POLLUTION

DOI: https:/doi.org/10.23649/jae.2019.3.11.1

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Received:22.07.2019; Accepted:20.08.2019; Published: 16.09.2019

ANALYSIS OF PHYTOTESTING OF SOILS POLLUTED BY PETROLEUM PRODUCTS AFTER BIOREMEDIATION

Research article

Abstract

The work is devoted to the problem of bioremidation of soils contaminated with petroleum products. The data on the effect of the ”Redetoil” biodestructor developed by us on oil-polluted soils are presented. Evaluation of soil restoration was carried out using phytotesting on the seeds of Brassica oleracea L. Phytotesting showed that after 30 days of remediation, the oil-contaminated soil becomes less toxic to the seeds of Brassica oleracea L., which indicates the rapid effectiveness of ”Remedoil” as an oil biodestructor. During the 8 months of remediation, there is a significant decrease in the toxicity of the soil for the seeds of Brassica oleracea L., which indicates the effectiveness of the drug ”Remedoil” as a biodestructor of petroleum products. On the studied soils, seedlings are formed, the length of which is on average 200 or more times longer than in the first variant of the experiment, the seeds are characterized by earlier germination, high germination rates and the presence of lateral roots in the seedlings.

Keywords: bioremidation, petroleum products, biodestructor, phytotesting.

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Получена: 22.07.2019; Доработана: 20.08.2019; Опубликована: 16.09.2019

АНАЛИЗ ФИТОТЕСТИРОВАНИЯ ПОЧВ ЗАГРЯЗНЕННЫХ НЕФТЕПРОДУКТАМИ ПОСЛЕ БИОРЕМИДИАЦИИ

Научная статья

Аннотация

Работа посвящена проблеме биоремидиации почв, загрязненных нефтепродуктами. Приводятся данные по действию разработанного нами биодеструктора «Ремедойл» на загрязненные нефтешламом почвы. Оценка восстановления почв проводилась с помощью фитотестирования на семенах *Brassica oleracea L.* Фитотестирование показало, что за 30 суток ремедиации загрязненный нефтепродуктами грунт становится менее токсичным для семян *Brassica oleracea L.*, что свидетельствует о быстрой эффективности препарата «Ремедойл» в качестве биодеструктора нефтепродуктов. За 8 месяцев ремидиации, происходит значительное снижение токсичности грунта для семян *Brassica oleracea L.* что свидетельствует эффективности препарата «Ремедойл» в качестве биодеструктора нефтепродуктов. На исследуемых почвах формируются проростки, длина которых в среднем в 200 и более раз больше, чем в первом варианте опыта, семена характеризуются более ранним прорастанием, высокими показателями всхожести и наличием боковых корней у проростков.

Ключевые слова: биоремидиация, нефтепродукты, биодеструктор, фитотестирование.

1. Introduction

Under normal conditions, the natural decomposition of petroleum and petroleum products occurs slowly, since elevated concentrations of hydrocarbons inhibit soil and water self-cleaning activity. As a result, hard-to-oxidize chemical products accumulate in the ecosystem that impede self-purification and self-healing [3]. The process of natural self-healing of the polluted environment is lengthy. At a pollution level of 5 g/kg of soil, it lasts from 2 to 30 years [4].

According to official data, more than 1.2 million hectares of land affected by various types of hydrocarbon pollution currently need reclamation in Russia [4]. Oil pollution by hydrocarbons causes irreversible changes in the morphology, physical and chemical properties of the soil [5].

When polluted by anthropogenic hydrocarbons in the soils, the morphological properties, coloring and addition, first of all change. The erasure of features of the natural profile is accompanied by the appearance of an intense black or brown color, the formation of a bituminous crust on the surface, and a thickening of the structure. This inevitably causes a decrease or loss of soil fertility, leads to a change in the ecological functions of the soil, accompanied by a decrease in biodiversity [6]. In the process of transformation, petroleum hydrocarbons form toxic compounds with carcinogenic, teratogenic and mutagenic properties, resistance to microbiological cleavage and the ability to pass into plants, which reduces the quality of cultivated crops and creates a threat to human and animal health [7, 8].

Thus, the soil degradation observed in areas polluted by oil and oil products allows scientists to attribute them to areas of ecological disaster. The problem of reclaiming oil-polluted soil acquires exceptional importance.

At present, bioremediation (bio-life, remedio-treatment) - purification of the natural environment using biological methods is promising for the purification of oil pollution. For example, stimulation of native microflora by fertilizing the polluted ecosystem or introducing specialized microorganisms preparations created to clean polluted ecosystems [1-2].

Previously it was assumed that microorganisms capable of decomposing and using hydrocarbons of petroleum and petroleum products are found only where oil fields, oil storages or oil pipelines are located, however, microorganisms oil destructors are widespread in nature and can be isolated from soil, sedimentary rocks, sea and river water. These heterotrophic microorganisms can assimilate a variety of organic compounds - carbohydrates, proteins, fats, etc.

Microorganisms oil destructors are able to effectively oxidize oil hydrocarbons with a C9-C30 chain length and aromatic hydrocarbons in a wide range of acidity of the medium (pH 4.5-9.5) and temperatures (5-45° C). The efficiency of hydrocarbon oxidation reaches 99%. As a result of biological treatment of oil pollution with biological products in the environment, easily decomposing bacterial protein remains, which does not require subsequent disposal, and non-toxic products of oil decomposition. The waste products of bacteria and the dead bacteria themselves are easily absorbed by the native microflora, giving the basis for the formation of humus (when using the drug to clean the soil) or forming bottom sludge (if used on water). The degree of purification depends on the initial value of pollution, the chemical nature of petroleum products, the mechanical composition of the soil.

The number of microorganisms oil destructors in natural biocenoses is largely determined by climatic conditions, the type of soil, the degree of their processing, and the depth of groundwater. Peculiarities of oil pollution utilization microorganisms are determined by the chemical and compositional composition of the individual components of the original oil. The chain length is crucial for the use of aliphatic hydrocarbons: as the chain of paraffins lengthens, the number of microorganisms species capable of using these compounds, as well as the activity of their use, increases. After the initial attack of the microorganisms in a very polluted environment, alkanes with a very long chain, polycyclic naphthenes, polyaromatic hydrocarbons and mixtures of substances that make up the fraction of resins and asphaltenes remain. All these substances cannot be metabolized by individual microorganisms. Their natural conditions are associated with the action of mixed populations of microorganisms or their consistent destructive action — the action of communities characterized by relations of cooperation and mutual assistance.

Under laboratory conditions, the degree of degradation of petroleum products ranged from 40.3% to 99.7% for different strains. In the microfield 60-day experiment, the destruction of petroleum products due to the activity of aboriginal microflora was 8.83%, whereas in the area with the introduction of a biological product - 48.37% [9-11].

Although numerous methods and technologies have been developed for the elimination of oil pollution, biological methods are put forward in the list of top priorities due to their high efficiency, environmental safety and economic benefits.

Despite the difficulties of microbiological methods of intensifying the processes of oil decomposition, sometimes contradictory results, technical difficulties in the production and use of microbial preparations, in general, only microorganisms can act as practically significant catalysts for the accelerated degradation of moderate scale oil pollution of natural ecosystems.

2. Materials and methods

For the biodegradation of oil-polluted soils, we proposed the biological preparation “Remedoyl”, which consisted of a consortium of yeast mushrooms of the native flora. The components of the biological product were grown under sterile conditions on special selective media in Petri dishes.

Preparing a liquid growth medium. At room temperature, the seed material was produced with constant stirring and growth control on a Den-1 densitometer for 7-10 days. The minimum concentration of cells in the resulting mother solution should be $4\text{-}6 \times 10^6$/ml.

In order to adapt the cells to the utilization of petroleum hydrocarbons, they were cultivated with intensive aeration with microcompressors (air flow not less than 2 liter/min).

To study the activity of the drug "Remedoyl" used sludge, which was initially analyzed.

Oil sludge is a multicomponent mixture of substances of different nature (asphaltenes, resins, hydrocarbons, alcohols and esters - dioctyl phthalates, mechanical impurities, heavy metals - Pb, Mn, Cr, Fe, Al, Cu, V).

To identify the composition of the organic part of the sludge, chromatography was carried out using a gas chromatography mass spectrometer GCMS-QP2010S Ultra from SHIMADZU (RestekRtx-5MS column, 30 mx 0.25 mmID, 0.25 μmdf, carrier gas — helium, ionization energy 70 eV) in a hexane solvent.

Analysis of the biodestructive activity of the drug ”Remedoil” was performed in the laboratory by the method of phytoindication on the seeds of *Brassica oleracea L.*

A 50 g sample of the soil was placed in a Petri dish and 5 ml of ”Remedoil” biodestructor was added. Then oil sludge was added in the following concentrations (relative to the weight of the soil): 1) 1%, 2) 2%, 3) 3%, 4) 4%, 5) 5%, 6) 6%, 7) 7%, 8) 8%, 9) 9%, 10) 10%. The biodegradator was added to all variants with thorough mixing of the soil. Evaluation of the results was carried out after 5 days and after 10 days of incubation (120 h of biotesting) and after 8 months, using seeds of *Brassica oleracea L.* for phytoindication. Noted the number of germinated seeds in the experimental and control variants on soils without bioremediation and treated with ”Remedoyl”. In each repetition, germination and length of seedlings were recorded.

3. Results and discussion

Seeds of *Brassica oleracea L.* showed high germination in the control in both the first and second variants of the experiment (after remitting the soil with an initial contamination with oil sludge 1% and 2%) (Table 1). The average germination (50-60%) have seeds in variants of experience 3,8,9,10, i.e. after bioremidation of soils with a fairly low level of initial pollution (3% of oil sludge) and a high level of initial pollution (8–10%). With an average level of initial contamination (4-7%), seeds of *Brassica oleracea L.* showed a rather low germination rate of 30-40%.

Table 1 – Indicators of seed germination of *Brassica oleracea L.*

Experience options	Germination, %	
	10 days (120 hours)	8 months
1	100	100
2	90	100
3	50	100
4	30	100
5	30	100
6	40	100
7	30	100
8	50	65
9	60	60
10	50	25
Control	100	100

However, when studying the dynamics of seed germination of *Brassica oleracea L.*, it can be noted that the seeds in the control germinate most actively on the first day of observation, and 100% germinate in three days. For the first five days in the variants of the experiment, seed germination is most intensive with initial contamination with oil sludge up to 5%, with higher initial contamination, seeds germinate in the later periods and have low germination and weak seedlings. Data on the length of seedlings on the 5th and 10th day are given in Table 2.

Table 2 – Length of seedlings of Brassica oleracea L. (120 hours of bioremidation)

Variants of experience	The length of seedlings, cm	
	5 days of germination	10 days of germination
1	4,7	6,2
2	4,3	5,1
3	3,1	3,8
4	-	1,4
5	1,1	1,5
6	0,9	1,3
7	0,6	1,1
8	1,2	1,5
9	0,3	0,6
10	-	0,3
Control	85,6	151

We studied samples of soils polluted with oil sludge after 8 months. During this period, the soil dried out completely, then it was watered with ordinary tap water until fully moistened and then dried again to completely dry condition in the air, in Petri dishes. The experiment was repeated 10 times. After the last moistening, phytotesting experiments were carried out on the seeds of *Brassica oleracea L.*

The seeds of *Brassica oleracea L.* showed a high germination rate in the control and in the first - the seventh variants of the experiment (after remitting the soil with an initial pollution of oil sludge 1 - 7%) (table 1). Sufficiently high germination (60-70%) have seeds in variants of experiment 8.9, i.e. after bioremidation of soils with a high level of initial pollution (8–9% of oil sludge). In the last version of the experiment, the seeds of Brassica oleracea L. They showed a rather low germination rate of 30%.

However, when studying the dynamics of seed germination of *Brassica oleracea L.*, it can be noted that the seeds in the control germinate most actively in the first days of observation, and in three days 100% germinate. In the first five days in the variants of the experiment, seed germination is most intensive with initial contamination with oil sludge up to 50%, with higher rates of initial contamination the seeds germinate in the later periods and have low germination and weak seedlings. Data on the length of seedlings on the 5th and 10th day are given in Table 2.

Table 3 – Length of seedlings of Brassica oleracea L. (8 months of bioremidation)

Variants of experience	The length of seedlings, cm	
	5 days of germination	10 days of germination
1	107	152
2	101	158
3	96	146
4	84	137
5	67	120
6	39	105
7	25	26
8	28	31
9	24	28
10	15	15
Control	87	146

4. Conclusion

Phytotesting showed that after 30 days of remediation, oil-contaminated soil becomes less toxic for *Brassica oleracea L.* seeds, which indicates the rapid effectiveness of ”Remedoil” as a biodestructor of petroleum products. During the 8 months of remediation, the oil-contaminated soil becomes less toxic for the seeds of *Brassica oleracea L.*, which testifies to the effectiveness of the drug ”Remedoyl” as a biodestructor of oil products. Compared with soil testing after 120 days of re-mediation, the latest data show an effective drug effect after 8 months. On the studied soils, seedlings are formed, the length of which is on average 200 or more times longer than in the first variant of the experiment, the seeds are characterized by earlier germination, high germination rates and the presence of lateral roots in the seedlings. We also noted a more active growth of seedlings in 1-3 variants of the experiment compared with the control.

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