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Research Article

Isolation of kerosene-degrading bacteria from soil samples and determination of optimal growth conditions

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ABSTRACT

Bacteria capable of decomposing hydrocarbon rocket power kerosene T-1 were isolated and studied. In the course of the studies, 30 isolates of microbial cultures were isolated from soils contaminated with hydrocarbon rocket fuel, of which 9 isolates were selected that actively assimilate kerosene T-1 as the only headwater of carbon. The strains that showed the best results in these screening analyses on four nutrient media with a concentration of T-1 kerosene 1% (10 g/kg) grow well 9 isolates of culture microorganisms: № 4, 8, 14, 23, 5, 18, 20, 25 and yeast № 12/5. Isolates on a medium with a concentration of T-1 kerosene 2% (20 g/kg) and 5% (50 g/kg) showed good growth in bacterial cultures № 5, 18, 20, 25, and yeast № 12/5.
The selected microorganisms were identified by physiological and biochemical characteristics: № 5 – *Arthrobacter sp.,* №18 – *Acinetobacter calcoaceticum,* №20 – *Bacillus sp.,* №25- *Micrococcus roseus,* № 12/5- *Candida sp.* Cultivation conditions for isolated microorganisms were created. It has been established that the optimal development temperature for the culture of *Arthrobacter sp. 5* is 25-30°C, *Acinetobacter calcoaceticum. 18* is 30-35 °C , *Micrococcus roseus. 25* is 25-37 °C. The optimal duration of cultivation of *Candida sp.* 12/5 is 1 day, for the rest of the studied cultures - 2 days.

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INTRODUCTION

Launches of launch vehicles (LV), using T-1 kerosene as rocket fuel, are accompanied by soil contamination in the areas where the separable parts (PF SP) of launch vehicles fall with fuel residues. With the increasing availability of commercial space flights, the environmental impact of space launches is becoming significant. The factors negatively affecting the environmental situation during the operation of rocket and space technology, first of all, include emissions of gases, ozone-depleting substances, pollution by fragments of the separating parts of launch vehicles (SP LV), and residues of components of rocket fuels of the Earth's surface, pollution of natural land ecosystems (soil, water, air, and plants) toxic components of rocket fuel and products of its decomposition [1].

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Hydrocarbon combustibles are stable and persist in soils for a long time. The content in the soil of high concentrations of various chemical compounds - toxicants adversely affects the vital activity of soil organisms, while the ability of the soil to self-purify itself from pathogens and other undesirable microorganisms is lost, which is fraught with serious consequences for humans, flora and fauna. Because soil microorganisms are sensitive to soil pollution, changes in their quantitative and qualitative composition can serve as an indicator of the state of soils. A large amount of pollutants during the first years will be contained in the surface soil layer (0.0 - 20.0 cm). Chemical compounds entering the soil accumulate and lead to a gradual change in the chemical and physical belongings of the soil, reducing the number of living organisms, and worsening their fertility [2,3].

In this regard, the restoration of soils in places of straits of hydrocarbon rocket fuel T-1 kerosene is very relevant. At present, the re-cultivation of soils contaminated with oil products is carried out with the help of soil microorganisms. The biological remedy of oil-contaminated ecosystems has been described by many researchers [4-7]. The microbiological method of reclamation based on the use of highly effective strains of hydrocarbon-oxidizing microorganisms is widely used in the world practice of reclamation activities. It is apparent that the chemical arrangement of pollution, the time elapsed since the moment of pollution, as well as soil and climatic situations, have a great mark on the vital activity of these microorganisms introduced in the soil. Effective destruction of various hydrocarbons by microorganisms introduced into the soil with the preparation is possible only in those cases when they find favorable conditions for life and development in the soil (food sources, the necessary thermal and water regimes, etc.), i.e. a microorganism or a group of microorganisms needs to create a favorable ecological niche in which they will develop. This indicates the expediency of isolating microorganisms adapted to the natural and climatic conditions of pollution and successfully utilizing hydrocarbons, which, in turn, will ensure the most effective soil purification by the biological method [8-10]. Currently, to clean the environment from oil pollution, biological preparations based on actively decomposing oil microorganisms are used: *D. acidovorans Cd11 and A. johnsonii Sb01, Acinetobacter sp. T4 and P. putida PB4, Bacillus, Pseudomonas, Vibrio, Micrococcus and Alcaligenes, Bacillus, Williamsia, Citricoccus, Rhodococcus, Arthrobacter, Ochrobactrum, Pseudomonas u Sphingomonas* [11-13].

Thus, for the production of oil-oxidizing biological products, either one strain or a mixture of strains of hydrocarbon-oxidizing bacteria, mainly aerobes, is used. These preparations effectively oxidize oil products, and aromatic hydrocarbons mainly in the temperature range of 15°C - 45°C with significant initial concentrations of contaminants in the soil and require mineral supplementation (nitrogen, phosphorus), moistening, and loosening. Known biological products are obtained in the shape of suspensions, emulsions, pastes, and powders. However, each form has its advantages and disadvantages. Dry preparations have an undeniable advantage: they are more convenient for transportation to the places of their application and have a longer shelf life. At the same time, the number of living microorganisms in them is usually low, since plants for the production of dry forms, primarily non-medicinal drugs, practice spray drying, which is less energy intensive and cheap compared to lyophilization, but there are large losses of viable cells. Considering the low number of microorganisms surviving after spray drying, the authors of the developed dry forms of biological products recommend activation of the microorganisms that make up their composition before use [14,15].

Compounds derived from hydrocarbons are essential in industry and our daily lives, but spills or leaks that occur regularly cause serious environmental impacts. In this review, we sought to isolate and study strains of T-1 kerosene capable of degrading hydrocarbon rocket fuel on different types of soils. For experimental work, soil samples were selected from the position area of the Baikonur Cosmodrome (sites 31 and 196) and the Ulytau region, zone U-25. The creation of highly effective biological preparations for the purification of environmental objects from oil and oil products is possible only based on active hydrocarbon-oxidizing microorganisms and associations adapted to the conditions of pollution and the development of an original technology for their production, taking into account the physiological characteristics of strains. One of the important points for the development of any form of preparation is the selection of optimal conditions for deep cultivation of hydrocarbon-oxidizing microorganisms in a liquid nutrient medium.

The purpose of the research was to separate and study bacteria capable of decomposing hydrocarbon rocket fuel kerosene T-1, as well as to select the conditions for cultivating the isolated microorganisms.

MATERIALS AND METHODS

Soil Image

For experimental work, soil samples (layer 0-20 cm) were taken from the position area of the Baikonur Cosmodrome; sandy desert soils from site 31, gray-brown desert soils from site 196, and brown semi-desert soils, brown saline heavy loamy soils from the Ulytau region, zone U-25 (Table 1).

Kerosene T-1

Fuel "Kerosin T-1" in appearance is a colorless or slightly yellowish liquid with the smell of petroleum products, obtained by direct distillation of low-sulfur crude oil. The average elemental and group composition of the fuel

Table 1. Location of soil samples

Type of soils	Soil samples	Locations	GPS coordinates
Sandy desert soils	Site 31	Positional area of the Baikonur Cosmodrome	45°59.55' N 63°34.15 E
Gray-brown desert soils	Site 196	Positional area of the Baikonur Cosmodrome	46°04.23' N 62°55.11 E
Brown semi-desert soils, brown saline heavy loamy soils	Zone U-25	Positional area of the Baikonur Cosmodrome	47°27.00' N 67°42.00 E

Table 2. Elementary and group composition of fuel Kerosene T-1

Chemical elements					Hydrocarbon groups			
C	H	O	S	N	Alkanes	Cyclans	Arena	Alkenes
86.3%	13.6%	0.04%	0.06%	0.02%	30.0-50.0%	40.0-60.0%	15.0-20.0%	1.0-1.5%

is given i Table 2 [14]. When released into water, T-1 gives a sharp specific odor, which is felt at a concentration of 0.1 mg/dm^3. Hydrocarbon fuels are very stable in soil and water. Biochemically, they are practically not destroyed. The T-1 odor perception threshold in water at 20°C is 0.03 mg/dm^3. They adversely affect the sanitary regime, enhance the process of BOD (biochemical oxygen demand), and inhibit nitrification at a concentration of 20.0 mg/dm^3 or more. Fuel "T-1" is nearly insoluble in water. Refers to low-hazard substances (4th class of toxicity), the features of its use are as follows.

Method for Isolating Bacterial Cultures

To isolate bacterial cultures, soil samples were sown on the following nutrient media:

1. Meat peptone agar (MPA) of the following composition (g/l): peptone - 5.0; sodium chloride - 5.0; meat extract - 1.5; yeast extract - 1.5; agar - 20.0.
2. Starch-ammonia agar (SAA), (g/l): dibasic potassium phosphate - 1.0; ammonium sulfate - 1.0; magnesium sulfate - 1.0; sodium chloride - 1.0; calcium carbonate, 1.0; insoluble starch - 10.0; agar-agar - 20.0.
3. For the isolation of myxomycetes, Chapek's nutrient medium (g/l) was used: sucrose, 30.0; sodium nitrite - 2.0; dibasic potassium phosphate, 1.0; magnesium sulfate - 0.5; potassium chloride, 0.5; iron sulfate - 0.01; agar-20.0.
4. To select microorganisms capable of assimilating kerosene T-1, they were grown on a dense nutrient medium Voroshilova-Dianova (VD) of the following composition (in g/l): NH_4NO_3 – 1.0; K_2HPO_4 – 1.0; KH_2PO_4 – 1.0; $MgSO_4$ – 0.2; $CaCl_2$ – 0.02; $FeCl_3$ – 2.0 drops of a concentrated solution; NaCl, 10.0; twin 80 - 10.0; agar, 20.0; distilled water - up to 1.0 l [15]. The cultivation of microorganisms was carried out in a thermostat at a temperature of 28°C - 30°C for 3 days (bacteria) - 7 days (micromycetes). Screening of the grown colonies was carried out on nutrient agar shoals.

The only source of carbon was kerosene T-1 (S-0.817), which was added to the molten agar nutrient medium in amounts of 1.0%, 2.0%, and 5.0%, i.e., at concentrations of 10.0, 20.0 and 50.0 g/kg. The experiments were repeated three times. Physiological and biochemical signs were determined according to standard methods [16,17]. The identification of selected strains of microorganisms was carried out using Bergi's determinant [18,19] as well as using commercial test systems with chromogenic substrates NEFERMtest 24, CANDIDAtest 21, STAPHYtest 16 (Micro-la-test, Erba Lachema). For this purpose, suspensions of appropriate density were prepared from isolated colonies of pure culture and poured into 0.1 ml wells of strips with chromogenic substrates. To study reactions under anaerobic conditions, 2 drops of sterile paraffin oil were added to the wells. The strips were incubated for 18-24 hours at the appropriate temperature conditions: 37°C for bacteria and 22°C for fungi. After the incubation time, the strips were placed in a device - a Multiskan Ascent analyzer (Thermo Labsystems, Finland). Measurements were carried out by selecting the Microbe-Automat program to determine whether the isolate belonged to a particular genus or species.

Methods for Researching the Development of Conditions for the Cultivation of Isolated Microorganisms

Studies have been carried out to develop the conditions for cultivating isolated microorganisms (selection of a nutrient medium, temperature regime, and duration of cultivating microorganisms of the optimal aeration regime). The objects of research were cultured *Micrococcus roseus.*

25, Bacillus sp.20, Arthrobacter sp.5., Acinetobacter calcoaceticum. 18, Candida sp. 12/5.

Cultures were grown on Petri dishes with dense MPA nutrient medium. The inoculation of the liquid nutrient medium was carried out with the mentioned initial cultures of microorganisms - by flushing from the Petri dishes of MPA (n × 108 CFU/ml) in an amount of 1.0%. Cultivation was carried out for 24h and 48h on a shaker at a temperature of 30°C. The determination of viable bacterial cells was carried out by seeding from the appropriate dilution of the culture into Petri dishes with a dense nutrient medium MPA. The hydrocarbon-oxidizing activity of bacteria was determined by the gravimetric method by their assimilation of kerosene T-1 (2% concentration) in a liquid nutrient medium during cultivation on a shaker for 10 days. The experiments were repeated three times.

Conditions for the Cultivation of Isolated Microorganisms

For the cultivation of hydrocarbon-oxidizing microorganisms, the following variants of nutrient media (g/l) were tested:

1. Meat peptone broth (MPB): peptone - 5.0; sodium chloride - 5.0; meat extract - 1.5; yeast extract - 1.5, pH 7.0-7.2.
2. Corn extract - 10.0; yeast ferment lysate – 3.0; glucose - 10.0; NaCl, 10.0; water - up to 1.0 l, pH 7.2-7.5.
3. KH_2PO_4 – 2.0; Na_2HPO_4 – 4.0; $MgSO_4$ – 0.5; $(NH_4)2SO_4$ – 2.0; CaC_{12} – 0.01; FeC_{13} – 0.05; wheat flour - 5.0; sucrose -10.0; water - up to 1.0 l, pH 6.8-7.2.
4. Voroshilova Dianov (VD) modified medium: NH_4NO_3 – 1.0; K_2HPO_4 - 1.0; KH_2PO_4 - 1.0; $MgSO_4$ - 0.2; $CaCl_2$ - 0.02, $FeCl_3$ - 2.0 drops of concentrated solution, NaCl -10.0; glucose - 10.0; nutrient broth - 10.0; distilled water - up to 1.0l. Cultivation was carried out on a shaker for 2 days. Then the number of viable cells was determined.

RESULTS AND DISCUSSION

Research on the Isolation of Native Strains (Microorganisms) Capable of Destroying Hydrocarbon Rocket Fuel - Kerosene T-1 in the Soil

The isolation of microorganisms was carried out from the soil samples described above after their inoculation on Meat Peptone Agar (MPA), Starch Ammonia Agar (SAA), Chapek's nutrient medium, and Voroshilova-Dianova's (VD) medium.

As a result of the studies, 30 isolates of microbial cultures were isolated from soils contaminated with T-1 hydrocarbon rocket fuel. After inoculation of the isolates on a dense nutrient medium VD, containing kerosene T-1 as the only source of carbon, microorganisms were identified that assimilate this type of T-1 fuel well. Out of 30 isolates, 9 isolates showed high activity.

On the medium, depending on the concentration of kerosene T-1 1% (10 g/kg), 9 isolates of microorganism cultures grow well: №. 4, 8, 14, 23, 5, 18, 20, 25, and in yeast №. 12/5.

On the medium, depending on the concentration of kerosene T-1 2% (20 g/kg) and 5% (50 g/kg), good growth was noted in bacterial cultures № 5, 18, 20, 25 and yeast № 12/5 (Figure 1-Figure 5).

The identification of the most active cultures of microorganisms by physiological and biochemical characteristics was also carried out.

Culture № 25 is cocci, solitary, in pairs, and clusters of 1.25-2.0 microns. Gram-positive, non-acid-resistant, do not form spores. On MPA and FHN (fish hydrolyzate of the nutrient medium), the culture forms rounded, convex red colonies, 1-2 mm in diameter. The edge of the colonies is even and does not release pigments into the medium. Aerobe. When growing in MPB the medium becomes cloudy, a precipitate forms, and an annular film forms on

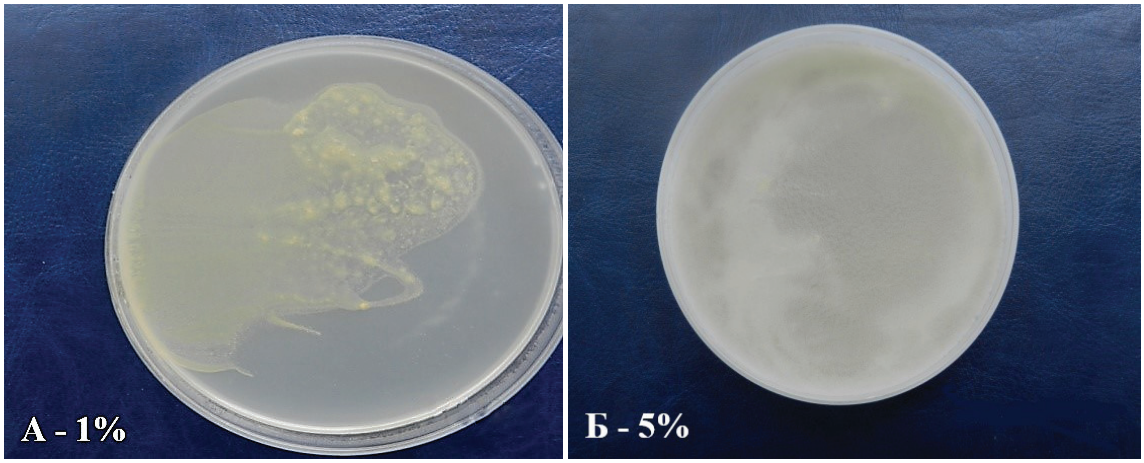

Figure 1. The growth of the culture of *Arthrobacter sp.* on a solid medium Voroshilova-Dianova with the addition of kerosene T-1.

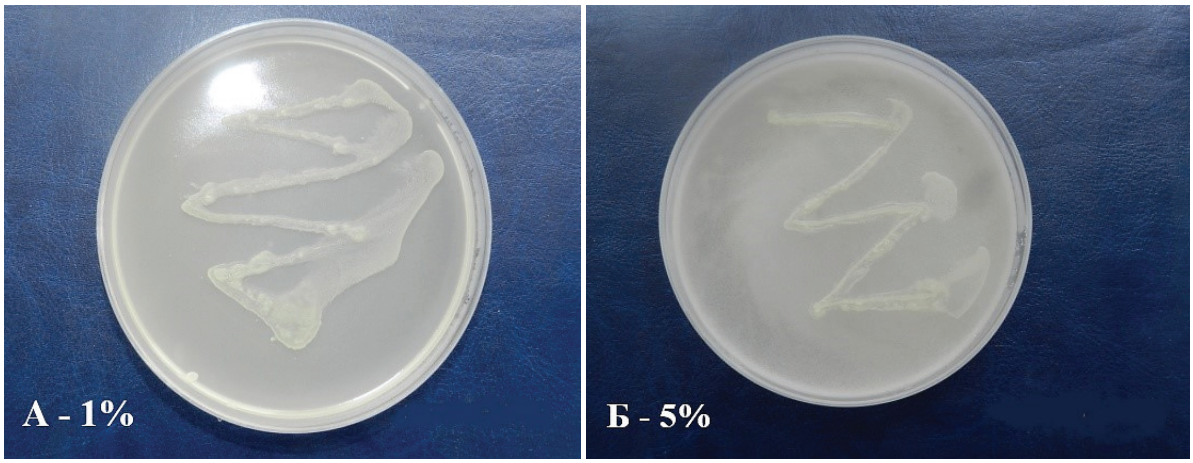

Figure 2. The growth of the culture of *Acinetobacter calcoaceticum* on a solid medium Voroshilova-Dianova with the addition of kerosene T-1.

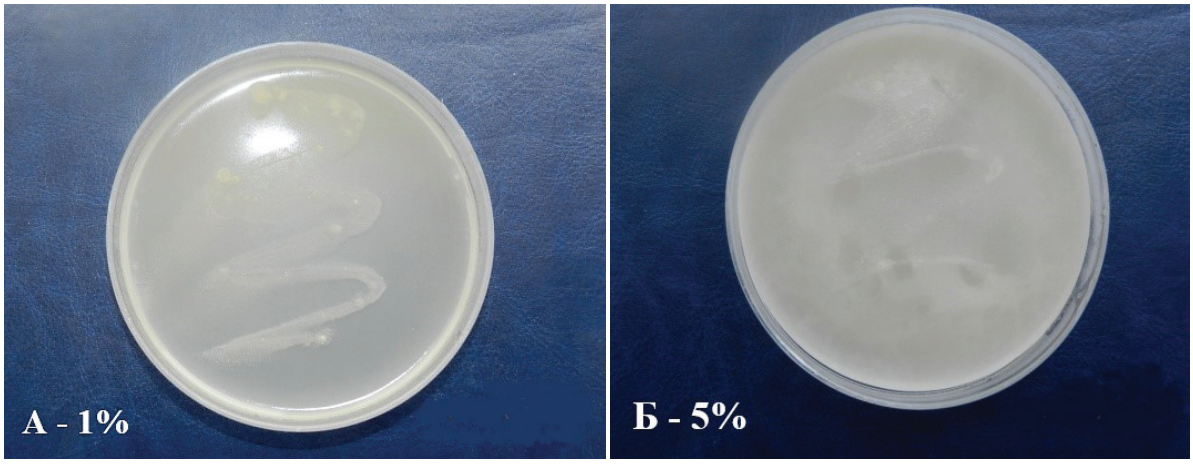

Figure 3. Growth of the culture of *Micrococcus roseus* on a solid medium Voroshilova-Dianova with the addition of kerosene T-1.

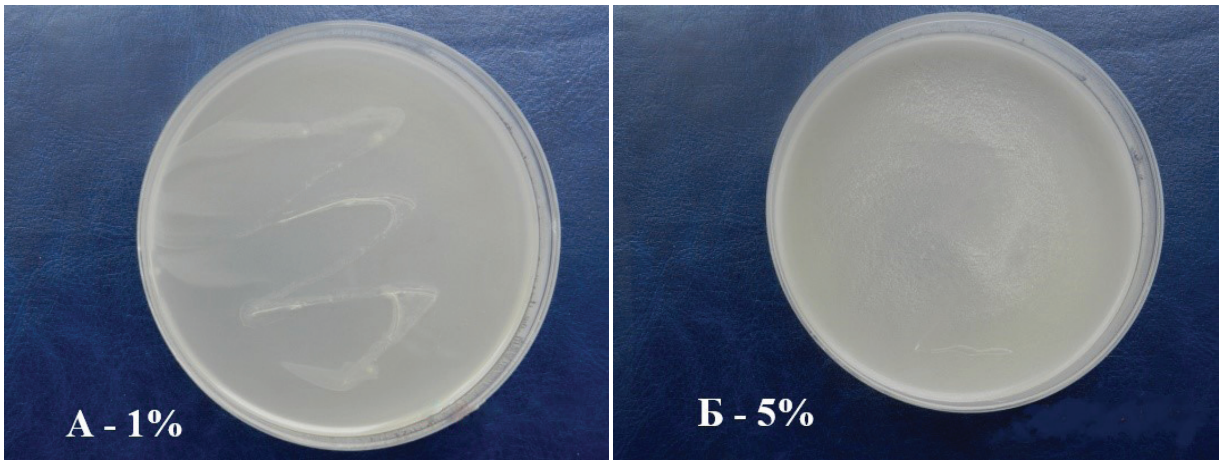

Figure 4. Growth of *Bacillus sp.* on a solid medium Voroshilova-Dianova with the addition of kerosene T-1.

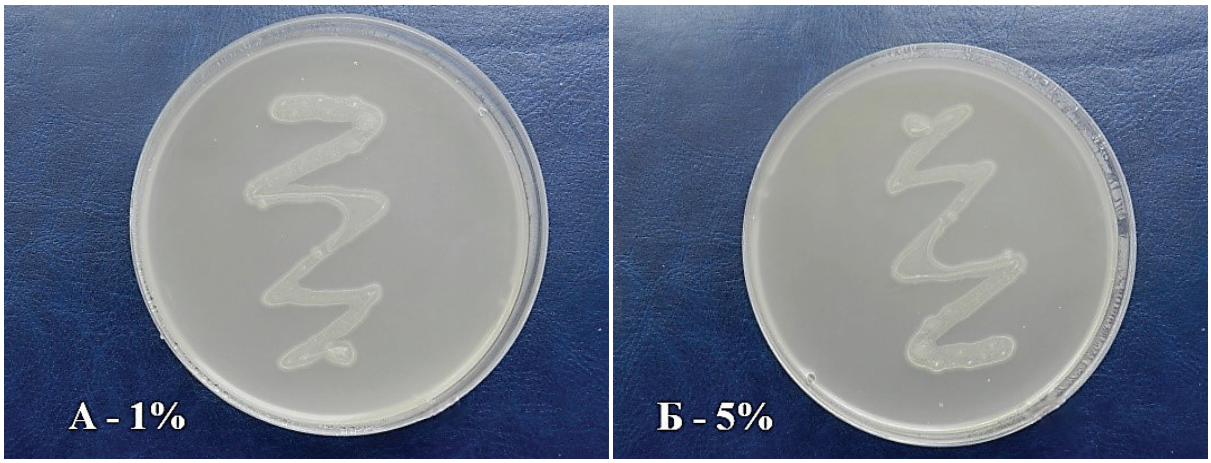

Figure 5. Growth of Candida sp. on a solid medium Voroshilova-Dianova with the addition of kerosene T-*1*.

the surface. It uses glucose, lactose, and mannose as a carbon source. Starch does not hydrolyze, and does not liquefy gelatin. Hydrogen sulfide and indole do not form. Nitrates are reduced to nitrites. Gives a positive reaction to catalase. The optimum growth temperature is 28°C. Selected from the soil sample of the Baikonur Cosmodrome, site 31 (0.0-50.0 cm). According to morphological and physiological-biochemical characteristics, the culture is assigned to *Micrococcus roseus.*

Culture № 20 is an upbeat spore-forming rods, located singly and in chains. Aerobe. When growing on MPA and FHN, the colonies are rounded, the surface is smooth and shiny, and the edges are even. Colonies are light beige, diameter of 3-4 mm, homogeneous structure, and oily consistency. The optimum growth temperature is 28°C. Selected from the soil sample of the site.31 (0-25cm). According to morphological and physiological-biochemical characteristics, the culture was assigned to *Bacillus sp.*

Culture № 5 is an upbeat, immobile cell. In young cultures, rods are irregularly shaped, which, with further development of the culture, break up into small cocci. Does not form a dispute. On agar, medium forms are rounded, and convex, with a smooth edge, and white-yellow colonies. Aerobe. Gives a positive reaction to catalase. The optimum growth temperature is 28°C. Selected from the soil sample of the site 196 (0-50cm). According to morphological and physiological-biochemical characteristics, the culture is assigned to *Arthrobacter sp.*

Culture № 18 is a Gram-positive, immobile cell. Does not form a dispute; aerobe. When growing on a solid nutrient medium MPA forms small, opaque, whitish, round, with a smooth edge of the colony. Growth on liquid nutrient media: MPB forms uniform turbidity. Utilizes maltose, sorbitol, inositol, glucose. Gives a negative reaction to lactose, sucrose, mannitol, sodium citrate, indole, and hydrogen sulfide. Gives a positive reaction to catalase. The optimum growth temperature is 28°C. Selected from the soil sample of the site 196 (0-50cm). According to morphological and physiological-biochemical characteristics, the culture was assigned to *Acinetobacter calcoaceticum.*

Culture № 12/5 represents single cells, round, sometimes oval, size 2-4 x 3-4 microns. Grows well on Raymond's agar medium (with 1% acetate and 0.25% yeast extract) containing up to 8% NaCl. Assimilates n-alkanes (C10-C30), acetate, propionate. Weak development on ethanol, and propanol. Does not ferment glucose. Uses ammonium nitrogen. Optimum growth temperature at 28°C. Selected from a soil sample of the U-25 zone, from the Ulytau region (0-25 cm). According to morphological and physiological-biochemical characteristics, the culture was assigned to *Candida sp.*

Selection of Optimal Nutrient Media for the Cultivation of Bacteria

The results of the experiment on the selection of optimal nutrient media for the cultivation of bacteria are presented in Table 3 and Figure 6-Figure 10.

The initial concentration of isolate cells before inoculation into the broth was controlled using an optical density measuring device - a DEN-1 densitometer (Biosan, Latvia). A suspension equal to 1.5×10^8 CFU/ml (0.5 McFarland units) was prepared from the isolated colony. Then a series of Koch dilutions was prepared, for which 1 ml of the prepared suspension was diluted in 9 ml of 0.9% sodium chloride solution. Thus, dilutions were obtained - 1.5×10^7 CFU/ml, 1.5×10^6 CFU/ml, 1.5×10^5 CFU/ml, 1.5×10^4 CFU/ml, 1.5×10^3 CFU/ml and 1.5×10^2 CFU/ml.

Growth of isolates in mineral salt broth containing various concentrations of kerosene. After 7 days of incubation at 35°C, the broth became cloudy due to microbial growth. A gradual increase in turbidity was observed, showing maximum turbidity at 3% kerosene and decreasing again at concentrations above 3% kerosene. It has been established that the optimal duration of cultivation of *Candida sp. 12/5* is 1 day, for the rest of the studied crops - 2 days. The best nutrient media for the culture of *Candida sp.12/5* are the

Table 3. Growth of hydrocarbon-oxidizing bacteria on various nutrient media

Number of culture medium	Strain of bacteria	Bacterial cell content, CFU/ml		
		Before inoculation	1 day	2 days
№ 1	*Candida sp. nov. 12/5*	1.5×10^2	1.0×10^6	3.0×10^6
№ 2	*Candida sp. nov. 12/5*	1.5×10^2	2.0×10^7	1.7×10^7
№ 3	*Candida sp. nov. 12/5*	1.5×10^2	1.6×10^7	9.3×10^7
№ 4	*Candida sp. nov. 12/5*	1.5×10^2	1.2×10^8	1.3×10^8
№ 1	*Bacillus sp. 20*	1.5×10^2	2.0×10^6	4.8×10^7
№ 2	*Bacillus sp. 20*	1.5×10^2	1.2×10^6	2.2×10^7
№ 3	*Bacillus sp. 20*	1.5×10^2	1.0×10^7	8.4×10^7
№ 4	*Bacillus sp. 20*	1.5×10^2	3.4×10^7	2.4×10^8
№ 1	*A. calcoaceticum.18*	1.5×10^2	2.4×10^7	3.5×10^8
№ 2	*A. calcoaceticum.18*	1.5×10^2	4.1×10^7	2.5×10^8
№ 3	*A. calcoaceticum.18*	1.5×10^2	3.8×10^7	4.4×10^8
№ 4	*A. calcoaceticum.18*	1.5×10^2	4.4×10^7	1.4×10^8
№ 1	*M. roseus. 25*	1.5×10^2	5.2×10^6	3.2×10^8
№ 2	*M. roseus. 25*	1.5×10^2	1.2×10^7	4.3×10^8
№ 3	*M. roseus. 25*	1.5×10^2	2.2×10^7	5.1×10^8
№ 4	*M. roseus. 25*	1.5×10^2	2.2×10^7	4.8×10^8
№ 1	*Arthrobacter sp. 5*	1.5×10^2	1.3×10^7	2.0×10^8
№ 2	*Arthrobacter sp. 5*	1.5×10^2	1.8×10^8	2.6×10^9
№ 3	*Arthrobacter sp. 5*	1.5×10^2	4.0×10^7	2.0×10^8
№ 4	*Arthrobacter sp. 5*	1.5×10^2	1.4×10^8	3.0×10^9

modified medium № 4 with the addition of nutrient broth and glucose, on which the bacterial titer reaches 1.3×10^8 CFU/ml, and medium № 3 - titer 9.3×10^7 CFU/ml. Less accumulation of bacterial cells was noted on medium № 1 - titer 3.0×10^6 CFU/ml.

For cultivation of culture *Acinetobacter calcoaceticum. 18* all tested nutrient media are suitable. At the same time, the titer of bacteria is in the range of $1.4\text{-}4.4\times10^8$ CFU/ml. The same picture was noted in the culture of *Micrococcus roseus. 25*, the titer of which on the indicated nutrient media is $3.2–5.1\times10^8$ CFU/ml.

Culture of *Bacillus sp. 20* also accumulates the largest number of cells on the modified medium № 4 - the titer is 2.4×10^8 CFU/ml, on the other tested media the bacterial titer is $2.2\text{-}8.4\times10^7$ CFU/ml.

For the culture of *Arthrobacter sp.* The top 5 nutrient media are № 2 based on corn extract and modified № 4, on which the number of bacterial cells was: 2.6×10^9 and 3.0×10^9 CFU/ml, respectively.

The optimal temperature for growing cultures of microorganisms was determined by their growth intensity on medium № 1 at various temperature conditions - from 10°C to 37°C. It has been established that the optimal growth temperature for the culture of *Arthrobacter sp. 5* is 25-30°C, *Acinetobacter calcoaceticum. 18* is 30-35 °C, *Micrococcus roseus. 25* is 25-37 ° C., *Bacillus sp.* 20 is 30-35°C, *Candida sp. 12/5* is 25-30 °C. Considering that the selected strains of hydrocarbon-oxidizing bacteria are aerobes, it is necessary to select the optimal aeration regime for their deep cultivation. The need for microorganism cultures in aeration was determined by changing the filling volume of 750 ml shake flasks with modified nutrient medium № 4 in the range

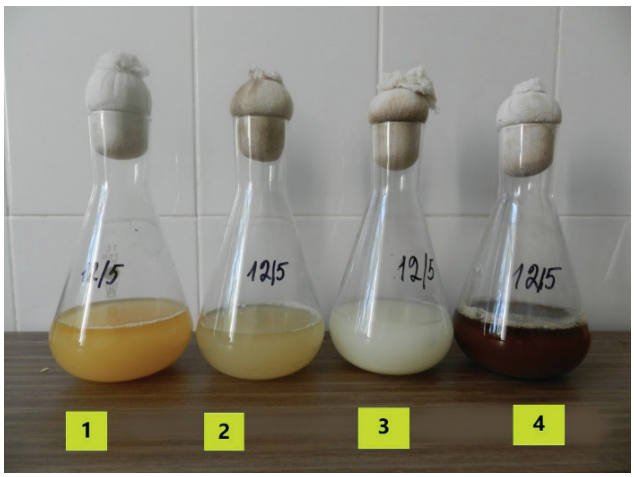

Figure 6. Growth of *Candida sp*. 12/5 on various types of nutrient media.

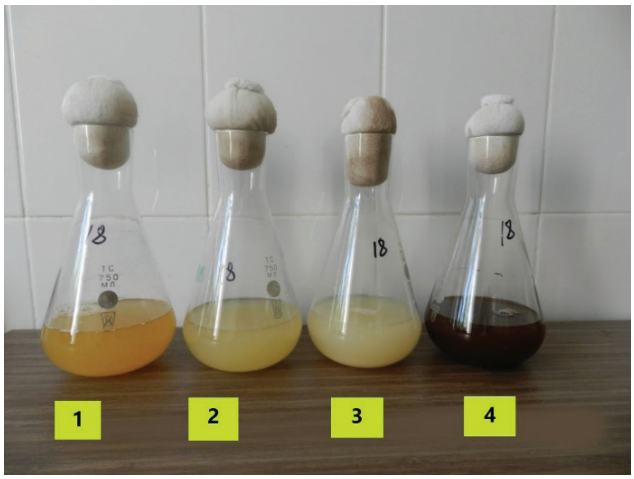

Figure 7. Growth of *Acinetobacter calcoaceticum. 18* on various nutrient media.

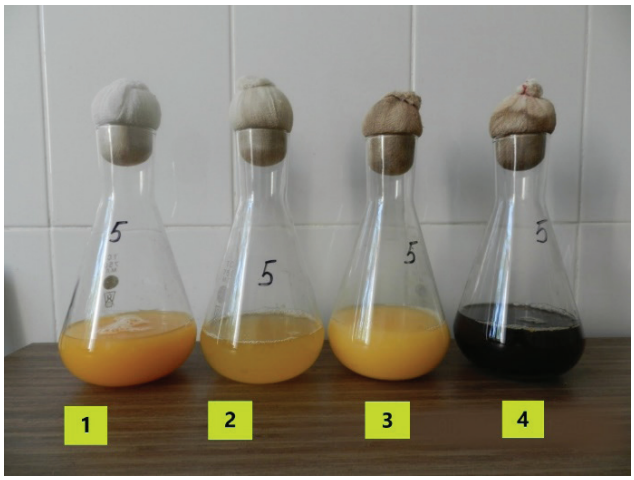

Figure 9. Growth of *Arthrobacter sp. 5* on various nutrient media.

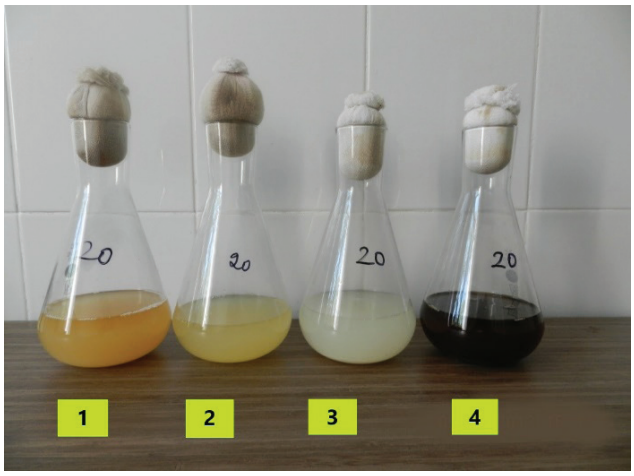

Figure 8. Growth of *Bacillus sp. 20* on various nutrient media.

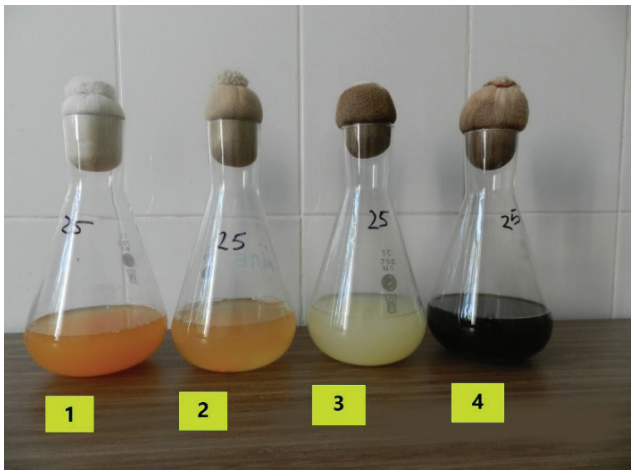

Figure 10. Growth of Micrococcus roseus. 25 on various nutrient media.

Table 4. Accumulation of biomass of oil-oxidizing bacteria depending on aeration

Strains of	Titers, CFU/ml, depending on the volume of the nutrient medium					
bacteria's	50 ml	100 ml	150 ml	200 ml	250 ml	300 ml
12/5	5.0×10^8	6.0×10^8	3.0×10^8	4.0×10^8	5.0×10^8	2.0×10^8
20	2.0×10^8	3.0×10^8	4.0×10^8	3.0×10^8	4.0×10^8	3.0×10^8
18	4.0×10^8	5.0×10^8	3.0×10^8	7.0×10^8	5.0×10^8	2.0×10^8
25	4.0×10^8	5.0×10^8	9.0×10^8	8.0×10^8	7.0×10^8	4.0×10^8
5	9.0×10^8	11.0×10^8	12.0×10^8	10.0×10^8	11.0×10^8	8.0×10^8

from 50 to 300 ml. Cultures were grown on a shaker for 2 days at 30°C (Table 4).

It was established that during the growth of the test cultures in various volumes (from 50 to 250 ml) of the modified nutrient medium № 4, no significant changes in the accumulation of biomass were noted. This indicates that when growing the studied cultures of hydrocarbon-oxidizing microorganisms in the fermenter, the flow rate of process air will not exceed 0.6–0.8 V/min per volume of the nutrient medium.

Table 5. Utilization of T-1 kerosene by hydrocarbon-oxidizing microorganisms grown on various nutrient media

Strains of bacteria	Utilization of kerosene T-1 (%)1			
	Culture medium № 1	Culture medium № 2	Culture medium № 3	Culture medium № 4
Control	63.00±0.03^{f}	62.30±0.03^{f}	64.03±0.03^{f}	63.27±0.03^{f}
12/5	96.23±0.07^{a}	91.57±0.03^{b}	98.73±0.03^{a}	87.37±0.03^{c}
20	69.97±0.03^{d}	65.67±0.03^{e}	72.80^{d}	73.20^{e}
18	82.70^{b}	68.20^{d}	84.40^{c}	84.00^{d}
25	65.90^{e}	97.80^{a}	71.60^{e}	90.90^{b}
5	72.20^{c}	84.20^{c}	96.17^{b}	92.47±0.03^{a}

[1]Data represent mean ± standard error (SE). Different superscript letters (a, b, c, d, e, f) in the columns indicate significant differences between means (Tukey's test, $p < 0.05$).

To determine the percentage of utilization of kerosene T-1 by selected cultures of microorganisms, they were grown under optimal conditions on various nutrient media for two days, then they were inoculated in an amount of 10.0% into liquid nutrient medium № 4 with 2.0% kerosene and cultivated for 10 days on a shaker. Then the percentage of kerosene utilization was determined by the one-way ANOVA and Tukey's test (Table 5).

According to experimental data, up to 62.30 % of the introduced kerosene is weathered in the control without the participation of microorganisms. Utilization of kerosene up to 98.73% occurred using the culture of *Candida sp. 12/5* grown on a medium with wheat flour. At 96.23% and 91.60%, the utilization of kerosene by this culture occurred when using MPB nutrient media for cultivation and with corn extract. The lowest hydrocarbon-oxidizing capacity was noted in the culture grown on modified medium № 4 (87.37%). Low hydrocarbon-oxidizing activity was noted in *Bacillus sp. 20* (69.97-73.20%), especially grown on corn extract medium (65.67%). A strain of *Acinetobacter calcoaceticum. 18* utilized kerosene T-1 by 82.70–84.40% when grown on various nutrient media, except for the variant with the medium with corn extract, in which the kerosene content decreased by 68.20%.

When using the bacteria *Micrococcus roseus. 25*, grown on media with corn extract and modified № 4, a decrease in the content of kerosene occurred by 97.80 and 90.90%, respectively. Lower oil utilization occurred when using the culture grown on medium №4 (65.90%) and medium №2 (71.60%). Culture of *Arthrobacter sp. 5* grown on medium № 3 with wheat flour and modified medium № 4 reduced the content of kerosene by 96.17 and 92.47%, respectively. A lower result was obtained when culture media № 4 (72.20%) and № 2 (84.20%) were used for bacterial cultivation.

Comparison of the results on the accumulation of the biomass of microorganisms on various nutrient media and their hydrocarbon-oxidizing activity made it possible to select the optimal nutrient media: *for Candida sp. 12/5* - modified medium №3 with wheat flour; for *Bacillus sp. 20* - modified medium №4; for *Acinetobacter calcoaceticum. 18* and *Arthrobacter sp. 5* - modified medium №4 and modified medium №3, for *Micrococcus roseus. 25* - modified medium №4 and №2 with corn extract.

CONCLUSION

Oil pollution is a global problem that destroys living organisms and causes potential harm to the environment and humanity. Existing physical and chemical methods proved to be less effective and therefore the emergence of biological methods became an intensive solution.

In the decomposition of oil in the soil, the main and decisive role is played by the functional activity of the complex of soil microorganisms, which ensure the complete mineralization of oil and oil products [3], [20,21]. Since hydrocarbon-oxidizing microorganisms are a constant component of soil biocenoses, it is natural that their catabolic activity is used to restore oil-contaminated soils. There are two ways to speed up the purification of soils from pollution: to activate the metabolic activity of the natural soil microflora by changing the corresponding physicochemical conditions of the environment or to introduce specially selected active oil-oxidizing microorganisms into the polluted environment [22-24]. The microbiological method of reclamation based on the use of highly effective strains of hydrocarbon-oxidizing microorganisms is widely used in the world practice of reclamation activities.

The expediency of isolating microorganisms adapted to the conditions of pollution and successfully utilizing hydrocarbons has been revealed, which in turn will ensure the most effective cleaning of soils by a biological method. With this in mind, the problem of developing destructor drugs for local conditions becomes especially relevant.

Therefore, this preliminary study was carried out to isolate different types of soil strains with a tendency to be degraded by T-1 kerosene hydrocarbon propellant, which is used by bacteria with the potential for degradation. Selected microorganisms were analyzed and identified according to physiological and biochemical characteristics: №5

– *Arthrobacter sp.,* №18 – *Acinetobacter calcoaceticum,* №20 – *Bacillus sp.,* №25- *Micrococcus roseus,* №12/5- *Candida sp.*

In this study, on a medium with a T-1 kerosene concentration of 1% (10 g/kg), 9 isolates of microorganism cultures grow well: № 4, 8, 14, 23, 5, 18, 20, 25 and in yeast № 12/5. The study showed that with a concentration of T-1 kerosene 2% (20 g/kg) and 5% (50 g/kg), good growth was noted in bacterial cultures *Arthrobacter sp.5, Acinetobacter calcoaceticum 18, Bacillus sp.20, Micrococcus roseus 25 and yeast Candida sp. 12/5.* Other studies have reported primary screening of kerosene degraders using a conventional accumulative culture containing 1% kerosene as the sole carbon source. n-alkanes, including fractions C_{11}-C_{15}, C_{17}-$C_{19.}$ Bacterial isolates of *Staphylococcus aureus* Ba01, *Delftia acidovorans* Cd11, *Acinetobacter calcoaceticus* Fe10, *Pseudomonas koreensis* Hg11, and *Acinetobacter johnsonii* Sb01, sharing this characteristic degradation pattern, demonstrated the ability to degrade gradations by 70–84% within 21 days of incubation [9]. There were similar results to study the growth characteristics of eight isolates from soil samples. When eight different isolates were cultured in mineral salt broth containing 1% (v/v) kerosene for 7 days at 35°C and 120 rpm, *Pseudomonas sp.* showed the highest growth, as evidenced by the high turbidity of the culture, followed by *Bacillus sp.* [24].

In the current study, it was found that the optimal duration of cultivation of *Candida sp.* 12/5 is 1 day, and for the rest of the studied crops - 2 days. The best nutrient media for the accumulation of biomass of microorganisms on various nutrient media and their hydrocarbon-oxidizing activity made it possible to select the optimal nutrient media: for *Candida sp.* 12/5 – modified medium № 3; for *Bacillus sp.* 20 - modified medium № 4; for *Acinetobacter calcoaceticum.* 18 and *Arthrobacter sp.* 5 - modified medium № 4 and №3, for *Micrococcus roseus.* 25 - modified medium № 4 and № 2. Subsequent inoculation of diluted 100 µl of each culture on mineral salt agar also gave results consistent with visualized turbidity, *Pseudomonas sp.* giving the highest growth of 8.38 log CFU/mL, and two species of *Bacillus sp*. giving almost similar results of 6.70 log10 CFU/mL and 6.04 log10 CFU/mL [24].

In the current culture of *Acinetobacter calcoaceticum.*18, all culture media tested are suitable. At the same time, the titer of bacteria is in the range of 1.4-4.4×10^8 CFU/ml. The same picture was noted in the culture of *Micrococcus roseus.*25. The top 5 cultures of *Arthrobacter sp.,* modified media are № 2 based on corn extract and modified № 4, on which the number of bacterial cells was: 2.6×10^9 and 3.0×10^9 CFU/mL, respectively. In a similar study using a Pseudomonas sp. isolate which showed maximum growth in the presence of kerosene. The isolate was found to tolerate kerosene concentrations up to 6% (v/v) and showed optimal growth at 3% kerosene (v/v) with bacterial counts of 5.53 log10 CFU/ml and 9.43 log10 CFU/ml, respectively [24].

Subsequent inoculation of diluted 100 µl of each culture on mineral salt agar also gave results consistent with visualized turbidity, *Pseudomonas sp.* giving the highest growth of 8.38 log CFU/ml, and two species of *Bacillus sp.* giving almost similar results of 6.70 log10 CFU/ml and 6.04 log10 CFU/ml. High concentrations of hydrocarbons, especially volatile hydrocarbons, can be detrimental to bacterial growth. It has been established that the selected microorganisms can be grown at a temperature of 30°C and aeration in the range of 0.6-0.8 V air/min per volume of the nutrient medium.

In conclusion, five activities of the bacterial community were analyzed, simulating natural conditions in the laboratory, and the biodegradability of complex oil components by the obtained bacteria was investigated. Our results provide a reasonable strategy for using the microbial community to treat an environment contaminated with T-1 kerosene hydrocarbon propellant in different seasons to obtain the best recovery effect. Thus, our results suggest that the strains belong to the same species, the formation of microbial consortia can contribute to the process of recovery of soil contaminated with hydrocarbons. With this result, it will not only be possible to clean up spills of rocket fuel T-1 kerosene but it can also be used to decompose those containing these compounds.

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AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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