*Pol. J. Environ. Stud. Vol. 32, No. 3 (2023), 2455-2464*
DOI: *10.15244/pjoes/160198* ONLINE PUBLICATION DATE: 2023-03-07

*Original Research*

# Isolation, Selection, and Use of Oil-Degrading Microorganisms for Biological Treatment of Contaminated Soil

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*Received: 5 October 2022*
*Accepted: 2 February 2023*

**Abstract**

The intensive use of oil products is one of the most significant problems of environmental pollution. They pollute water, soil, and air. For these reasons, the search for effective methods of oil biodegradation is very important for solving environmental problems. The research aimed to isolate and select oil-degrading microorganisms and to investigate their effectiveness in oil-contaminated soil "ex-situ". Using microorganisms isolated from the contaminated soil, a biopreparation was developed consisting of bacteria, yeast and filamentous fungi. Microorganism isolates were identified using MALDI-TOF mass spectrometry (Bruker, USA). The biopreparation was tested in the soil treatment sites of GVT LT, Ltd (Kiškėnai village, Dovilai eldership, Klaipėda district) in two test variants. The count of microorganisms (CFU/g) in the soil was determined at the beginning of the research, after two weeks and every month thereafter until the end of the cleaning season by serial dilution method in media with 1% of diesel. The content of oil products (g/kg) was determined with the same periodicity according to LST EN ISO 16703. An effective biopreparation for the degradation of oil hydrocarbons has been developed. Within 5 months, it reduced the amount of oil products to 61,7% in variant No. 1 and 58,7% in variant No. 2.

**Keywords**: oil, biodegradation, bacteria, yeast, filamentous fungi

## Introduction

Oil is the backbone of the global fuel and energy economy. The extraction, transport, and use of oil and its products pollute the environment – soil, water, and air – endangering living organisms in ecosystems and human health.

Between 0.08% and 0.4% of the world's annual oil production is estimated to be released into the environment as pollutants. The amount of oil released into the environment is increasing every year and causing more and more environmental problems. Many methods of cleaning the environment from these pollutants are known, but not all of them are effective

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enough. The search for and development of new biological ways of cleaning the environment is one of the most important environmental problems [1].

Microorganisms are widespread in the biosphere and have astounding metabolic properties. They can grow over a very wide range of environmental conditions. The nutritional versatility of microorganisms can also be used for the biodegradation of various contaminants. This process is called bioremediation. It is based on the ability of microorganisms to convert, transform and utilize hazardous pollutants, obtain energy, and increase biomass in the process. Instead of simply accumulating and retaining contaminants, bioremediation is used as a way to break down and transform contaminants into less toxic or completely non-toxic forms [2].

A lot of the oil hydrocarbons released into the environment are degraded or metabolized by microorganisms naturally present in the environment, which use them to meet their carbon demand and the needs of the reproduction process, as well as to reduce the physiological stress caused by oil pollutants. The work of various scientists shows that the environment polluted by oil hydrocarbons contains significant amounts of oil-degrading microorganisms and their amounts and prevalence are closely related to the types of oil products and environmental conditions. Many species of normal and extreme microorganisms have been isolated and used for the biodegradation of oil hydrocarbons [3-4].

Many scientists around the world are studying the role of microorganisms in the biodegradation of oil products. Studies have shown that the most common fungi involved in the biodegradation process belong to the following genera: *Alternaria, Aspergillus, Candida, Cephalosporium, Cladosporium, Fusarium, Geotrichum, Gliocladium, Mucor, Paecilomyces, Penicillium, Pleurotus, Polyporus, Rhizopus, Rhodotorula, Saccharomyces, Talaromyces* and *Torulopsis*. Bacterial genera involved in the biodegradation of oil hydrocarbons: *Achromobacter, Acinetobacter, Alkanindiges, Alteromonas, Arthrobacter, Burkholderia, Dietzia, Enterobacter, Kocuria, Marinobacter, Pandoraea, Pseudomonas, Staphylococcus, Streptobacillus, Streptococcus* and *Rhodococcus* [3, 5].

Various studies have shown that the biodegradation process of oil hydrocarbons is affected by a variety of environmental processes. When oil hydrocarbons enter the environment, they are affected by the environmental factors: physical-dispersion, physicochemical-evaporation, dissolution, absorption, chemical-photooxidation, auto-oxidation, and biological-plant and microbial catabolism. Environmental conditions can strongly affect the potential of oil biodegradation, and the extent of biodegradation and the type of environmental conditions can vary depending on the location of the contaminated site [6-8].

One of the main factors influencing degradation is the availability of microorganisms capable of performing pollutant catabolism. In the environment affected by oil pollution (oil spills, shipping lanes, ports, oil fields, gas stations, and other similar facilities), microorganisms use oil hydrocarbons in large quantities as readily available nutrients [9-11].

Bioavailability is understood as the amount of a chemical present in the soil that can be transformed by microorganisms living in it. Also, this term describes the influence of physical, chemical, and microbiological factors on the biodegradation process. Oil hydrocarbons have low bioavailability and are classified as hydrophobic organic pollutants. These substances are sparingly soluble in water, making them resistant to photolytic, chemical, and biological degradation [9-11]. There are also other ways to clean up an oil-contaminated environment. Biological (bioremediation and phytoremediation), chemical (chemical oxidation and electrokinetic remediation), thermal (incineration, thermal desorption, and microwave heating), and physicochemical (solvent extraction, soil vapor extraction, flotation technology, and sonication) can be used to clean the oil-contaminated environment [12].

The goal of our research was to isolate and select efficient oil hydrocarbon-degrading microorganisms and to compile a biopreparation for the cleanup of contaminated soil.

## Material and Methods

### Sample Collection, Isolation, Screening, and Identification of Oil-Degrading Microorganisms

Soil samples (100 g) from surface soil (0-15 cm depth) were collected from 7 different locations (40 sampling points) in the territories of the Republic of Lithuania where there is increased pollution with oil products: Klaipėda fuel base (6 samples), Klaipėda city (5 samples), Radviliškis fuel base (9 samples), Kaunas fuel base (5 samples), Vaidotai fuel base (4 samples), Vilnius city (7 samples) and Vilnius fuel base (4 samples).

Samples were taken from 3-4 random locations per plot, mixed, and transferred into sterile plastic bags using a sterile spatula. Samples were stored in a refrigerator at 5ºC before delivery to the laboratory. In the lab, stones and other unwanted soil debris were removed using 3 millimeters sieve. The cultivable microorganisms were isolated and enumerated using the serial dilution plate technique. 0.1 ml of the suspension was inoculated into an agar medium with 1% diesel plates and spread on the surface of the medium with a spreader in three replications [13]. The media used were: solid medium for yeasts with 1% of diesel ($(NH_4)_2SO_4$ – 5.0 g/l, $K_2HPO_4$ – 0.15 g/l, $KH_2PO_4$ – 0.85 g/l, $MgSO_4$ – 0.5 g/l, NaCl – 0.1 g/l, $CaCl_2$ – 0.1 g/l), medium for bacteria with 1% of diesel ($NH_4Cl$ – 2.0 g/l, NaCl – 5.0 g/l, $Na_2HPO_4$ – 3.0 g/l, $KH_2PO_4$ – 0.1 g/l, $MgSO_4$ x $7H_2O$ – 2.0 g/l, $CaCl_2$ x $6H_2O$

– 0.01 g/l, $MnSO_4$ x $5H_2O$ – 0.02 g/l, $FeSO_4$ x $7H_2O$ – 0.01 g/l), Czapak agar ($KH_2PO_4$ – 1.00 g/l , KCl – 0.50 g/l , $NaNO_3$ – 3.00 g/l , $MgSO_4 \cdot 7H_2O$ – 0.50 g/l, $FeSO_4$ – 0.01 g/l) with 1% of diesel (for filamentous fungi).

The plates were incubated in the incubator at 25-28ºC (for yeast and fungi) and 30ºC (for bacteria). Yeast colonies were counted after 2-3 days of incubation, bacteria – after 1-2 days of incubation, and filamentous fungi – after 5-7 days of incubation [13-15].

The biodegradability activity of the microorganism isolates was tested by growing them in a liquid medium for bacteria, filamentous fungi and yeasts supplemented with 1,0% of diesel (the same media that was used to isolate the microorganisms without agar) for 72 hours at the same temperature as during the isolation of the microorganisms. The initial microorganism count was $10^5$ CFU/ml. Isolates were selected based on the count of colony-forming units (CFU) (grown in the same media that was used to isolate the microorganisms) per milliliter (CFU/ml) of culture fluid.

Microorganism isolates were identified using the Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry method (Bruker, USA). Samples were prepared by formic acid extraction (for filamentous fungi) and direct transfer (for yeast and bacteria) protocols [16].

### Determination of Oil Hydrocarbon Degradation Activity and Residual Content of Oil Hydrocarbons

The research lasted five months, beginning in June 2021 and ending in November 2021.

The biodegradation activity of microorganisms was investigated in two variants, which differed in the amount of oil hydrocarbons. The initial amount of oil hydrocarbons in variant No.1 was 50 g/kg and in variant No.2 - 48 g/kg. Fertilizers were added to the soil (g/kg): ammonium nitrate (0.36), "Azofoska" NPK 16-16-16 (0.216), potassium chloride (0.084).

Control variants were also formed from these soils (variants No.1 and No. 2), where the extent of self-cleaning was observed.

The suspension (400 ml) of selected microorganisms was prepared and multiplied in a fermenter with a capacity of 3l and then in a fermenter with a capacity of 3 $m^3$ until it reached $10^9$ CFU/ml concentration. Microorganisms were incubated for three days at 25ºC under aeration. The composition of the culture medium (g/l) was: ammonium nitrate (1.50), "Azofoska" NPK (16-16-16) (0.50), potassium chloride (0.25), diesel (4.00).

The resulting suspension of microorganisms was sprayed on a total of 470 tons of soil contaminated with oil products. During the spraying of the biopreparation, the soil was mixed and later aerated every two weeks.

The initial count of microorganisms (CFU/g) in the contaminated soil before and after the application of the biopreparation was estimated. The count of microorganisms in the test variants was determined after two weeks and every month thereafter until the end of the cleaning season. It was done by analyzing soil samples by the serial dilution method (on the media described in section Sample collection, isolation, screening, and identification of oil-degrading microorganisms).

The residual content of oil hydrocarbons (g/kg of soil) in the dry soil samples was determined according to LST EN ISO 16703 (LAND 89-2010) at the GVT LT, Ltd.

### Statistical Analysis

Data analysis was performed with IBM SPSS Statistics 27 software (IBM Corporation, USA). The obtained results were considered statistically significant when $P<0.05$.

## Results and Discussion

### Isolated Microorganisms, Selected Strains, and Identified Microorganisms

Microorganisms of various systemic groups (yeasts, filamentous fungi, bacteria) were isolated from various oil-contaminated soils. A total of 36 microorganism isolates were isolated, of which eighteen were bacterial, ten yeast, and eight filamentous fungi isolates. The count of oil-degrading microorganisms (CFU/g) in oil-contaminated soils and the most abundant species of oil-degrading microorganisms are shown in Table 1.

Analyzing the results of the study, it was determined that the highest count of microorganisms degrading oil hydrocarbons was: 2.1 x $10^4$ CFU/g of yeasts in the soil samples of the Klaipėda fuel base, 1.6 x $10^4$ CFU/g of bacteria in soil samples from Radviliškis fuel base and 6.2 x $10^4$ CFU/g of bacteria in the soil samples collected in Vilnius city. The lowest count of microorganisms was found in soil samples from the Vaidotai fuel base (2.2 x $10^2$ CFU/g of yeasts). It was also observed that yeasts were found only in the soil samples from Klaipėda, Radviliškis, and Vaidotai fuel bases, while fungi and bacteria were found in all soil samples.

Two strains of *Sporobolomyces roseus* yeast were isolated that showed the ability to grow at lower ambient temperatures. *Bergauer* et al. (2005) show that these yeasts can grow at 1.0-15.0ºC ambient temperature. This property is unique to psychrophilic yeasts adapted to survive at low ambient temperatures. *Sporobolomyces roseus* does not belong to the group of true psychrophilic yeasts because the maximum temperature at which they can grow is 25.0ºC [22]. Our isolated yeasts also had this property.

During the selection, the ability of all tested microorganisms to grow in a medium with 1,0% of diesel was evaluated. Thirty-six microorganism isolates were tested and 6 strains of bacteria, 5 yeasts, and

Table 1. Content of oil-degrading microorganisms and the most abundant species of oil-degrading microorganisms in examined soil (CFU/g soil).

| Soil description | The average count of oil-degrading microorganisms (CFU/g) | | | The most common species of oil-degrading microorganisms | | |
|---|---|---|---|---|---|---|
| | Bacteria | Filamentous fungi | Yeasts | Bacteria | Filamentous fungi | Yeasts |
| Soil potentially contaminated with oil hydrocarbons (Klaipėda fuel base) | 1.8 (± 0.02) x $10^3$ | 1.5 (±0.08) x $10^3$ | 2.1 (±0.09) x $10^4$ | *Micrococcus luteus*, *Rhodococcus fascians*, *Pseudomonas brassicacearum* | *Acremonium kiliense* (current name: *Sarocladium kiliense)* | *Rhodosporidium diobovatum*, *Rhodotorula minuta*, *Rhodotorula mucilaginosa* |
| Soil potentially contaminated with oil hydrocarbons and heavy metals (chrome) (Klaipėda city) | 3.5 (± 0.07) x $10^3$ | 2.5 (±0.12) x $10^3$ | 0 | *Pseudomonas putida*, *Micrococcus luteus* | *Aspergillus niger* | - |
| Soil potentially contaminated with oil hydrocarbons (Radviliškis fuel base) | 1.6 (± 0.03) x $10^4$ | 1.9 (±0.05) x $10^3$ | 2.0 (±0.12) x $10^3$ | *Klebsiella oxytoca*, *Acinetobacter baumanii*, *Pseudomonas kilonensis*, *Acinetobacter johnsonii* | *Trichoderma longibrachiatum* | *Rhodosporidium diobovatum*, *Yarrowia lipolytica*, *Sporobolomyces roseus* |
| Soil potentially contaminated with oil hydrocarbons (Kaunas fuel base) | 2.2 (± 0.08) x $10^3$ | 4.6 (±0.12) x $10^3$ | 0 | *Pseudomonas cedrina* | *Fusarium solani* | - |
| Soil potentially contaminated with oil hydrocarbons (Vaidotai fuel base) | 3.5 (± 0.07) x $10^3$ | 1.1 (±0.06) x $10^3$ | 2.2 (±0.10) x $10^2$ | *Pseudomonas brassicacearum*, *Acinetobacter calcoaceticus*, *Pseudomonas corrugata* | *Fusarium oxysporum* | *Sporobolomyces roseus*, *Candida tropicalis*, *Aureobasidium pullulans*, *Rhodotorula mucilaginosa* |
| Soil potentially contaminated with oil hydrocarbons (Vilnius fuel base) | 2.5 (± 0.08) x $10^3$ | 1.8 (±0.06) x $10^3$ | 0 | *Pseudomonas koreensis*, *Pseudomonas pleoglossicida* | *Cladosporium cladosporioides* | - |
| Soil potentially contaminated with oil hydrocarbons and heavy metals (Vilnius city, Geležinis vilkas street) | 6.2 (± 0.15) x $10^4$ | 7.1 (± 0.13) x $10^3$ | 0 | *Pseudomonas jessenii*, *Pseudomonas frederiksbergensis*, *Pseudomonas trivialis* | *Trichoderma harzianum* | - |

3 strains of filamentous fungi with the most abundant growth were selected. The results of microorganism selection are shown in Fig. 1.

The species of microorganisms that we have been able to isolate for use in the biodegradation of oil products have also been described in the publications of other authors. According to the literature, many species of microorganisms are involved in the biodegradation of oil hydrocarbons, including *Acinetobacter, Aeromonas, Bacillus, Corynebacterium, Flavobacterium, Klebsiella, Micrococcus, Nocardia, Pseudomonas, Rhodococcus, Acremonium, Aspergillus, Candida, Cladosporium, Fusarium, Saccharomyces, Trichoderma, Yarrowia* and other genera [17-24].

When evaluating the ability of microorganisms to grow in a medium with 1% diesel, it was observed that *Trichoderma harzianum* GTC BTL VLN 7 had the least abundant growth (3.6 x $10^4$ CFU/ml) among filamentous fungi, and *Trichoderma longibrachiatum* GTC BTL RDV 12 had the most abundant growth (5.5 x $10^7$ CFU/ml). Among yeasts, the growth of *Rhodotorula minuta* GTC BTL KL 45 was the least abundant (1.2 x $10^4$ CFU/ml), and *Sporobolomyces roseus* GTC BTL RD 169 had the most abundant growth (5.0 x $10^7$ CFU/ml). Of the bacteria, *Pseudomonas putida* GTC BTL KLP 3 had the least abundant growth (1.1 x $10^5$ CFU/ml) and *Acinetobacter baumanii* GTC BTL RDV 7 had the most abundant growth (6.5 x $10^{10}$ CFU/ml).

Microorganisms with the most abundant growth were selected and included in the biopreparation which consisted of 6 bacteria (*Klebsiella oxytoca, Acinetobacter baumanii, Micrococcus luteus,*

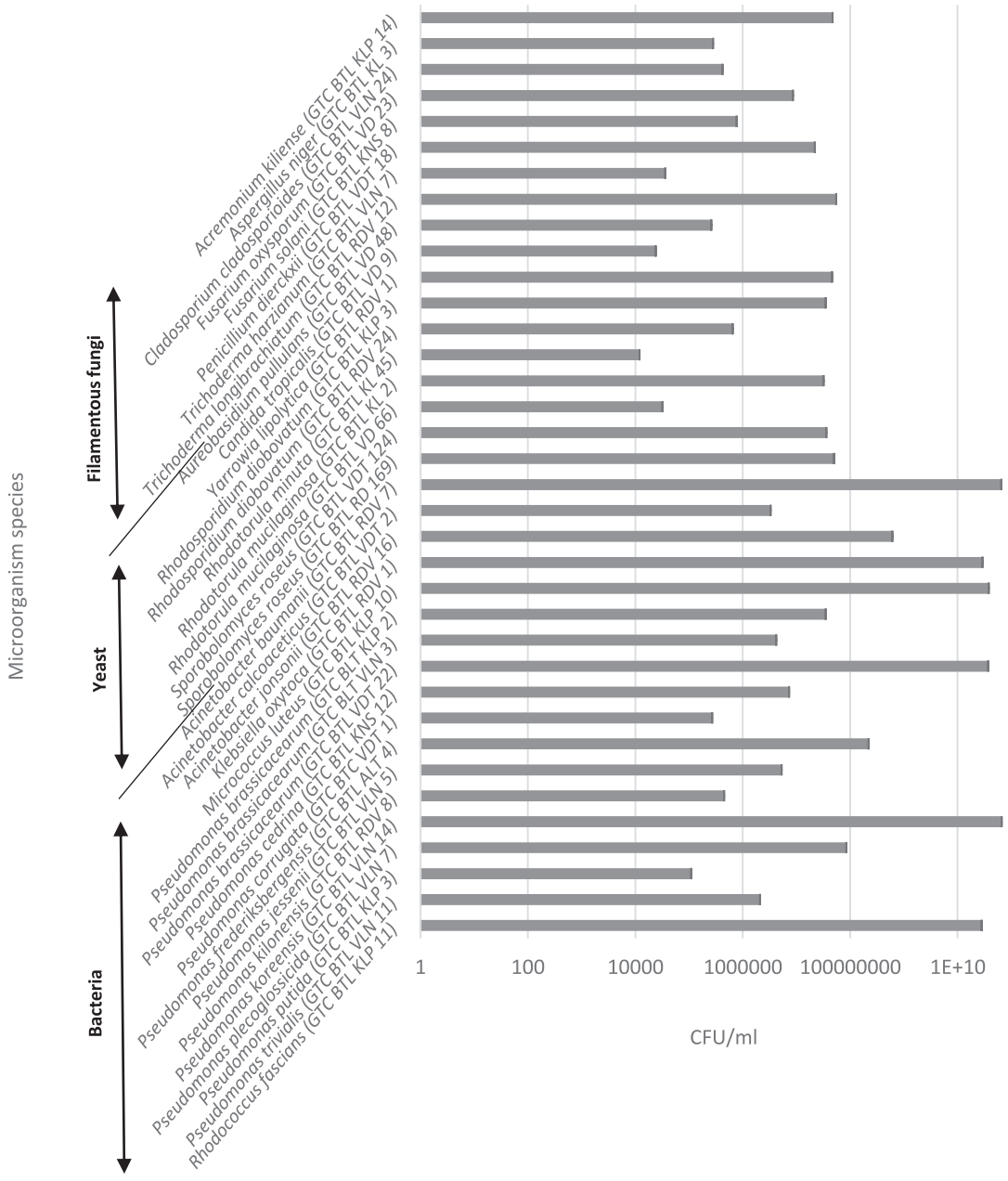


Fig. 1. Count of oil-degrading microorganisms (CFU/ml) in selection media with 1.0% diesel.

*Rhodococcus fascians, Pseudomonas koreensis, Pseudomonas brassicacearum*), 4 yeasts (*Yarrowia lipolytica, Rhodotorula mucilaginosa, Rhodosporidium diobovatum, Sporobolomyces roseus* (2 strains)) and 3 species of filamentous fungi (*Trichoderma longibrachiatum, Acremonium kiliense, Penicillium dierckxii*).

## Oil Hydrocarbon Degradation

### *Characterization and Change of Environmental Conditions during the Biodegradation of Oil Hydrocarbons*

The soil pH during this research remained close to neutral throughout the experiment and was in the pH range of 6.0-7.0 at both experimental variants. Most heterotrophic microorganisms grow best at neutral to alkaline pH. Also, according to the literature, the biodegradation of some oil hydrocarbons is most intense in the pH range of 6.5-8.0 [25].

The average monthly ambient temperature during the research period ranged from 10.5 to 22.5ºC (Fig. 2). According to the literature, ambient temperature influences changes in soil temperature. In summer soil temperature is lower than the ambient temperature. In the deeper layers of the soil (from a depth of 1 meter), the temperature is more constant and remains in the range of about 12.0-13.0ºC all year round [26].

Soil temperatures ranged from 15.0ºC to 34.0ºC during the research period (Fig. 2). The average soil temperature for the entire study period was 26.3ºC. According to the literature, the optimal temperature for the growth of filamentous fungi and bacteria is 25.0-30.0ºC [27]. The highest levels of hydrocarbon metabolism are achieved at a soil temperature of 30.0-40.0ºC. At lower ambient temperatures, the extent of degradation usually decreases, presumably due to decreasing enzyme activity [25]. The soil temperature in Lithuania reaches 25.0-30.0ºC degrees only in June-August, and for the rest of the year it is lower [28], therefore, it can be assumed that biodegradation rates should be highest during summer.

Soil humidity during the research period in both experimental sites and control sites ranged from 12.0% to 27.0%. According to the literature, the optimum humidity for bacterial growth is 20.0% and for growth of filamentous fungi is 60.0%. However, the highest activity of microorganism enzymes is achieved at a humidity level of 20.0% [29]. Assessing the soil moisture fluctuations during the research, it can be stated that the soil moisture was close to optimal, but care should be taken to ensure its stability. Depending on the soil humidity, it was additionally irrigated every two weeks. There were no significant differences in soil humidity between the test and control variants.

### *Changes in the Count of Microorganisms in the Treated Soil*

The count of oil-degrading bacteria (CFU/g) in the soil with applied biopreparation increased until week 2 of the research and then decreased throughout the research period and ranged from $10^{11}$ CFU/g to $10^5$ CFU/g (Fig. 3). Before the application of the biopreparation to the oil-contaminated soil, the count of oil-degrading bacteria was $10^4$-$10^7$ CFU/g.

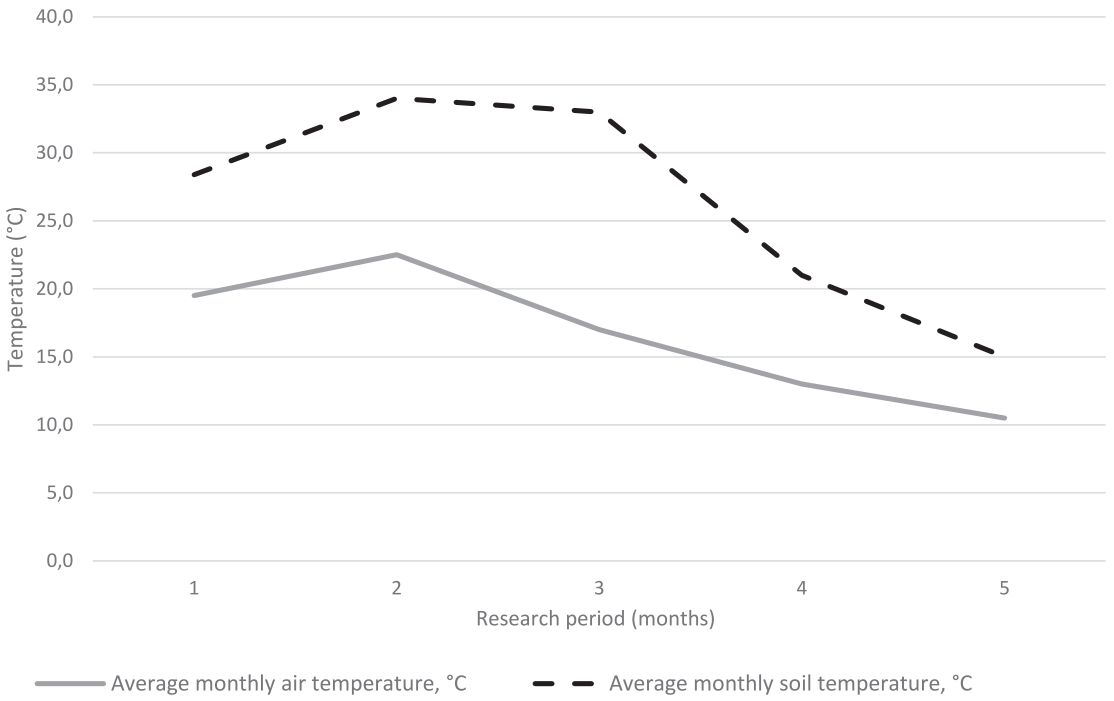


Fig. 2. Average monthly air and soil temperatures.

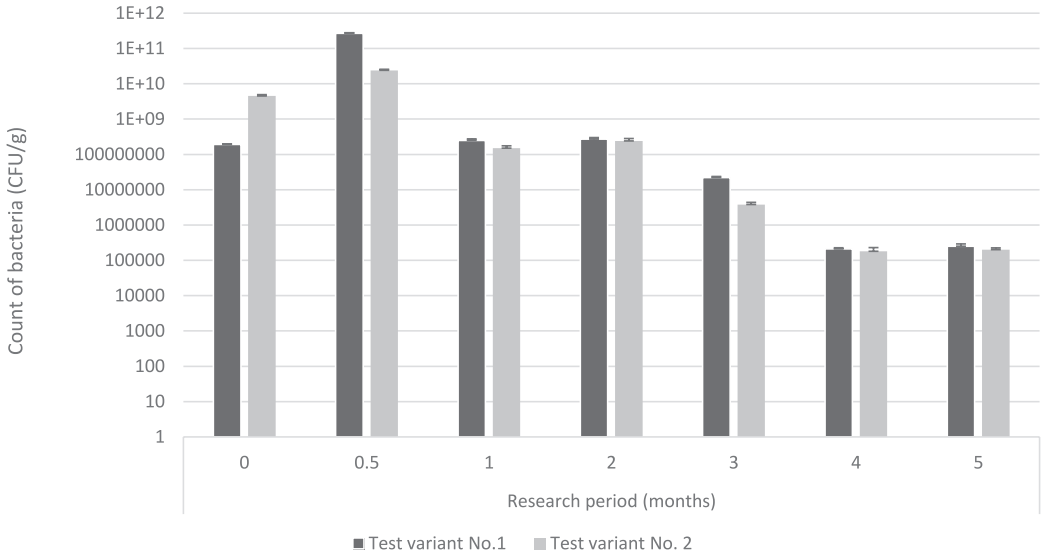


Fig. 3. Changes in the count of bacteria in test variants (CFU/g).

The count of oil-degrading yeasts remained stable until the 5th month of the research and differed little from the initial yeast count. From the third month of the research, the count of yeasts started to decrease and ranged from $10^4$ to $10^3$ CFU/g (Fig. 4). Before the application of the biopreparation to the oil-contaminated soil, the count of oil-degrading yeast was $10^2$-$10^3$ CFU/g.

The count of oil-degrading filamentous fungi remained in the range of $10^4$-$10^5$ CFU/g until the 5 month of the research with slight fluctuations (Fig. 5). Before the application of biopreparation to oil-contaminated soil, the count of oil-degrading filamentous fungi was $10^3$-$10^4$ CFU/g.

According to the literature, the counts of indigenous microorganisms in the contaminated soil can be $10^4$-$10^8$ CFU/g of bacteria, $10^3$-$10^4$ CFU/g of yeast and $10^3$-$10^4$ CFU/g of filamentous fungi depending on the time of year and other environmental conditions [30-34].

*Changes in the Residual Amount of Oil Hydrocarbons*

In the control soil samples, the amount of oil hydrocarbons decreased from 50 g/kg to 46 g/kg at control No.1 and from 48 g/kg to 44 g/kg at control No.2 over five months. The amount of oil hydrocarbons in the test variants decreased steadily throughout the research period. After the first month of testing, a higher amount of oil hydrocarbons was found in variant No. 2 compared to the test results obtained during the first two weeks of the test. This change in

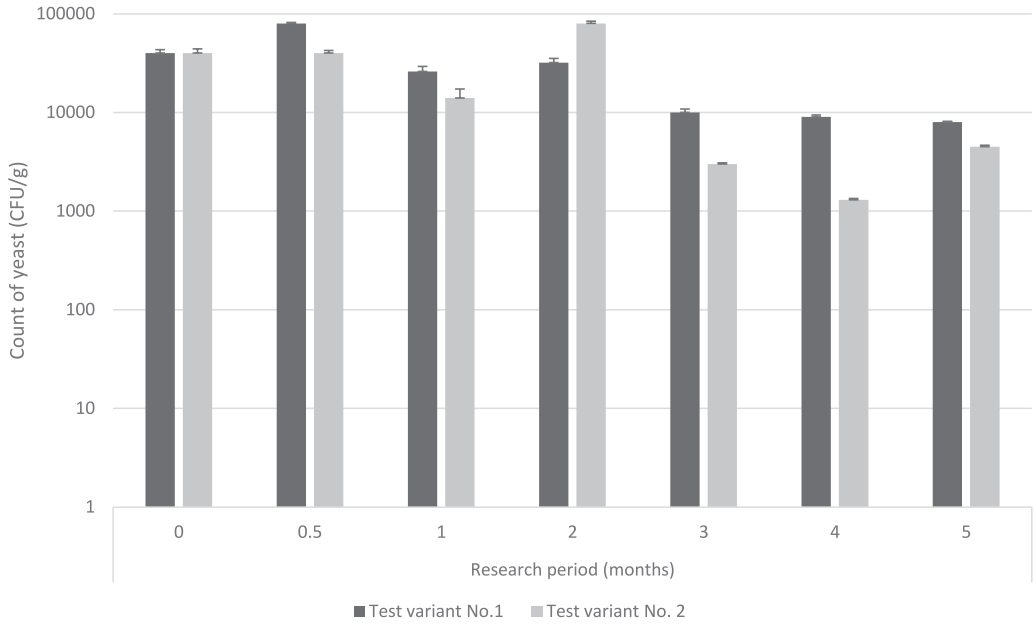


Fig. 4. Changes in the count of yeast in test variants (CFU/g).

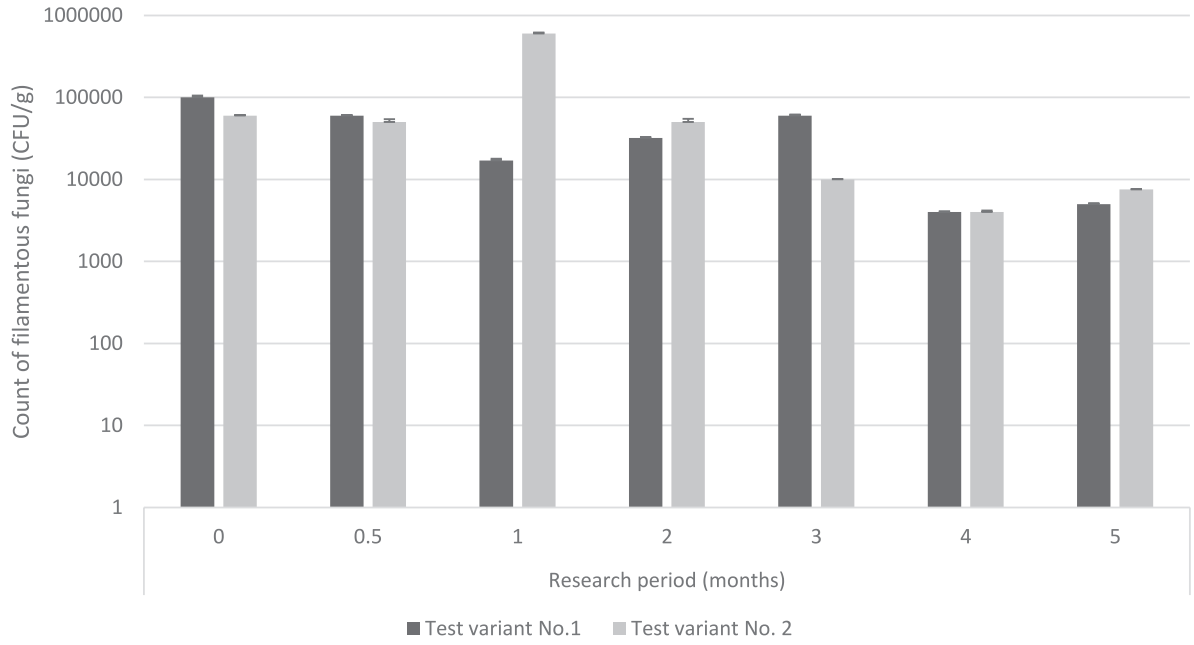


Fig. 5. Changes in the count of filamentous fungi in test variants (CFU/g).

the amount of oil hydrocarbons may have been due to an insufficiently homogenized soil sample. When this discrepancy was observed, other soil samples were given more attention to ensure their homogeneity. The amount of oil hydrocarbons in the soil with biopreparation compared to control variants decreased by 60.9% and 56.8%, respectively (P<0,05) (Fig. 6).

The results of the studies obtained by various authors are very different, the extent of biodegradation ranges between 7.4% and 76.7% of the total amount of biodegraded oil hydrocarbons. In the mentioned studies, the initial amount of oil hydrocarbons differed, which ranged between 1.0-5.0% of oil hydrocarbons per kilogram of soil. The authors also studied the biodegradation of different oil hydrocarbons, some of them described only the results of biodegradation of light oil fractions, while others described the decrease in the total amount of oil hydrocarbons. As is well known, the extent of biodegradation of oil hydrocarbons varies greatly depending on which of the oil fractions

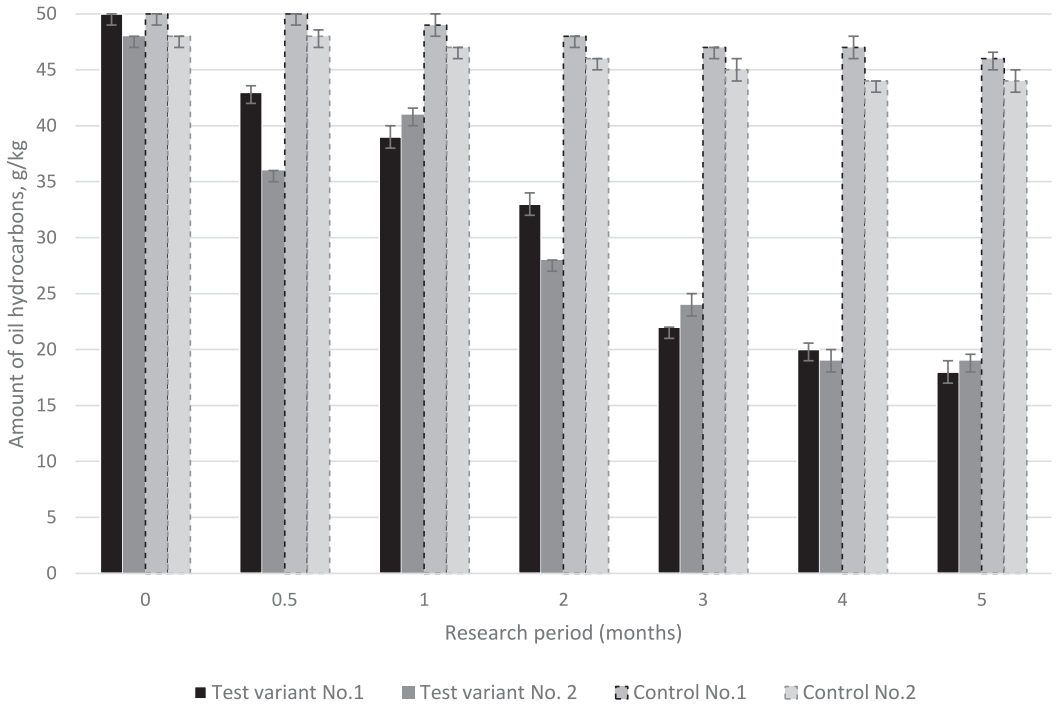


Fig. 6. Decline of oil hydrocarbons in the soil during the research period.

the soil is contaminated with. The microorganisms and their mixtures (biopreparations) also differed [18, 22, 35]. Therefore, it is quite difficult to compare the results of these studies.

Fan et al. (2014) found that increasing the concentration of oil hydrocarbons in the soil decreases biodegradation activity. At a concentration of 5.0% in oil hydrocarbons in the soil, the biodegradation efficiency was 42.0% [36].

The rates of "self-cleaning" of the soil in this research were 8.0% (control No. 1) and 8.3% (control No. 2) over the whole research period, or about 2.0% each research month on average. These results are consistent with those obtained by other authors. Liu et al. (2010) found that soil "self-cleaning" rates can reach up to 15.6% per year. "Self-cleaning" of soils from oil hydrocarbons is caused by microbiological processes of oxidative degradation of the pollutants, evaporation of light-end split products and their filtration into the lower layers of the soil [37].

In this research, the contaminated soil contained as much as 50 g/kg (variant No. 1) and 48 g/kg (variant No. 2) of oil hydrocarbons (5.0 and 4.8%, respectively). According to the literature, the highest rates of biodegradation are achieved in the soil with less than 5.0% of oil hydrocarbons, and 5.0% and more of them in the soil can be toxic to microorganisms [19]. Therefore, it can be stated that the results obtained in this research are unique in that the microorganisms were able to survive in such unfavorable conditions and significantly ($P<0.05$) reduce the total amount of oil hydrocarbons in the soil.

## Conclusions

The results of the research showed that microorganisms isolated from various oil-contaminated soils in this research were capable of cleaning the soil contaminated with high amounts of oil hydrocarbons. An effective biopreparation degrading oil hydrocarbons was developed, which consisted of 6 bacteria (*Klebsiella oxytoca, Acinetobacter baumanii, Micrococcus luteus, Rhodococcus fascians, Pseudomonas koreensis, Pseudomonas brassicacearum*), 4 yeasts (*Yarrowia lipolytica, Rhodotorula mucilaginosa, Rhodosporidium diobovatum, Sporobolomyces roseus* (2 strains)) and 3 species of filamentous fungi (*Trichoderma longibrachiatum, Acremonium kiliense, Penicillium dierckxii*). The obtained results showed that within 5 months the amount of oil hydrocarbons in test variant No. 1 decreased by as much as 60.9% and in test variant No. 2 - by 56.8%. Bearing in mind that the test variants had a high or even toxic amount of oil hydrocarbons, it can be concluded that the biopreparation performed particularly efficient degradation of petroleum hydrocarbons and significantly reduced their amount in the soil.

## Acknowledgments

This work has been supported by the funding of GVT LT, Ltd.

## Conflict of Interest

The authors declare no competing interests.

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