International Journal of Design & Nature and Ecodynamics
Vol. 19, No. 4, August, 2024, pp. 1139-1150
Journal homepage: http://iieta.org/journals/ijdne

Hydrocarbon-Oxidizing Activity of the New Rhodococcus sp. Strain 1D/1 Promising for Cleaning the Environment from Oil Pollution

Elmira Faizulina[1*], Larissa Tatarkina[1], Gulzhan Spankulova[1], Svetlana Aitkeldiyeva[1], Gul Baimakhanova[1], Irina Smirnova[1], Zlatka Alexieva[2], Kazhybek Ashimuly[1]

[1] Research and Production Center of Microbiology and Virology LLC, Almaty 050010, Kazakhstan
[2] Stephan Angeloff Institute of Microbiology, Sofia 1113, Bulgaria

Corresponding Author Email: fayzulina.e.r@mail.ru

Copyright: ©2024 The authors. This article is published by IIETA and is licensed under the CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0/).

https://doi.org/10.18280/ijdne.190405

Received: 28 March 2024
Revised: 24 July 2024
Accepted: 30 July 2024
Available online: 28 August 2024

Keywords:
biodegradation, bioremediation, oil, oil-oxidizing microorganisms, petroleum products, temperature

ABSTRACT

This study investigated the oil-degrading properties of strain 1D/1 of Rhodococcus sp., isolated from oil-contaminated soil in Kazakhstan's Kyzylorda region. Using accumulative cultures, researchers isolated the strain and confirmed its classification within the genus Rhodococcus through 16S ribosomal RNA analysis. Strain 1D/1 demonstrated effective decomposition of various petroleum products, such as crude oil, fuel oil, and diesel fuel, and showed versatility in utilizing cresol, naphthalene, anthracene, and phenanthrene isomers as sole carbon and energy sources. Gas chromatography analyses revealed its capability to degrade n-alkanes ranging from C11 to C28, highlighting its potential for bioremediation efforts in addressing oil contamination challenges. The strain effectively decomposed crude oil, fuel oil, and diesel fuel, degrading n-alkanes with C11-C28 chain lengths. Gas chromatographic analysis showed that the strain degraded up to 100% of certain n-alkanes. The strain grew on both light and heavy oil and could degrade oil at different temperatures, with biodegradation rates of 60.9% at 10°C and 60.6% at 30°C for Dossor oil. At 50°C, the activity of the strain decreased slightly, with a biodegradation rate of 56.1%. The strain is capable of degrading both light and heavy oils at low and elevated temperatures, making it a versatile candidate for environmental bioremediation technologies.

1. INTRODUCTION

The daily life of modern society largely depends on oil and petroleum products [1]. However, environmental pollution due to accidental oil spills, leaks, and damage to oil pipelines is common and causes serious concern to governments, environmentalists, and the public [2-4]. Crude oil damages life in all ecosystems, and its spills are becoming more frequent as the demand for petroleum products around the world is steadily growing [5]. During the extraction, storage, transportation, and processing of oil, spills and discharges of petroleum hydrocarbons are possible [6]. Accidents with oil emissions occur during field development and leaks from oil pipelines and storage tanks. One can note accidents with oil leakage from tankers, at oil wells, as well as during major repairs of equipment of oil refineries and petrochemical production [7, 8]. Oil and petroleum product spills should be processed or eliminated to the maximum extent possible. However, in some cases, their collection can be difficult, especially in extreme or unique conditions, such as polar regions, deep-sea areas, deserts, and wetlands [9]. As a result, they remain in the affected area and pose a constant risk to the environment. Such hydrocarbon pollution can lead to soil infertility and changes in its physicochemical and microbiological properties, plant growth retardation, and contamination of groundwater, which poses a significant risk to human and animal health because it has carcinogenic, mutagenic, and immunotoxic effects [10, 11]. The impact of oil can lead to a decrease in agricultural productivity and, thus, negatively affect socio-economic life [12, 13].

Given these severe consequences, the issue of cleaning the environment from oil pollution is increasingly crucial. Traditional methods of remediation, such as mechanical and physicochemical cleaning, although effective to some extent, are often prohibitively expensive and environmentally unsustainable. These methods can also pose additional environmental hazards, as they sometimes involve the use of harmful chemicals and generate significant amounts of waste.

Mechanical cleaning, which includes techniques like skimming, dredging, and manual removal, is labor-intensive and not always effective for large-scale or deep-seated contamination. Physicochemical methods, such as using dispersants, detergents, and absorbents, can mitigate surface spills but often fail to address sub-surface pollution and can introduce new contaminants into the ecosystem.

As a result, there is a growing need for more sustainable and cost-effective solutions. Bioremediation, for example, leverages natural processes involving microorganisms that can degrade and detoxify pollutants, presenting a promising alternative. This method is generally more environmentally

friendly, less disruptive, and can be applied over larger areas and varied terrains [14].

2. LITERATURE REVIEW

Oil located on an open surface can undergo chemical and, partially, photo-oxidation [15]. However, abiotic oxidation is too long a process [16]. In nature, the destruction of petroleum hydrocarbons is primarily driven by the process of biological oxidation, which is facilitated by oil-oxidizing microorganisms. These microorganisms, such as certain bacteria and fungi, have evolved metabolic pathways that enable them to use hydrocarbons as a source of energy and carbon.

The process begins with the microorganisms attaching to oil droplets or hydrocarbon molecules. They produce enzymes that break down the complex hydrocarbon structures into simpler compounds [17, 18]. Such microorganisms appeared as a result of existence close to sources of petroleum hydrocarbons. Thus, soil with a high content of hydrocarbons contains more destructive microbes than soil with a low content of hydrocarbons [19-22]. Microbes isolated from such soils can be used for bioremediation [23-25]. In recent years, the method of biological purification from oil pollution has attracted increasing attention from environmentalists. It is based on the use of microorganisms capable of utilizing petroleum hydrocarbons as their sole source of carbon is an effective and sustainable method for reducing oil contamination to background levels at relatively low operating costs. This approach leverages the natural metabolic capabilities of specific microorganisms that can degrade complex hydrocarbon molecules into simpler, less harmful substances [26, 27]. Bioremediation is considered the most thorough method of mineralization of crude oil, where microorganisms turn environmental pollutants into harmless end products. This method is more economical than physicochemical reclamation. This is a simple and easy-to-maintain method, applicable over large areas and environmentally friendly. It does not lead to the formation of waste and contributes to the destruction of pollutants [28-30].

Among all known microorganisms, bacteria are recognized as the most important microbes from the point of physiology, ecology, and biotechnology, because of their universality and prevalence [31, 32]. Many bacterial species from both normal and extreme ecosystems have been identified as effective biological destructors of petroleum hydrocarbons. These bacteria are capable of degrading a wide range of hydrocarbon compounds, making them valuable for bioremediation efforts. According to the latest data, more than 79 genera of bacteria have been recognized for their ability to decompose various petroleum hydrocarbons [33]. For example, *Alcanivorax* and *Acinetobacter* decompose alkanes well [34]; *Cycloclasticus* decomposes aromatic hydrocarbons with high efficiency [35]; and *Dietzia* can decompose n-alkanes, branched alkanes, and aromatic hydrocarbons (naphthalene, phenanthrene, pyrene, and fluoranthene) [36]. Representatives of the *Rhodococcus* genus play an important role in the decomposition of various chemical compounds of an organic nature [37-39].

However, the effectiveness of biological purification technology, or bioremediation, is influenced by numerous environmental factors that can hinder its practical application. Key factors such as temperature, salinity, pH (hydrogen index), nutrient availability, electron acceptors, and substrate concentration play crucial roles in bioremediation and impact the biological degradation reactions of hydrocarbons. Understanding and managing these factors is essential for optimizing bioremediation processes [40-42]. For example, Ke et al. [43] reported that an increase in temperature from 10 to 40°C improved the biodegradation of oily sludge by a bacterial consortium. At 50°C, the biodegradation of the sediment began to decrease, and the optimal temperature was about 30-40°C. Thus, any deviation from the optimal temperature leads to a decrease in the biodegradation of oil. Besides, it largely depends on the temperature limit of a particular region [44]. Researchers have observed that many bacteria capable of decomposing petroleum hydrocarbons achieve excellent results in laboratory conditions but demonstrate unsatisfactory results in field tests. This discrepancy can be attributed to several factors that differ significantly between controlled laboratory environments and the more complex and variable conditions found in natural field settings [45, 46]. For example, the climate in the oil-producing regions of Kazakhstan is continental. It is characterized by extremely arid, hot, and dry summers and cold, short, snow-free winters. The temperature can rise to +48°C and in some winters fall to -42°C [47]. The soil is a saline desert, subject to secondary salinization [48].

Currently, the technology of microbial purification from pollution by petroleum hydrocarbons is undergoing continuous development and improvement, attracting a lot of attention from scientists and the public [49, 50]. One of the methods is bioaugmentation, which involves collecting oil-destroying microbes with high resistance to environmental stresses from a contaminated site and cultivating them for re-introduction [51]. The success of this method in the decomposition of a wide range of hydrocarbon compounds is reported by Devinny and Chang [52], Alisi et al. [53], and Li et al. [54].

Kazakhstan has a high rank in global oil production. Therefore, there is an urgent problem of soil contamination with oil and petroleum products. Comprehensive research, namely, the isolation of new forms of microorganisms capable of degradation of petroleum hydrocarbons at high and low temperatures and the study of their physiological, biochemical, and molecular genetic properties are relevant.

The purpose of this work was to study the properties and oil-oxidizing potential of the new Rhodococcus sp. strain 1D/1, which was isolated from oil-contaminated soil in Kazakhstan. The study aimed to evaluate the strain's capabilities in degrading petroleum hydrocarbons and its potential application in bioremediation efforts to clean up oil-contaminated environments.

3. MATERIALS AND METHODS

3.1 Soil samples

Soil samples were taken at the Kumkol deposit in the Kyzylorda region from a 0-30 cm deep layer. The samples were collected in sterile kraft paper bags and delivered to the laboratory.

3.2 Chemicals and nutrient media

Chemicals: All analytical-grade mineral salts used in the experiments were supplied by AppliChem, ensuring high

purity and consistency. Phenol, cresol isomers, naphthalene, anthracene, and phenanthrene were obtained from Sigma-Aldrich, with purities ranging from 98% to 99%, guaranteeing the reliability of the chemical analyses and reactions.

Nutrient Media: For the preparation of nutrient media, nutrient agar was sourced from Titan Biotech Ltd. (India), and agar-agar was provided by Himedia (India). The mineral medium was carefully formulated with the following composition per liter: NH_4NO_3: 1.0 g, K_2HPO_4: 1.0 g, KH_2PO_4: 1.0 g, $MgSO_4$: 0.2 g, $CaCl_2 \times 6H_2O$: 0.02 g, $FeCl_3$: in trace amounts, NaCl: 10.0 g, and maintained at a pH range of 7.0 to 7.2. This precise formulation supports optimal growth conditions for microbial cultures.

Oil: The oil samples were sourced from various fields in the Kyzylorda and Atyrau regions, each with distinct characteristics:

- *Kumkol Field, Kyzylorda Region:* This oil has a density ranging from 0.812 to 0.819 kg/m³ and varies in sulfur content from 0.11 to 0.52%. It contains paraffins at 10.8 to 11.5%, asphaltenes at 0.11 to 0.92%, and resins between 4.8 to 8.42%. These parameters provide a comprehensive profile for evaluating the oil's properties and behavior.
- *Dossor Field*: The oil from this field has a density of 0.84 g/cm³. It contains paraffins ranging from 0.31% to 2.07%, sulfur at 0.22%, and resins at 7%. The composition reflects the oil's potential for various applications and its impact on environmental processes.
- *West Prorva Field*: With a density of 0.899 g/cm³, this oil features paraffin content from 2.05% to 4.22%, sulfur ranging from 0.33% to 1.46%, asphaltenes at 1.12%, and resins at 15.6%. These characteristics highlight the oil's complexity and its potential influence on remediation efforts.
- *Karsak Field*: This oil has a density of 0.933 g/cm³, paraffin content varying from 0.17% to 2.19%, sulfur ranging from 0.1% to 0.6%, and resins between 9% and 52%. The wide range of resins, in particular, suggests a significant variability in the oil's chemical composition and behavior.

Fuel Oil: Fuel oil was acquired from the thermal power plant located in Kyzylorda. This source provides a specific grade of fuel oil used for energy production and its characteristics were carefully considered for experimental purposes.

Diesel Fuel: Diesel fuel was sourced from a gas station in Almaty. This type of fuel was chosen to represent typical commercial diesel used in various applications, ensuring that the experiments reflect real-world conditions.

3.3 Isolation and identification of the strain

The isolation of the microbial strain was carried out using the accumulative cultures method, a systematic approach designed to enrich and isolate microorganisms with specific capabilities. The procedure began by collecting 10 grams of soil contaminated with oil from the Kyzylorda region. This soil sample was introduced into a flask containing 100 milliliters of mineral medium. To create a conducive environment for microbial growth, 1% of Kumkol oil was added to the mineral medium, ensuring that the oil served as a carbon source for the microorganisms.

The flask was then placed on an orbital shaker, which was set to maintain a temperature of 30°C and a shaking speed of 180 revolutions per minute (rpm). This incubation setup was maintained for a period of 14 days, allowing sufficient time for the growth of oil-degrading microorganisms. The shaking motion facilitated the even distribution of nutrients and enhanced aeration, promoting the proliferation of bacteria capable of metabolizing the oil.

Following this initial incubation period, 10 milliliters of the culture fluid from the flask was carefully transferred to a new flask containing fresh mineral medium, once again supplemented with 1% Kumkol oil. This step aimed to further enrich the culture with microorganisms that are adept at degrading oil. The new flask was incubated under the same conditions—30°C and 180 rpm—for another 14-day cycle.

This process of transferring and incubating was repeated multiple times to continuously enrich the culture with oil-oxidizing bacteria. Each cycle progressively increased the concentration of oil-degrading microorganisms, thereby enhancing their population and activity.

To isolate specific oil-oxidizing strains from the enriched culture, a portion of the suspension was subjected to serial dilutions. Specifically, 1 milliliter of the enriched culture was used for creating serial dilutions, ranging from 10^{-1} to 10^{-7}. These dilutions were then plated onto Petri dishes containing nutrient agar. After incubation, individual colonies that developed on the agar plates were isolated using the depleting streak method to ensure that pure, single colonies were obtained. These pure cultures were subsequently transferred onto nutrient agar slants for further analysis.

These identification procedures followed the protocols outlined in Bergey's Manual of Systematic Bacteriology, a comprehensive reference for bacterial classification and identification. Cell morphology was observed using a TESCAN scanning electron microscope LYRA series (Czech Republic). 16S ribosomal RNA (rRNA) sequence analysis was conducted for taxonomic classification [55].

3.4 Determination of the spectrum of utilized aromatic substrates of the studied strain

To conduct experiments on the destruction of aromatic hydrocarbons, the culture of microorganisms was cultivated in a nutrient broth with 1% glucose. Then 1 ml of the suspension was introduced into flasks with 30 ml of liquid mineral medium and hydrocarbons were added. Monoaromatic hydrocarbons (phenol, ortho-, para-, meta-cresol) were added at a concentration of 0.3 g/l. Cultivation was carried out on an orbital shaker at 180 rpm and at a temperature of 30°C. The concentration of hydrocarbons in the medium was determined every 24 hours to zero readings on a spectrophotometer (UV-VIS 6305 JENWAY) at a wavelength of 620 nm and a cuvette thickness of 0.5 cm. The growth characteristics of the strain were studied by the optical density of the culture on a spectrophotometer at a wavelength of 540 nm and a cuvette thickness of 0.5 cm.

3.5 Biodegradation of Kumkol oil and petroleum products

In Erlenmeyer flasks containing 100 ml of mineral medium, 5 ml of a day-old culture suspension of the isolated microbial strain was introduced. The medium was supplemented with different petroleum products (oil, fuel oil, and diesel fuel) as the sole carbon source, at concentrations of 1%, 3%, and 5% (v/v). These flasks were then incubated in thermostatically

controlled shakers set to 30°C for a period of 14 days to facilitate microbial growth and hydrocarbon degradation.

3.6 Biodegradation of oil from different fields of Kazakhstan

In this experiment, Erlenmeyer flasks containing 100 ml of mineral medium were each inoculated with 5 ml of a day-old culture suspension of the isolated microbial strain. The purpose was to assess the strain's ability to degrade oil from three different oil fields: Dossor, West Prorva, and Karsak. The oils from these fields were introduced into the medium as the sole carbon and energy source at a concentration of 1% (v/v). The experiment was designed to evaluate the microbial degradation of oil at different temperatures and to quantify the residual oil content.

3.7 Analytical methods

3.7.1 Determination of the degree of degradation of n-alkanes

The concentration of petroleum hydrocarbons was measured using a 7890-gas chromatograph (GC) combined with a mass spectrometer (MS) (Agilent, Santa Clara, USA). For this analysis, we utilized the DB-35MS column (30m x 0.25mm, film thickness 0.25 microns) from J&W Scientific Inc., Folsom, California, USA, as the stationary phase. Helium with a purity of >99.995% (from Orenburg-Techgas, Russia) was used as the mobile phase, flowing at a rate of 1.0 ml/min. An injection volume of 1.0 µl was employed with a split ratio of 10:1 to ensure proper sample introduction. The GC furnace temperature was programmed to start at 40°C and hold for 5 minutes, then ramp up to 150°C at a rate of 7°C/min, and finally increase to 300°C at 5°C/min for an additional 5 minutes. The injector and transport line temperatures were set to 250°C and 280°C, respectively, to optimize the separation and analysis of the hydrocarbons. Mass spectrometry (MS) detection was performed at an ionization energy of 70 eV, with the mass spectrometer scanning a range from m/z 34 to 850 to identify and quantify the petroleum hydrocarbons effectively. Calibration curves were established using known concentrations of hydrocarbons to ensure accurate measurements and reproducibility of the results.

Data management and analysis, including control and processing of GC system data, were performed using Agilent MSD ChemStation software (version 1701EA). This involved determining retention times, peak areas, and processing spectral data obtained from the MS detector. Interpretation of mass spectra utilized the Wiley 7th edition program alongside the NIST'02 library, which contains over 550 thousand spectra.

3.7.2 Determination of residual oil content in the medium

The amount of residual oil in the medium was measured using a gravimetric method. Chloroform was used to extract the remaining oil, and the extract was placed in pre-weighed cups. The chloroform was then evaporated at room temperature in a fume hood, and the cups were weighed again using OHAUS ExplorerEX 124 analytical scales (USA). To determine the quantity of oil and petroleum products consumed, the following formula was applied:

$$A = \frac{A1 - A2}{A1} \times 100\% \quad (1)$$

where, A represents the percentage of oil consumed, A1 is the initial amount of oil added, and A2 is the amount of residual oil.

All experiments included appropriate controls to ensure reliability and reproducibility of the results. Control samples without bacterial inoculation were incubated under identical conditions to account for abiotic degradation. Reproducibility was ensured by conducting each experiment in triplicate.

4. RESULTS AND DISCUSSION

4.1 Characteristics of the strain

The microbial strain used in this study was isolated from oil-contaminated soil sampled at the Kumkol field in the Kyzylorda region. The isolation process involved using the accumulative cultures method to enrich for oil-degrading bacteria. After isolation, the strain underwent a series of identification tests to confirm its taxonomic position and potential for hydrocarbon degradation.

Figure 1 shows the phylogenetic tree of the strain, illustrating its genetic relationship with other Rhodococcus species. Comparative analysis revealed that the strain had 99% similarity with the typical Rhodococcus sp. strain 7B-577 (GenBank reference code: KF441686.1) [56].

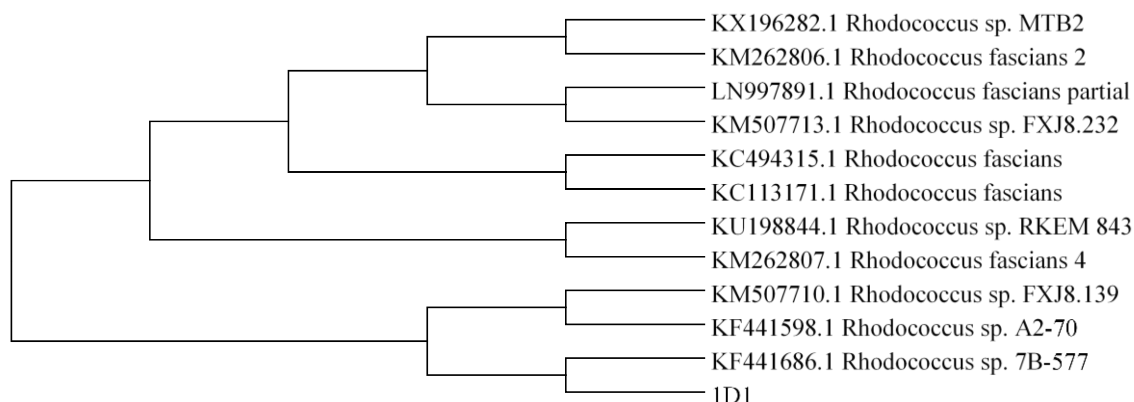

Figure 1. Phylogenetic tree

4.2 Cultural/morphological and physiological/biochemical signs

To characterize the isolated bacteria decomposing oil, morphological and biochemical characterization was carried out using Bergey's Manual of Systematic Bacteriology.

Figure 2. Colonies of the strain

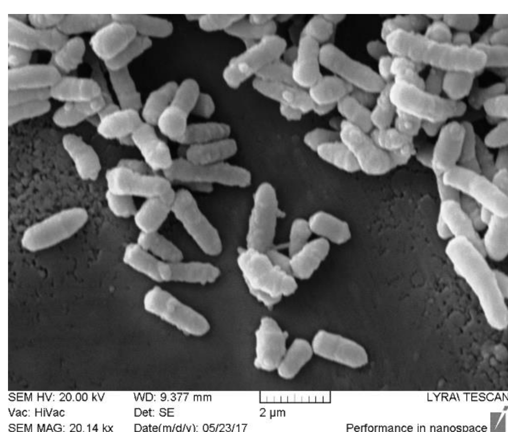

Figure 3. Strain cells on the nutrient agar (scanning microscopy)

The strain forms colonies of orange color, shiny, with an even edge (Figure 2). The cells are represented by Gram-positive stationary rods (Figure 3). They are aerobic, catalase-positive, and oxidase-negative. This strain does not reduce nitrates, produce indole, or hydrolyze starch. Additionally, it demonstrates a high tolerance for varying environmental conditions, which supports its effectiveness in bioremediation applications. The strain's ability to thrive in oxygen-rich environments and its lack of nitrate reduction suggest a metabolic pathway distinct from those of many other bacteria. These characteristics are crucial for identifying its potential utility in specific environmental cleanup processes. It assimilates glucose, sucrose, arabinose, xylose, and mannitol. It grows at a temperature of 10-50°C, and the optimum growth temperature is 30°C. It withstands NaCl concentrations up to 10%.

4.3 Growth of the strain on aromatic hydrocarbons

Preliminary screening showed that the strain grew well on isomers of cresol, naphthalene, anthracene, phenanthrene, oil, fuel oil, and diesel fuel. Statistical analysis indicated significant differences in the degradation rates of these compounds ($p < 0.05$).

An experiment was conducted using the strain cultivated in a mineral medium where 0.3 g/l of phenol, along with m-, o-, and p-cresol, served as the sole sources of carbon and energy. The goal was to assess the strain’s ability to utilize these compounds for growth. It was observed that phenol exerted an inhibitory effect on the strain’s growth. This inhibition suggests that phenol may interfere with the metabolic processes or physiological functions of the strain. The presence of phenol at this concentration likely affects the strain’s ability to effectively metabolize the other carbon sources, thereby impacting its overall growth and activity. In contrast, the effect of m-, o-, and p-cresol on the strain’s growth was also noted, and understanding these interactions is essential for optimizing conditions for the strain's use in bioremediation applications [57].

Figure 4 shows the growth and degradation of o-, m-, and p-cresol by the strain. In 72 hours, the doubling of the initial biomass was observed with growth on m-cresol. Spectrophotometric analysis showed the total consumption of m-cresol in the medium during this period. The results were statistically significant with p-values < 0.01. The doubling of initial biomass was observed with m-cresol, and confidence intervals for degradation rates were calculated at 95% CI. p-Cresol was completely absorbed in 96 h, while o-cresol was more resistant to microbial decomposition. Its complete degradation was observed after 120 hours.

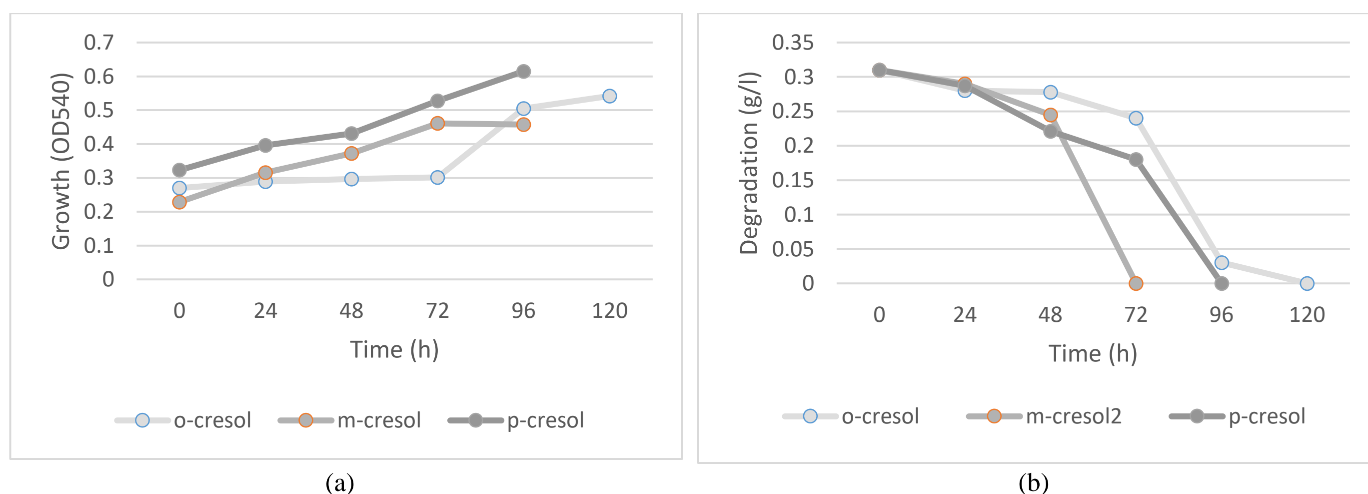

Figure 4. Growth (a) and degradation (b) of o-, m-, and p-cresol by the strain

Previously conducted experiments with naphthalene, anthracene, and phenanthrene showed the ability of the strain to grow and completely utilize 0.2 g/l of each compound in 4 days [58].

4.4 Growth of the strain on Kumkol oil and petroleum products

Experiments were carried out on the biodegradation of Kumkol oil, fuel oil, and diesel fuel in the amount of 1%, 3%, and 5% (v/v) as the only carbon source. Authors indicated significant differences in degradation efficiency at different concentrations ($p < 0.05$). Cultivation was carried out in a liquid mineral medium at 30℃ in a thermostatically controlled shaker for 14 days. The strain showed good degradation potential. During 14 days of cultivation, the amount of 1 and 3% oil decreased by 50.2 and 49.6% relative to the control variant, respectively (Figure 5). With an increase in the oil content in the medium to 5%, the degree of biodegradation of oil decreased slightly and amounted to 47.9%.

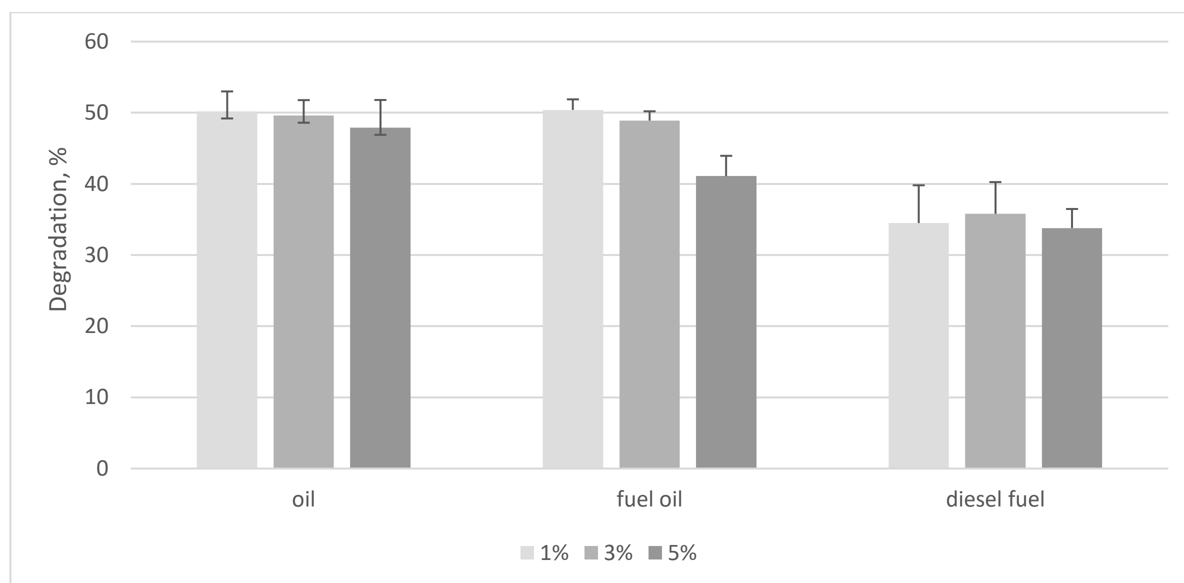

Figure 5. Degradation of oil, fuel oil, and diesel fuel by the strain at different concentrations

At the same level, the strain disposed of fuel oil in the amount of 1 and 3%, and biodegradation was 50.4 and 48.9%. With a fuel oil content of 5%, the destruction was 41.1%.

The strain grew worse on diesel fuel. At all studied concentrations, its utilization was approximately at the same level, 33.8 to 35.8%. Statistical analyses confirmed the differences ($p < 0.01$), and confidence intervals were reported at 95%.

4.5 Biodegradation of n-alkanes by the strain

Most of the oil fields in Kazakhstan are concentrated in the Atyrau region. The soil and climate of the Kyzylorda and Atyrau regions have similar indicators. Therefore, the strain was selected as a promising option for further study of the growth of oil fields located in this area.

Table 1. Biodegradation of n-alkanes after cultivation of the strain in a medium with 1% oil

n-Alkanes	Peak Area, 10^{-6}		Biodegradation, %
	Control Variant	1D/1	
Undecane	4.1±0.13	not defined (n/d)	100
Dodecane	28.3±2.15	n/d	100
Tridecane	58.7±5.40	n/d	100
Tetradecane	58.9±5.74	n/d	100
Pentadecane	54.4±5.25	n/d	100
Hexadecane	51.9±6.91	21.6±0.61	57.6
Heptadecane	44.6±3.42	n/d	100
Octadecane	35.6±2.99	n/d	100
Nonadecane	35.9±5.56	n/d	100
Eicosane	31.4±3.18	n/d	100
Heneicosane	28.5±5.20	7.7±0.12	73
Docosane	24.6±3.70	n/d	100
Tricosane	19.5±3.51	n/d	100
Tetracosane	15.5±2.92	n/d	100
Pentacosane	13.4±1.81	n/d	100
Hexacosane	13.9±1.82	n/d	100
Heptacosane	11.3±1.92	n/d	100
Octacosane	8.5±1.26	n/d	100

Table 1 presents data on the decomposition of n-alkanes after the growth of the strain on Dossor oil. The results of GC analysis showed that the strain under study could efficiently utilize n-alkanes with a short (C11-C13), medium (C14-C25), and long (C26-C28) chain. Among the n-alkanes of oil, hexadecane and heneicosane were more resistant to the effects of the strain, and their degree of destruction equaled 57.6 and 73%, respectively.

GC analysis showed the strain could utilize n-alkanes with chain lengths of C11-C28, achieving 100% degradation for several n-alkanes (e.g., undecane, dodecane, tridecane). Statistical significance was confirmed ($p < 0.05$), with 95% confidence intervals provided.

4.6 Growth of the strain in the oil fields of the Atyrau region at different temperatures

Since the oils of various fields differ in their physicochemical parameters, the strain was selected for further study of the growth of the Dossor, West Prorva, and Karsak oil fields.

An experiment on the destruction of three oils from the Atyrau region in a liquid mineral medium was carried out. Oil was added in an amount of 1% (v/v). Cultivation experiments were conducted to observe the activity of a particular microbial strain at three different temperatures: 10℃, 30℃, and 50℃. The cultivation process lasted for a duration of 14 days, during which the microbial strain was allowed to grow and metabolize under controlled conditions at each specified temperature. The results of the gravimetric analysis revealed that the activity levels of the strain at 10℃ and 30℃ were similar, indicating that the strain exhibited comparable growth and metabolic activity at these two temperatures (Figure 6). During this period, it disposed of 60.9 and 60.6% of the Dossor oil relative to the control, respectively. At elevated temperatures, the activity of the strain was slightly lower, and the biodegradation of oil was 56.1%.

When using West Prorva oil, the destruction at 10 and 30℃ exceeded 61% and at 50℃ 48.3% in 14 days. The oil of the Karsak field was slightly worse destroyed. At 10 and 30℃, the destruction of oil was 56.9 and 57.6%, respectively. At 50℃, the strain reduced the amount of oil by 46%. This can be explained by the higher density of this oil (0.933 g/cm^3) and a large amount of resins (up to 52%). These results were statistically significant ($p < 0.01$) with 95% confidence intervals.

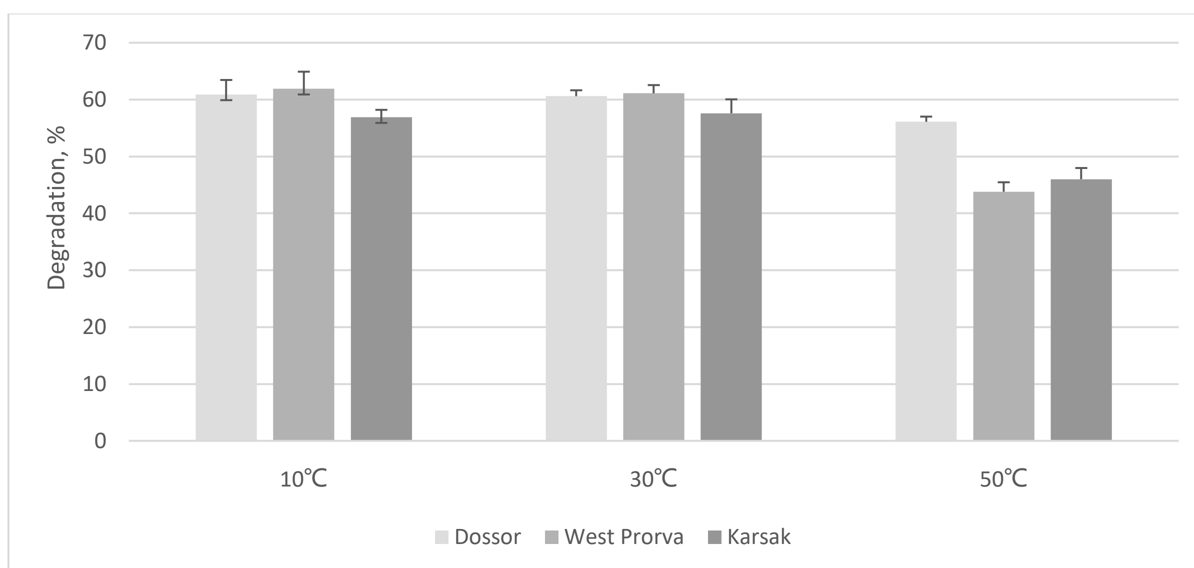

Figure 6. Degradation of oil from different fields by the strain at different temperatures

Hydrocarbon pollution, largely resulting from petrochemical industry activities, is a significant environmental problem. Accidental emissions of petroleum products pose a particular threat. Traditional mechanical and chemical methods for removing hydrocarbons are often limited in effectiveness and costly. Bioremediation, which uses microorganisms to degrade pollutants, is a promising alternative due to its cost-effectiveness and potential for complete pollutant mineralization [58].

The Rhodococcus genus, a group of Nocardia-like actinomycetes, is widespread in various environments, including soil, water, plants, and animals. Rhodococcus species are ideal for bioremediation due to their ability to decompose many organic compounds, produce surfactants, and resist environmental stress. These qualities make them excellent candidates for enhancing bioremediation efforts to clean contaminated areas effectively. In terms of their ecological significance, metabolic versatility, and potential for biotechnological use, *Rhodococci* are in some respects similar to *Pseudomonadaceae* and related bacteria [59, 60].

For example, Larkin et al. [59] reported that Rhodococcus sp. JZX-01 degraded 65.27% of crude oil in 9 days. In comparison, our strain degraded up to 61.9% of Dossor oil in 14 days, showing similar efficacy under laboratory conditions. Furthermore, the strain's ability to degrade both light and heavy oils at low and elevated temperatures is comparable to the findings of Aitkeldiyeva et al. [61], who demonstrated thermotolerant microorganisms' effectiveness in oil degradation at varying temperatures.

A new strain, Rhodococcus sp. 1D/1, was isolated from oil-contaminated soil, demonstrating the ability to decompose oil and petroleum products, including fuel oil and diesel fuel. Comparative analysis revealed enhanced biodegradability of oil and fuel oil over diesel fuel, potentially attributable to diesel's higher toxicity. For example, in experiments investigating the effects of diesel fuel on ecosystems, researchers found significant impacts on biodiversity. The main toxic effects observed included a decrease in species

richness, which refers to the number of different species present in a given area. Additionally, there was a reduction in species uniformity, indicating an imbalance in species abundances, and a decline in phylogenetic diversity, which measures the evolutionary relationships among species. These findings highlight the detrimental effects of diesel fuel contamination on ecological health and biodiversity [62].

The ability of microorganisms to biodegrade oil and petroleum products is influenced by the concentration and composition of hydrocarbons present. When petroleum hydrocarbon levels are extremely high, they can severely inhibit bacterial growth. This inhibition results in low biodegradation efficiency, as the microorganisms responsible for breaking down the hydrocarbons struggle to survive and function effectively. In extreme cases, the high toxicity of petroleum hydrocarbons can lead to bacterial death, further diminishing the biodegradation potential and exacerbating the environmental impact of the pollution [63]. Hossain et al. [64] showed that with an increase in the concentration of diesel fuel in the medium, the growth rate of three bacterial isolates decreased. On the contrary, the growth rate increased with an increase in the concentration of used engine oil. All isolates demonstrated their efficiency in the decomposition of diesel fuel with a concentration of 4% v/v and spent engine oil with a concentration of 8% v/v [64]. The concentration of oil and petroleum products (1, 3, and 5%) did not have a significant effect on the destructive activity of the strain. Biodegradation of oil was 47.9 to 50.2%, of fuel oil 41.1 to 50.4%, and of diesel fuel 33.8 to 35.8%.

Crude oil is a mixture of aliphatic and aromatic compounds, and n-alkanes make up 60% of most forms of crude oil [65]. Aliphatic hydrocarbons are partially oxidized by a wide range of microorganisms, but only a select few species are capable of fully metabolizing these compounds. Notable among these are species from the genera Pseudomonas, Nocardia, Xanthomonas, Bacterium, Corynebacterium, Mycobacterium, and Acinetobacter. These microorganisms possess the specialized enzymatic systems necessary to completely break down aliphatic hydrocarbons into non-toxic end products.

Research indicates that the biodegradation of aromatic hydrocarbons, which are characterized by one or more benzene rings, is influenced by the number of rings and the complexity of their molecular structure. According to studies [66-68], the more benzene rings an aromatic hydrocarbon contains, and the more complex its structure, the more challenging it is for bacteria to degrade it. This is due to the increased stability and complexity of the molecular structure of aromatic hydrocarbons, which makes them less accessible to microbial enzymatic action.

Most bacteria are limited in their ability to decompose or utilize only specific components of petroleum hydrocarbons. While some bacteria can effectively break down certain simpler components, others remain completely inaccessible to microbial degradation. This variability in biodegradation capabilities highlights the need for selecting appropriate microbial strains or engineering them to enhance their ability to degrade complex and recalcitrant hydrocarbons in environmental cleanup efforts [69, 70]. The 1D/1 strain can efficiently utilize n-alkanes with a chain length of C11-C28, cresol isomers, and polycyclic aromatic hydrocarbons, such as naphthalene, anthracene, and phenanthrene. However, phenol had an inhibitory effect on it.

Oil is a complex mixture made up of many different components. The primary constituents of oil are hydrocarbons, which are molecules made up solely of carbon and hydrogen. Depending on the source, crude oil consists of 82 to 85% carbon, 10 to 14% hydrogen, 0.01 to 7% sulfur, 0.02 to 2% nitrogen, and 0.1 to 1% oxygen. By composition, oil can be divided into light (density from 0.65 to 0.87 g/cm^3), medium (density from 0.87 to 0.91 g/cm^3), and heavy (from 0.91 to 1.05 g/cm^3) [71]. The oils of the Atyrau region used differed in density and content of sulfur compounds and resins. The oils from the Karsak and West Prorva fields are heavy oils, and the Dossor oil is light. The research showed that the 1D/1 strain can degrade both light and heavy oils. When cultured on the three oils at 30°C for 14 days, no significant difference in oil-oxidizing activity was observed. Biodegradation of oil was 57.6-61.1%.

Temperature plays a crucial role in hydrocarbon biodegradation, impacting the composition of pollutants, microbial physiology, and diversity [57]. Typically, degradation rates of hydrocarbons decrease as temperatures decline. This trend is observed because lower temperatures often slow down the metabolic activities of microorganisms, leading to reduced degradation efficiency. For example, strains such as Acinetobacter sp. JLS1 and Pseudomonas aeruginosa JLC1, which were isolated from the Momoge wetlands in Jilin Province, China, demonstrated varied responses to temperature changes during the degradation of the alkane C16.

These strains showed significant differences in their ability to degrade alkane C16 at different temperatures, underscoring how temperature can dramatically influence the rate at which hydrocarbons are broken down. At higher temperatures, these microorganisms generally exhibit increased metabolic activity and, consequently, higher degradation rates. Conversely, as temperatures drop, the enzymatic processes involved in hydrocarbon degradation become less efficient, leading to slower rates of breakdown. This variability highlights the critical role of temperature in optimizing bioremediation strategies and selecting appropriate microbial strains for effective environmental cleanup [46, 72, 73].

Since the temperature range in the oil-producing regions of Kazakhstan is wide (from +48 to -42°C, depending on the season), the resistance of strains of destructive microorganisms to temperature fluctuations is of great importance during bioremediation. Earlier, the *Rhodococcus fascians K-3* strain, isolated and studied for its characteristics, exhibited high oil-oxidizing activity at temperatures of 35 and 50°C [61]. The 1D/1 strain showed good destructive activity about oil from different fields both at low and elevated temperatures. At 10 and 30°C, the biodegradation of oil was at the same level. With an increase in temperature to 50°C, the activity of the strain decreased slightly. The data are consistent with the results obtained by Ullrich and Hofrichter [68]. The strain *Rhodococcus sp.* JZX-01 isolated by them had a good ability to decompose oil at medium and low temperatures.

5. CONCLUSIONS

The new *Rhodococcus sp*. 1D/1 strain was isolated from the oil-contaminated soil of the Kyzylorda region. It is capable of degrading oil and petroleum products. Morphological and physiological/biochemical signs of the strain were studied. Based on the analysis of 16S RNA, the strain was assigned to the species *Rhodococcus sp*. The strain can use n-alkanes with a chain length of C11-C28, isomers of cresol, naphthalene,

anthracene, and phenanthrene as the only source of carbon and energy. The strain effectively degraded oil, fuel oil, and diesel fuel. The strain is capable of degrading both light and heavy oils at low and elevated temperatures. The new strain can be recommended for the development of biotechnologies related to the elimination of environmental pollution by oil and petroleum products.

Limitations of the study include the fact that it was focused solely on Rhodococcus sp. 1D/1. In natural environments, biodegradation often involves consortia of microorganisms working synergistically. This single-strain approach may not fully capture the complexities of in situ biodegradation. Future research should explore microbial consortia, including this strain, to enhance degradation efficiency. Testing with various oil types from different regions and conducting long-term studies to assess sustained performance and ecological impacts will provide a comprehensive understanding of its capabilities and safety.

FUNDING

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No.: AP19677718).

ACKNOWLEDGMENTS

We express our gratitude to the staff of the Laboratory of Fungal Genetics of the S. Angeloff Institute of Microbiology, Bulgaria, for their assistance in conducting scanning microscopy of cells, PCR analysis, and experiments on biodegradation of aromatic compounds.

REFERENCES

[1] Yessenbayeva, G., Kenenbayev, S., Dutbayev, Y., Salykova, A. (2024). Organic farming practices and crops impact chemical elements in the soil of Southeastern Kazakhstan. International Journal of Agriculture and Biosciences, 13(2): 222-227. https://doi.org/10.47278/journal.ijab/2024.106

[2] Han, G., Cui, B.X., Zhang, X.X., Li, K.R. (2016). The effects of petroleum-contaminated soil on photosynthesis of *Amorpha fruticosa* seedlings. International Journal of Environmental Science and Technology, 13: 2383-2392. https://doi.org/10.1007/s13762-016-1071-7

[3] Ikuesan, F.A. (2017). Evaluation of crude oil biodegradation Potentials of some indigenous soil microorganisms. Journal of Scientific Research and Reports, 13(5): 1-9. https://doi.org/10.9734/JSRR/2017/29151

[4] Xue, J., Yu, Y., Bai, Y., Wang, L., Wu, Y. (2015). Marine oil-degrading microorganisms and biodegradation process of petroleum hydrocarbon in marine environments: A review. Current Microbiology, 71: 220-228. https://doi.org/10.1007/s00284-015-0825-7

[5] Chandra, S., Sharma, R., Singh, K., Sharma, A. (2013). Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. Annals of Microbiology, 63: 417-431. https://doi.org/10.1007/s13213-012-0543-3

[6] Semikhina, L., Kovaleva, I., Semekhin, D. (2023). Phase transitions in oil disperse systems and their influence on the thermochemical process of oil dehydration. Qubahan Academic Journal, 3(4), 469–475. https://doi.org/10.48161/qaj.v3n4a299

[7] Chen, M., Xu, P., Zeng, G., Yang, C., Huang, D., Zhang, J. (2015). Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols, and heavy metals by composting: Applications, microbes and future research needs. Biotechnology Advances, 33(6): 745-755. https://doi.org/10.1016/j.biotechadv.2015.05.003

[8] Wang, C., Liu, X., Guo, J., Lv, Y., Li, Y. (2018). Biodegradation of marine oil spill residues using aboriginal bacterial consortium based on Penglai 19-3 oil spill accident. China. Ecotoxicology and Environmental Safety, 159: 20-27. https://doi.org/10.1016/j.ecoenv.2018.04.059

[9] Sapanov, M.K., Elekesheva, M.M., Sizemskaya, M.L., Kolesnikov, A.V. (2024). Ecological condition and invasiveness of Fraxinus pennsylvanica in different ecotopes of the arid Caspian region. Brazilian Journal of Biology, 84: e284645. https://doi.org/10.1590/1519-6984.284645

[10] Chukwura, E.I., Ojiegbu, M.N., Nwankwegu, A.S. (2016). Hydrocarbon degradation potentials of fungi associated with oil-contaminated soil from selected mechanic workshops in Awka, Anambra State, Nigeria. Frontiers in Environmental Microbiology, 2(6): 38-44. https://doi.org/10.11648/j.fem.20160206.13

[11] Eze, V.C., Onwuakor, C.E., Orok, F.E. (2014). Microbiological and physicochemical characteristics of soil contaminated with used petroleum products in Umuahia, Abia State, Nigeria. Journal of Applied and Environmental Microbiology, 2(6): 281-286. https://doi.org/10.12691/jaem-2-6-3

[12] Ali Abdul-Ameer, W. (2019). Biodegradation and phytotoxicity of crude oil hydrocarbons in an agricultural soil. Chilean Journal of Agricultural Research, 79(2): 266-277. https://doi.org/10.4067/S0718-58392019000200266

[13] Chorom, M., Shariffi, H.S., Mutamedi, H. (2010). Bioremediation of a crude-oil polluted soil by application of fertilizers. Iranian Journal of Health Science Engineering, 7(4): 319-326.

[14] Jain, P.K., Gupta, V.K., Gaur, R.K., Lowry, M., Jaroli, D.P., Chauhan, U.K. (2011). Bioremediation of petroleum oil contaminated soil and water. Research Journal of Environmental Toxicology, 5(1): 1-26. https://doi.org/10.3923/rjet.2011.1.26

[15] Makhanbetova, B., Shevko, V., Aitkulov, D. (2024). Producing a Ferroalloy and Zinc Concentrate from the Shalkiya Deposit Sulfide and Oxidized Ores' Mixture. Journal of Applied Science and Engineering, 28(1): 175-182. https://doi.org/10.6180/jase.202501_28(1).0017

[16] Korotkih, P.S., Neverov, E.N., Korotkiy, I.A. (2023). Assessment of carbon dioxide exchange processes between water bodies and the atmosphere. Caspian Journal of Environmental Sciences, 21(5): 1239-1245. https://doi.org/10.22124/CJES.2023.7419

[17] Kleindienst, S., Paul, J.H., Joye, S.B. (2015). Using dispersants after oil spills: Impacts on the composition and activity of microbial communities. Nature Reviews

Microbiology, 13: 388-396. https://doi.org/10.1038/nrmicro3452
[18] Prischepa, O.M., Morgunov, P.A., Nefedov, Y.V. (2019). Biodegradation of oil and oil products by hydrocarbon-oxidizing microorganism strains. International Journal of Civil Engineering and Technology, 10(4): 152-164.
[19] Fuentes, S., Barra, B., Caporaso, J.G., Seeger, M. (2015). From rare to dominant: A fine-tuned soil bacterial bloom during petroleum hydrocarbon bioremediation. Applied and Environmental Microbiology, 82: 888-896. https://doi.org/10.1128/AEM.02625-15
[20] Olajire, A.A., Essien, J.P. (2014). Aerobic degradation of petroleum components by microbial consortia. Journal of Petroleum and Environmental Biotechnology, 5(5): 195. https://doi.org/10.4172/2157-7463.1000195
[21] Varjani, S.J., Gnansounou, E. (2017). Microbial dynamics in petroleum oilfields and their relationship with physiological properties of petroleum oil reservoirs. Bioresource Technology, 245: 1258-1265. https://doi.org/10.1016/j.biortech.2017.08.028
[22] Yang, Y., Wang, J., Liao, J., Xie, S., Huang, Y. (2015). Abundance and diversity of soil petroleum hydrocarbon-degrading microbial communities in oil exploring areas. Applied Microbiology and Biotechnology, 99: 1935-1946. https://doi.org/10.1007/s00253-014-6074-z
[23] Guerra, A.B., Oliveira, J.S., Silva-Portela, R.C., Araujo, W., Carlos, A.C., Vasconcelos, A.T.R., Freitas, A.T., Domingos, Y.S., de Farias, M.F., Fernandes, G.J.T., Agnez-Lima, L.F. (2018). Metagenome enrichment approach used for selection of oil-degrading bacteria consortia for drill cutting residue bioremediation. Environmental Pollution, 235: 869-880. https://doi.org/10.1016/j.envpol.2018.01.014
[24] Lea-Smith, D.J., Biller, S.J., Davey, M.P., Cotton, C.A., Sepulveda, B.M.P., Turchyn, A.V., Scanlan, D.J., Smith, A.G., Chisholm, S.W., Howe, C.J. (2015). Contribution of cyanobacterial alkane production to the ocean hydrocarbon cycle. In Proceedings of the National Academy of Sciences, 112(44): 13591-13596. https://doi.org/10.1073/pnas.1507274112
[25] Ron, E.Z., Rosenberg, E. (2014). Enhanced bioremediation of oil spills in the sea. Current Opinion in Biotechnology, 27: 191-194. https://doi.org/10.1016/j.copbio.2014.02.004
[26] Abbasian, F., Lockington, R., Mallavarapu, M., Naidu, R. (2015). A comprehensive review of aliphatic hydrocarbon biodegradation by bacteria. Applied Biochemistry and Biotechnology, 176(3): 670-699. https://doi.org/10.1007/s12010-015-1603-5
[27] Ghosal, D., Ghosh, S., Dutta, T.K., Ahn, Y. (2016). Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review. Frontiers in Microbiology, 7: 1369. https://doi.org/10.3389/fmicb.2016.01369
[28] Cui, J., Huang, L., Wang, W., Xu, P., Zanaroli, G., Tang, H. (2020). Maximization of the petroleum biodegradation using a synthetic bacterial consortium based on minimal value algorithm. International Biodeterioration & Biodegradation, 150: 104964. https://doi.org/10.1016/j.ibiod.2020.104964
[29] McGenity, T.J. (2014). Hydrocarbon biodegradation in intertidal wetland sediments. Current Opinion in Biotechnology, 27: 46-54. https://doi.org/10.1016/j.copbio.2013.10.010
[30] Omotayo, A.E., Ojo, O.Y., Amund, O.O. (2012). Crude oil degradation by microorganisms in soil composts. Research Journal of Microbiology, 7: 209-218. https://doi.org/10.3923/jm.2012.209.218
[31] Xia, M., Liu, Y., Taylor, A., Fu, D., Khan, A., Terry, N. (2017). Crude oil depletion by bacterial strains isolated from a petroleum hydrocarbon impacted solid waste management site in California. International Biodeterioration & Biodegradation, 123: 70-77. https://doi.org/10.1016/j.ibiod.2017.06.003
[32] Xia, M., Fu, D., Chakraborty, R., Singh, R.P., Terry, N. (2019). Enhanced crude oil depletion by a constructed bacterial consortium comprising bioemulsifier producer and petroleum hydrocarbon degraders. Bioresource Technology, 282: 456-463. https://doi.org/10.1016/j.biortech.2019.01.131
[33] Tremblay, J., Yergeau, E., Fortin, N., Cobanli, S., Elias, M., King, T.L., Lee, K., Greer, C.W. (2017). Chemical dispersants enhance the activity of oil-and-gas condensate-degrading marine bacteria. The ISME Journal, 11: 2793-2808. https://doi.org/10.1038/ismej.2017.129
[34] Lin, M., Liu, Y., Chen, W., Wang, H., Hu, X. (2014). Use of bacteria-immobilized cotton fibers to absorb and degrade crude oil. International Biodeterioration & Biodegradation, 88: 8-12. https://doi.org/10.1016/J.IBIOD.2013.11.015
[35] Chen, W., Kong, Y., Li, J., Sun, Y., Jun, M., Hu, X. (2020). Enhanced biodegradation of crude oil by a constructed bacterial consortium comprising salt-tolerant petroleum degraders and biosurfactant producers. International Biodeterioration & Biodegradation, 154: 105047. https://doi.org/10.1016/j.ibiod.2020.105047
[36] Wang, X.B., Chi, C.Q., Nie, Y., Tang, Y.Q., Tan, Y., Wu, G., Wu, X.L. (2011). Degradation of petroleum hydrocarbons (C6-C40) and crude oil by a novel Dietzia strain. Bioresource Technology, 102(17): 7755-7761. https://doi.org/10.1016/j.biortech.2011.06.009
[37] Nie, Y., Liang, J.L., Fang, H., Tang, Y.Q., Wu, X.L. (2014). Characterization of a CYP153 alkane hydroxylase gene in a gram-positive Dietzia sp. DQ12-45-1b and its “team role” with alkw1 in alkane degradation. Applied Microbiology and Biotechnology, 98: 163-173. https://doi.org/10.1007/s00253-013-4821-1
[38] Sarkar, P., Roy, A., Pal, S., Mohapatra, B., Kazy, S.K., Maiti, M.K., Sar, P. (2017). Enrichment and characterization of hydrocarbon-degrading bacteria from petroleum refinery waste as potent bioaugmentation agent for in situ bioremediation. Bioresource Technology, 242: 15-27. https://doi.org/10.1016/j.biortech.2017.05.010
[39] Sharma, S. (2012). Bioremediation: Features, strategies and applications. Asian Journal of Pharmacy and Life Science, 2(2): 202-213.
[40] Abed, R.M.M., Al-Kharusi, S., Al-Hinai, M. (2015). Effect of biostimulation, temperature, and salinity on respiration activities and bacterial community composition in an oil-polluted desert soil. International Biodeterioration & Biodegradation, 98: 43-52. https://doi.org/10.1016/j.ibiod.2014.11.018
[41] Jahangeer, J., Kumar, V. (2013). An overview on microbial degradation of petroleum hydrocarbon contaminants. International Journal of Engineering and Technical Research, 1(8): 34-37.

[42] Varjani, S.J., Upasani, V.N. (2016). Biodegradation of petroleum hydrocarbons by oleophilic strain of Pseudomonas aeruginosa NCIM 5514. Bioresource Technology, 222: 195-201. https://doi.org/10.1016/j.biortech.2016.10.006

[43] Ke, C.Y., Qin, F.L., Yang, Z.G., Sha, J., Sun, W.J., Hui, J.F., Zhang, Q.Z., Zhang, X.L. (2021). Bioremediation of oily sludge by solid complex bacterial agent with a combined two-step process. Ecotoxicology and Environmental Safety, 208: 111673. https://doi.org/10.1016/j.ecoenv.2020.111673

[44] van Beilen, J.B., Funhoff, E.G. (2007). Alkane hydroxylases involved in microbial alkane degradation. Applied Microbiology and Biotechnology, 74: 13-21. https://doi.org/10.1007/s00253-006-0748-0

[45] Head, I.M., Jones, D.M., Röling, W.F. (2006). Marine microorganisms make a meal of oil. Nature Reviews Microbiology, 4: 173-182. https://doi.org/10.1038/nrmicro1348

[46] Li, D., Xu, X., Zhai, Z., Yu, H., Han, X. (2017). Isolation and identification of an n-hexadecane bacterial degrader from soil polluted by petroleum oil in Momoge wetlands and its degradation characteristics. Wetland Science, 15: 85-91.

[47] Kyzylordinskaya oblast' [Kyzylorda Region]. Planet Earth. https://geosfera.org/aziya/kazaxstan/2660-kyzylordinskaya-oblast.html, accessed on Aug. 8, 2024.

[48] ElDala.kz. (2021). V Kyzylordinskoy oblasti zasoleny okolo 85% pochv [About 85% of soils in Kyzylorda region are saline]. https://eldala.kz/novosti/kazahstan/5735-v-kyzylordinskoy-oblasti-zasoleny-okolo-85-pochv, accessed on Aug. 8, 2024.

[49] Dombrowski, N., Donaho, J.A., Gutierrez, T., Seitz, K.W., Teske, A.P., Baker, B.J. (2016). Reconstructing metabolic pathways of hydrocarbon-degrading bacteria from the Deepwater Horizon oil spill. Nature Microbiology, 1: 16057. https://doi.org/10.1038/nmicrobiol.2016.57

[50] Dvořák, P., Nikel, P.I., Damborský, J., de Lorenzo, V. (2017). Bioremediation 3.0: Engineering pollutant-removing bacteria in the times of systemic biology. Biotechnology Advances, 35(7): 845-866. https://doi.org/10.1016/j.biotechadv.2017.08.001

[51] Kuppusamy, S., Palanisami, T., Megharaj, M., Venkateswarlu, K., Naidu, R. (2016). In-situ remediation approaches for the management of contaminated sites: A comprehensive overview. In: de Voogt, P. (ed) Reviews of Environmental Contamination and Toxicology, vol. 236. Springer, Cham, 1-115. https://doi.org/10.1007/978-3-319-20013-2_1

[52] Devinny, J.S., Chang, S.H. (2000). Bioaugmentation for soil bioremediation. In: Wise, D.L., Trantolo, D.J. (eds) Bioremediation of Contaminated Soils, pp. 465-488.

[53] Alisi, C., Musella, R., Tasso, F., Ubaldi, C., Manzo, S., Cremisini, C., Sprocati, A.R. (2009). Bioremediation of diesel oil in a co-contaminated soil by bioaugmentation with a microbial formula tailored with native strains selected for heavy metals resistance. Science of the Total Environment, 407(8): 3024-3032. https://doi.org/10.1016/j.scitotenv.2009.01.011

[54] Li, X., Lin, X., Li, P., Liu, W., Wang, L., Ma, F., Chukwuka, K.S. (2009). Biodegradation of the low concentration of polycyclic aromatic hydrocarbons in soil by microbial consortium during incubation. Journal of Hazardous Materials, 172(2-3): 601-605. https://doi.org/10.1016/j.jhazmat.2009.07.044

[55] Spankulova, G., Gerginova, M.., Peneva, N.., Alexieva, Z. (2018). Molecular identification of petroleum-degrading bacteria and characterization of their biodegradation potential related phenol. Comptes Rendus de l'Acade'mie Bulgare des Sciences, 71: 1473-1478. https://doi.org/10.7546/CRABS.2018.11.05

[56] Isina, Z.M., Koigeldina, A.K., Tursunova,, A.K., Kopzhassarov, B., Sardar, A., Boltaeva, L. (2024). Impact of environmental degradation on the development of moniliosis: A case study of apple orchards in the Almaty region, Kazakhstan. Caspian Journal of Environmental Sciences, 22(1): 211-220. https://doi.org/10.22124/cjes.2024.7513

[57] Das, N., Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. Biotechnology Research International, 2011: 941810. https://doi.org/10.4061/2011/941810

[58] Kim, D., Choi, K.Y., Yoo, M., Zylstra, G.J., Kim, E., (2018). Biotechnological potential of rhodococcus biodegradative pathways. Journal of Microbiology and Biotechnology, 28(7): 1037-1051. https://doi.org/10.4014/jmb.1712.12017

[59] Larkin, M.J., Kulakov, L.A.., Allen, C.C. (2006). Biodegradation by members of the genus Rhodococcus: Biochemistry, physiology, and genetic adaptation. Advances in Applied Microbiology, 59: 1-29. https://doi.org/10.1016/S0065-2164(06)59001-X

[60] Li, C., Zhou, Z.X., Jia, X.Q., Chen, Y., Liu, J., Wen, J.P. (2013). Biodegradation of crude oil by a newly isolated strain Rhodococcus sp. JZX-01. Applied Biochemistry and Biotechnology, 171(7): 1715-1725. https://doi.org/10.1007/s12010-013-0451-4

[61] Aitkeldiyeva, S.A., Faizulina, E.R., Tatarkina, L.G., Alimzhanova, M.B., Daugaliyeva, S.T., Auezova, O.N., Alimbetova, A.V., Spankulova, G.A., Sadanov A.K. (2020). Degradation of petroleum hydrocarbons with thermotolerant microorganisms. RASAYAN Journal of Chemistry, 13(2): 1271-1282. https://doi.org/10.31788/RJC.2020.1325580

[62] Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q., Jiang, P., Gao, X., Li, F., Li, H., Yu, H. (2018). Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis. Frontiers in Microbiology, 9: 2885. https://doi.org/10.3389/fmicb.2018.02885

[63] Ma, Y.L., Lu, W., Wan, L.L., Luo, N. (2015). Elucidation of fluoranthene degradative characteristics in a newly isolated Achromobacter xylosoxidans DN002. Applied Biochemistry and Biotechnology, 175: 1294-1305. https://doi.org/10.1007/s12010-014-1347-7

[64] Hossain, M.F., Akter, M.A., Sohan, M.S.R., Sultana, D.N., Reza, M.A., Hoque, K.M.F. (2022). Bioremediation potential of hydrocarbon-degrading bacteria: Isolation, characterization, and assessment. Saudi Journal of Biological Sciences, 29(1): 211-216. https://doi.org/10.1016/j.sjbs.2021.08.069

[65] Nejidat, A., Meshulam, M., Diaz Reck, D., Ronen, Z. (2023). Emergence of hydrocarbon-degrading bacteria in crude oil-contaminated soil in a hyperarid ecosystem: Effect of phosphate addition and augmentation with nitrogen-fixing cyanobacteria on oil bioremediation.

International Biodeterioration & Biodegradation, 178: 105556. https://doi.org/10.1016/j.ibiod.2022.105556
[66] Sui, X., Wang, X., Li, Y., Ji, H. (2021). Remediation of petroleum-contaminated soils with microbial and microbial combined methods: Advances, mechanisms, and challenges. Sustainability, 13(16): 9267. https://doi.org/10.3390/su13169267
[67] Varjani, S.J. (2017). Microbial degradation of petroleum hydrocarbons. Bioresource Technology, 223: 277-286. https://doi.org/10.1016/j.biortech.2016.10.037
[68] Ullrich, R., Hofrichter, M. (2007). Enzymatic hydroxylation of aromatic compounds. Cellular and Molecular Life Sciences, 64(3): 271-293. https://doi.org/10.1007/s00018-007-6362-1
[69] Chaerun, S.K., Tazaki, K., Asada, R., Kogure, K. (2004). Bioremediation of coastal areas 5 years after the Nakhodka oil spill in the Sea of Japan: Isolation and characterization of hydrocarbon-degrading bacteria. Environment International, 30(7): 911-922. https://doi.org/10.1016/j.envint.2004.02.007
[70] Varjani, S.J., Upasani, V.N. (2017). A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants. International Biodeterioration & Biodegradation, 120: 71-83. https://doi.org/10.1016/j.ibiod.2017.02.006
[71] Stepanova, A.Y., Gladkov, E.A., Osipova, E.S., Gladkova, O.V., Tereshonok, D.V. (2022). Bioremediation of soil from petroleum contamination. Processes, 10(6): 1224. https://doi.org/10.3390/pr10061224
[72] Xu, X., Zhai, Z., Li, H., Wang, Q., Han, X., Yu, H. (2017). Synergetic effect of bio-photocatalytic hybrid system: G-C_3N_4, and *Acinetobacter* sp. JLS1 for enhanced degradation of C16 alkane. Chemical Engineering Journal, 323: 520-529. https://doi.org/10.1016/j.cej.2017.04.138
[73] Delegan, Y., Petrikov, K., Frantsuzova, E., Rudenko, N., Solomentsev, V., Suzina, N., Travkin, V., Solyanikova, I.P. (2022). Complete genome analysis of *Rhodococcus opacus* S8 capable of degrading alkanes and producing biosurfactant reveals its genetic adaptation for crude oil decomposition. Microorganisms, 10: 1172. https://doi.org/10.3390/microorganisms10061172

NOMENCLATURE

AG	Analytic grade
DNA	Deoxyribonucleic acid
GC	Gas chromatography
MS	Mass spectrometry
MSD	Mass selective detector
NIST	National institute of standards and technology
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
UV-VIS	Ultraviolet-visible spectroscopy