based on the above research results, a biosorbent was developed for cleaning soils from oil pollution [10, 22-24].

Field experiments with the use of the preparation were conducted on permafrost heavy loam soil contaminated with oil.

The initial content of petroleum products in the soil of the experimental site before applying the biosorbent was 44319 mg/kg.

The efficiency of cleaning was assessed by the degree of degradation of oil pollution, by the dynamic pattern of the number of major groups of soil microorganisms and by soil phytotoxicity.

The oil content in the soil was determined by gravimetric method according to the instruction RD52.18.647-2003.

It was found that the degree of biodegradation of petroleum products in the experimental site for 1 growing period after the introduction of biosorbent into the soil was 76.7 % (Table 2).

Table 2. Degradation of petroleum products in the pilot area.

| Variant of experiment                      | Petroleum products content, mg/kg | Degree of degradation of petroleum products, % |
|--------------------------------------------|----------------------------------|-----------------------------------------------|
|                                            | before   | after   |                                            |
| Soil + oil pollution                       | 3446     | 2449    | 14.4                                        |
| Soil + oil pollution + biosorbent          | 44319    | 10339   | 76.7                                        |

The dynamics of biodegradation of oil pollution was consistent with the dynamics of accumulation of soil microorganisms, including oil destructors, whose number increased by an average of 2 orders of magnitude after the introduction of biosorbent into the soil. Microorganisms were recorded in the colony forming units (CFU) per 1 gram of absolutely dry weight (ADW) of the soil (Table 3).

Table 3. Dynamics of microbial accumulation, thousands CFU/g soil ADW.

| Variant of experiment                     | Total abundance of microorganisms | Hydrocarbon oxidizing microorganisms |
|------------------------------------------|----------------------------------|-------------------------------------|
|                                          | before   | after   | before | after |
| Soil + oil pollution                     | 21.9     | 404.4   | 0.03   | 0.6   |
| Soil + oil pollution + biosorbent        | 11.4     | 1645.4  | 0.08   | 126.3 |

Biotesting showed that phytotoxicity decreased in the process of clearing oil contamination in the soil of the experimental site, as evidenced by an increase in the percentage of germination of test plant seeds from 30 to 68%.
5. Conclusion
The obtained results indicate the effectiveness of the biosorbent for cleaning permafrost soils from oil pollution.

The advantage of the biosorbent is that due to the combination of unique properties of the carrier substrate (expanded vermiculite) and the strain of bacteria *Pseudomonas panipatensis* VKPM B-10593 immobilized on it, which is able to develop and destroy oil and petroleum products in a wide temperature range (from +8 °C to +37 °C), it has the ability to stimulate the biological degradation of petroleum products in the soil.

The method of preparation and use of biosorbent is cost-effective, since it does not require complex technological equipment for its implementation. And vermiculite, which serves as a carrier substrate in this biosorbent, is both an affordable and cheap raw material, since vermiculites have a surface occurrence and are developed in an open-cut way.

Cleaning the soil from oil contamination with the obtained biosorbent allows to prevent the spread of pollutants to the adjacent landscapes and to eliminate the spots of contaminated soil, which significantly improves the sanitary and environmental condition of the disturbed territory.

6. References
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