

PROSPECTS FOR THE USE OF THERMOTOLERANT MICROORGANISMS FOR REMEDIATION OF OIL-CONTAMINATED SOILS

Aitkeldiyeva S.A., Faizulina E.R., Tatarkina L.G., Alimbetova A.V.,
Auezova O.N., Spankulova G.A.

Research and Production Center for Microbiology and Virology
105, Bogenbai batyr str., Almaty, 050010, Kazakhstan
sa.kz@list.ru, ecomicrolab@gmail.com

ABSTRACT

Most of the world's oil fields are concentrated in geographic areas with high temperature conditions. One of the main problems of remediation of territories in hot climates is the fact that high temperatures reduce the viscosity of oil and, thus, accelerate its diffusion into the soil. This greatly complicates the processes of soil cover restoration using conventional methods. An alternative can be bioremediation using the oil-oxidizing microorganisms that are resistant to high temperatures and low humidity. The presented review is devoted to the analysis of studies of thermotolerant microorganisms by scientists from different countries with the aim of using them in the remediation of contaminated soils from oil and oil products. The data on the influence of the main environmental factors, such as temperature, oxygen, pH, salinity, aeration, the presence of nutrients, etc., on the viability and vital activity of thermotolerant hydrocarbon-oxidizing microorganisms are presented. Examples of biochemical and molecular genetic characteristics of thermotolerant microorganisms are given. Their taxonomic diversity is shown, and examples of the degradation of individual oil components by active strains are given.

Key words: thermotolerant microorganism, bioremediation, oil, oil destructor, temperature

Among the energy resources of our planet, oil and petroleum hydrocarbons are the main environmental pollutants. Starting from the exploration and production of oil, and ending with the use of petroleum products, all stages to one degree or another lead to severe environmental pollution and negative impact on human health. By entering the soil, oil products worsen the overall environmental situation, significantly changing the agrophysical and agrochemical properties of soils. The decomposition of oil and oil products in the soil under natural conditions is a biochemical process. The intensity of oil degradation is directly dependent on the biological (enzymatic) activity of the soil, the total amount of soil microflora and its physiological activity [1].

According to the United States Environmental Protection Agency (EPA USA), the amount of contaminated soil exceeds 1 billion cubic meters. Only in the EU there are more than one and a half million contaminated sites, the cleaning of which requires more than 85 billion Euros [2].

More than half of the world's oil is produced in the Persian Gulf, the coast of which belongs to the territories of Qatar, Bahrain, Oman, the United Arab Emirates, Saudi Arabia, Kuwait, Iraq and Iran. Kazakhstan takes 8th place in world proven oil reserves. Only Saudi Arabia, Iran, Iraq, Kuwait, the United Arab Emirates, Venezuela and Russia are ahead of him. The total forecast hydrocarbon resources are estimated at

12-17 billion tons [3]. According to forecasts, global oil demand by 2030 will increase by 37% compared to 2006 and will reach 118 million barrels per day. With such volumes of consumption, environmental problems caused by the release of oil into the environment will reach a critical level. The main risk of oil and oil products pollution is in the regions where the fields are located and oil is being extracted. About 60% of the world's oil reserves are located in countries with hot climates, where the specifics of natural, in particular, temperature conditions force a more careful approach to the choice of remediation method for contaminated lands [4].

The growing need for restoration of contaminated sites has led to the development of new technologies that focus on biological detoxification and the destruction of organic pollutants. Bioremediation is one of these technologies. Using this technology, various pollutants are destroyed or neutralized, while using the biological activity of certain groups of microorganisms. The use of bioremediation provides significant advantages in the fight against environmental pollution, however, due to the variability of pollutants and their possible biotransformation into uncontrolled metabolites, the risk of developing adverse effects on human health is possible [5]. Bioremediation technologies can be divided into two main categories: *ex situ* and *in situ*. *Ex situ* are methods that require the physical removal of contaminated material and its transportation to another area for further processing, for example, using bioreactors, farming and composting. *In situ* technologies include manipulations in which contaminated material is processed locally, for example, by bioventilation and biostimulation. In general, bioremediation is considered a very promising technology with great potential when working with certain types of contaminated sites.

The bioremediation method is based on the use of active microbial strains exhibiting the ability to degrade oil hydrocarbons. Hydrocarbon-oxidizing microorganisms (HOMs) decompose petroleum hydrocarbons at a very high speed, since they have high enzymatic activity, they multiply rapidly. They are able to use oil hydrocarbons as the sole source of carbon and bring the process of transformation of organic matter to full mineralization. As a result of biochemical processes, natural pollutants are converted into carbon dioxide, water, and other environmentally neutral compounds. That is why bioremediation of soils contaminated with oil and oil products, by active strains of hydrocarbon-oxidizing microorganisms is proposed as the most promising method of combating this pollution [6].

Since the problem of pollution of natural ecosystems with oil and oil products is extremely acute, research on the bioremediation of soil and water from these pollutants is being conducted in many countries. The degradation of petroleum products involves the use of both individual strains of microorganisms and their associations. In nature, a complex of consortium species formed in a given biotope is usually involved in oil biodegradation. For active oil degradation, a combination of bacterial strains with wide enzymatic capabilities is necessary – in this case, the process of removing the pollutant from the ecosystem is most effective [7]. It was noted that the use of consortia of hydrocarbon-oxidizing microorganisms leads to the most complete destruction of these compounds [8].

In addition, the introduction of hydrocarbon-oxidizing microorganisms into the existing microocenosis may prove to be ineffective due to possible antagonism with indigenous microflora. Therefore, it is preferable in the elimination of local pollution by oil products to isolate microorganisms - oil product destructors adapted to specific conditions.

The following main factors influence the activity of microorganisms: temperature, oxygen, pH, salinity, aeration, the presence of nutrients, etc. (Figure 1) [9].

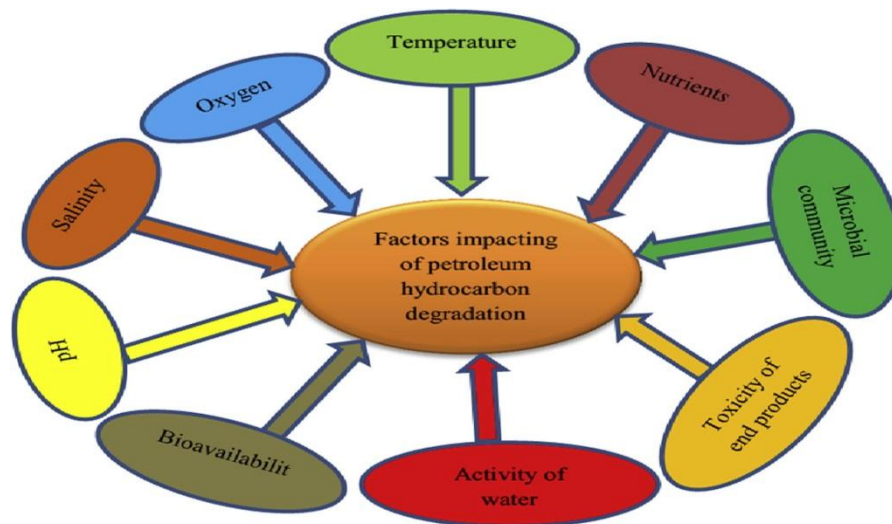


Fig. 1 Factors affecting the biodegradation of petroleum hydrocarbons

Under natural conditions, microorganisms are exposed to abiotic factors, significantly different in nature and mechanism of action. Nevertheless, the nature of the dependence of microorganism vital activity indicator on the level of various external factors is similar. For any abiotic factor, there is a range of changes within which the vital signs of the microorganism practically do not change, remaining at a level called optimal. Lower levels of the factor do not ensure the full functioning of the microorganism, limiting the intensity of its vital processes, and the corresponding interval is called the *limiting zone*. Excessive factor levels inhibit the activity of microorganisms, and this interval is called the *zone of inhibition*. There are extremely high and extremely low factor levels at which the vital activity of a microorganism becomes impossible. The extreme limits of the factor changes that the body is able to tolerate are commonly called *tolerance limits*. Different microorganisms have significantly different limits of tolerance to the same environmental factor [10].

Temperature is one of the key factors affecting the physical properties and chemical composition of petroleum hydrocarbons, and, consequently, their biodegradation. At low temperatures, a decrease in the rate of degradation is usually observed, which is believed to be the result of a decrease in the rate of enzymatic activity [11].

Aislabie J. et al. [12] and Okoh A.I. [13] by their studies proved that elevated temperature increases the solubility of hydrocarbon pollutants, reduces viscosity and transfers long chain n-alkanes from the solid phase to the aqueous phase. Thamer M. et al. showed that high salinity and temperature inhibit the growth of microorganisms and the release of their metabolites [14].

The rate of hydrocarbon biodegradation depends on the level of oxygen in the soil, the degree of oxygen consumption by microorganisms, and the type of soil. Studies have shown that anaerobic degradation of petroleum hydrocarbons by microorganisms can occur at an insignificant rate [15]. Mc Nally D.L. et al. [16] reported that aerobic biodegradation of petroleum hydrocarbons was higher compared to anaerobic biodegradation. It was proved that the rate of biodegradation of petroleum hydrocarbons under anaerobic conditions was not as high as under aerobic conditions [17]. Anaerobic degradation processes are less studied than aerobic ones [18, 19]. It should be noted that BTEX (benzene, toluene, ethylbenzene, xylene) compounds undergo degradation at approximately the same rate, both during aerobic and anaerobic metabolism [20]. During anaerobic metabolism, these aromatic compounds are first oxidized to phenols or organic acids and then converted to long chain volatile fatty acids, which are

ultimately metabolized to CH₄ and CO₂ [19-21]. During anaerobic degradation of oil hydrocarbons, electron acceptors are nitrate ions, ferrous, manganese or sulfate ions [21, 22].

Abed R.M.M. et al. [23] found that the coastline of the Persian Gulf is inhabited mainly by anaerobic sulfate-reducing microorganisms of the *Gamma*- and *Deltaproteobacteria* groups. It was found that oil pollution stimulates the microorganisms involved in the sulfur cycle, and sulfate reducers are often destructors of oil hydrocarbons [24]. The detection of such bacteria indicates anaerobic degradation of oil, which was confirmed by the selected samples of the microbial mat. At the same time, well-known aerobic destructors of such genera as *Pseudomonas* and *Alcanivorax*, Abed R.M.M. et al. not found [23].

In addition to temperature and aeration, salinity also has a significant effect on the process of bioremediation and biodegradation, as well as on the growth and diversity of microbes [25]. It was proved that salinity has an adverse effect on the activity of some key enzymes involved in the biodegradation of hydrocarbons [26]. Halophilic bacteria degrading hydrocarbons have been described by many researchers. Previously Ben Ali Gam Z. et al. [27] from an oil well in Tunisia isolated a strain of Lit2 belonging to the genus *Modicisalibacter* and capable of growth in the temperature range of 15-45 °C with an optimum of 37 °C. The strain withstood up to 25% of the salt in the medium, but the optimal concentration for it was 10% NaCl in the medium. Wang Y.N. et al. [28] isolated the strain DQS3-9A1 from a field in China and described a new genus *Amicollicoccus* and a new species *A. subflavus* on its basis. The strain grew in the temperature range 15-42°C with an optimum of 37°C, pH 6-11 (optimum 8) and in the presence of salt in the medium 1-12% (optimum 8%).

The pH can vary greatly in both soil and water and must be taken into account to improve biological treatment methods. The pH of the medium affects processes such as cell membrane transport, the balance of catalytic reactions, and enzyme activity [29]. Most heterotrophic bacteria prefer to grow at a pH of neutral to alkaline. Unlike the pH of most aquatic ecosystems, soil acidity can vary greatly from 2.5 to 11 pH in alkaline deserts [30]. In general, heterotrophic fungi and bacteria prefer an almost neutral pH, although fungi are tolerant to acidic conditions. Thavasi R. et al. [31] found that the maximum biodegradation of crude oil by *Pseudomonas aeruginosa* in water was at pH 8.0. R. Pawar [32] noted that a soil pH of 7.5 was most optimal for the degradation of virtually all petroleum hydrocarbons.

Bioavailability is the presence of a chemical in the soil that can be absorbed or converted by living organisms. The physicochemical properties and bioavailability of hydrocarbon pollutants play a very important role for successful bioremediation [33, 34]. Oil pollution has low bioavailability and is classified as hydrophobic organic pollutants. These chemicals have little solubility in water, which makes them resistant to photolytic destruction and chemical biological effects [35]. When all of the above conditions are favorable for microorganisms, the biodegradation of petroleum hydrocarbons reaches a maximum level.

Soil pollution with oil and oil products is an urgent problem for Kazakhstan. It is known that in the oil-producing regions of Kazakhstan, the climate is sharply continental, characterized by sharp seasonal and daily temperature differences, high rates of water evaporation and, as a result, salinity and low soil moisture. In hot climates, volatile hydrocarbon fractions evaporate quickly, and the remaining long-chain aliphatic and aromatic components are more difficult to degrade and can remain in the environment for a long time. Therefore, research related to the search and study of thermotolerant hydrocarbon-oxidizing oil-degrading microorganisms is currently very relevant.

For thermotolerant oil-oxidizing microorganisms, the temperature regime is one of the main factors for the oxidation of oil hydrocarbons. Depending on the temperature, bacterial activity and biodegradation rates may vary seasonally [36, 37].

One of the main problems of remediation of territories in hot climates is the fact that high temperatures reduce the viscosity of oil and, thus, accelerate its diffusion deep into the soil. In addition, the evaporation of light oil fractions at elevated temperatures leads to air pollution with toxic products, while the remaining non-volatile components with a high molecular weight form films that are poorly biodegradable. At the same time, the increased average daily temperatures are the reason for the rapid evaporation of water from the soil and from the surface of water bodies, which leads to their salinization. Therefore, microorganisms' resistant to a lack of water in the soil and an increased salt content in the treated area are promising agents for the remediation of oil-contaminated sites in high-temperature regions [38].

Temperature has a huge impact on many aspects of the life of organisms - the geography of their distribution, reproduction and other biological properties. The temperature range in which life can exist ranges from about -200°C to $+100^{\circ}\text{C}$, sometimes the existence of bacteria in hot springs is detected at a temperature of $+250^{\circ}\text{C}$. In fact, most organisms can exist at a narrower temperature range. All chemical processes in the organism depend on temperature. Changes in thermal conditions, often observed in nature, deeply affect the growth, development and other manifestations of the vital activity of animals and plants.

In relation to temperature, microorganisms are divided into the following groups: psychrophilic (minimum - about 0°C , maximum - below 20°C); psychroactive / psychrotrophic (minimum - about 0°C , optimum and maximum - above 20°C); mesophilic (minimum - above 0°C , maximum - up to 45°C); thermophilic (maximum above 45°C).

Thermophilic microorganisms are divided into 5 groups depending on the temperature range:

1. Thermotolerant (minimum - $+10^{\circ}\text{C}$; optimum - $+35-40^{\circ}\text{C}$, as in mesophiles; maximum - $+55-60^{\circ}\text{C}$): gram-negative bacteria *Methylococcus capsulatus*; red algae *Cyanidium caldarium*;

2. Optional thermophiles (minimum - below $+20^{\circ}\text{C}$; maximum - $+55-65^{\circ}\text{C}$): homoenzymatic lactic acid bacteria of the genus *Lactobacillus*, cyanobacteria *Thermosynechococcus elongatus*;

3. Obligatory thermophiles (minimum - $+40^{\circ}\text{C}$; optimum - adjacent to the upper boundary ($+65-70^{\circ}\text{C}$); maximum - above $+70^{\circ}\text{C}$): *Bacillus tearothermophilus*;

4. Extreme thermophiles (minimum - above $+40^{\circ}\text{C}$; optimum - $+70-75^{\circ}\text{C}$; maximum - above $+90^{\circ}\text{C}$): bacteria *Thermus*, *Thermomicrobium*, archaea *Thermoplasma*;

5. Hyperthermophiles (minimum - about $+70^{\circ}\text{C}$; optimum - above $+80^{\circ}\text{C}$; maximum - above $+100^{\circ}\text{C}$): archaea *Geogemma barossii*, *Pyrolobus fumarii*, *Methanopyrus kandleri*.

Upon adaptation to high temperature, more saturated fatty acids appear in the membrane of bacteria and eukaryotes; in gram-positive bacteria, an increase in the content of iso-branched fatty acids is observed. In some thermophilic bacteria, instead of a complex ether bond, a simple ether bond is present in lipids. Thermophiles show an increase in the content of G+C bases in the regions of RNA molecules that form secondary structures. In addition, thermophile proteins are more stable than those of mesophiles, due to an increase in the number of intramolecular interactions. In thermophilic archaea, histones stabilizing the DNA structure are present in the cell [10].

The effect of temperature on the growth of microorganisms is due to its effect on the rate of chemical reactions in the cell and the state of cellular macromolecules

(membrane viscosity, protein conformation, etc.). An increase in temperature above a critical level leads to irreversible inactivation of cellular components, primarily the denaturation of proteins and nucleic acids, and the death of a microorganism. To assess the lethal effect of temperature on the cells of microorganisms, quantitative parameters are used: the *thermal point of extinction* (TPE) - the temperature at which a given organism dies in 10 minutes, and the *thermal time of extinction* (TTE) - the time for which this organism dies at a certain temperature [10].

Thermal tolerance is a polygenic trait that promotes the survival and growth of cells at unusually high temperatures. Although some genes associated with high-temperature growth (Htg +) have been identified, the ability of cells to accumulate mutations to achieve long-term thermal tolerance remains poorly understood [39].

The taxonomic diversity of thermotolerant oil destructors covers many bacteria. So microbial communities in hot springs are widely studied around the world. In this sense, areas of the Indian Himalayas are valuable ecosystems for studying microbial diversity. In a study by Harmesh S. et al. [39] a total of 140 thermophilic bacteria were isolated from 12 sample samples collected from the hot springs of Manikaran and Yumtang in the Indian Himalayas. Growth properties were studied in bacterial isolates under various conditions and as potential sources of extracellular thermostable hydrolytic enzymes such as protease, amylase, xylanase and cellulase. Based on the isolation of extracellular hydrolases, 51 isolates were identified using 16S rRNA gene sequencing. They included 37 different species from 14 different genera, such as *Anoxybacillus*, *Bacillus*, *Brevibacillus*, *Brevundimonas*, *Burkholderia*, *Geobacillus*, *Paenibacillus*, *Planococcus*, *Pseudomonas*, *Rhodanobacter*, *Thermoactinomyces*, *Thermobacillus*, *Thermonema* and *Thiobacillus*. Out of 51 hydrolase producing bacteria, 24 isolates showed stability over a wide range of temperature and pH treatments. In the present study, it was found that three thermotolerant bacteria, namely *Thermobacillus sp* NBM6, *Paenibacillus ehimensis* NBM24 and *Paenibacillus popilliae* NBM68, produced cellulase-free xylanase. These extracellular thermostable hydrolytic enzymes have great commercial potential for use in the oil industry, medicine, and agriculture. In studies by David M.C. et al. [40] ten thermophilic bacterial strains were isolated from manure compost. Phylogenetic analysis based on 16S rRNA genes and biochemical characterization made it possible to identify four different species belonging to four genera: *Geobacillus thermodenitrificans*, *Bacillus smithii*, *Ureibacillus suwonensis*, and *Aneurinibacillus thermoaerophilus*. PCR-RFLP profiles of the 16S-ITS-23S rRNA region made it possible to distinguish two subgroups among *G. thermodenitrificans* isolates. The isolates were screened for thermo-tolerant hydrolytic activity (60-65 ° C). Thermo-tolerant lipolytic activity was detected for *G. thermodenitrificans*, *A. thermoaerophilus*, and *B. smithii*. In the group of *G. thermodenitrificans*, the activity of thermotolerant protease, α -amylase, and xylanase was also observed. The authors concluded that these species represent a source of potential new thermostable enzymes for industrial use.

The thermotolerant bacterium PHS1 isolated from a hot spring in Pohang, Korea, was able to grow on benzene, toluene, ethylbenzene and xylene (BTEX) as the sole carbon source. Strain PHS1 was a Gram-negative, rod-shaped cell with optimal growth at 42°C and pH 7.2. According to the analysis of 16S rRNA, the strain PHS1 showed the greatest similarity with *Ralstonia* (formerly called *Alcaligenes eutrophus*). However, unlike its closest known species, *Ralstonia*, strain PHS1 could use toluene, ethylbenzene, o-xylene and both m- and o-cresol. According to the authors, the degradation of o-xylene by strain PHS1 is especially important, since o-xylene is a compound of significant environmental interest due to its instability. And very few microorganisms use o-xylene as their sole carbon source. It was found that strain PHS1 transformed o-xylene into 2,3-dimethylphenol by direct oxygenation of the aromatic

ring. The unique properties of strain PHS1, such as heat resistance and degradability of o-xylene, may be important for the treatment of industrial effluents contaminated with BTEX [41].

From the oil-contaminated soil taken from the mud pit at the Khangiran oil and gas field (northeast of Iran), a thermally tolerant actinobacterial strain RIPIT degrading crude oil was isolated. RIPIT was strictly aerobic, catalase and oxidase-positive. The strain grew with NaCl (3-5%) at 25-55°C (opt. 45°C) and at pH 6.0-9.5 (opt. PH 7.0). Based on multiphase taxonomic data, the authors suggested that RIPIT is a new species of the genus *Prauserella*, which was given the name *Prauserella oleivorans* sp. [42].

Raza C. et al. [43] when studying indigenous oil-oxidizing microorganisms in Pakistan soils, revealed that the main oil destructors in the soil of the Rajan field are pseudomonads and bacilli, as well as representatives of the genera *Klebsiella*, *Acinetobacter*, *Clostridium*, *Erwinia*, and *Streptococcus*. At the same time, the authors isolated strains of the genera *Acinetobacter*, *Amphibacillus*, *Arthrobacter*, *Bacillus*, *Corynebacterium*, *Flavobacterium*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Moraxella*, *Providencia* and *Pseudomonas* from soil samples from the Missa Kasval deposit. An experiment on the destruction of oil by bacterial monocultures showed that at 37°C and an initial oil concentration of 1%, the representatives of *Pseudomonas* and *Bacillus* were the most effective oil destructors: after 15 days of cultivation, they oxidized to 20% and up to 16% of oil, respectively. Mnif S. et al. [44] isolated a number of oil-oxidizing bacteria from various Tunisian deposits, attributed by the authors to the genera *Geobacillus*, *Bacillus*, *Brevibacillus*, *Pseudomonas*, *Lysinibacillus*, *Achromobacter*, and *Halomonas*. Strains grew on oil in the presence of various salt concentrations (1-10%) and in the temperature range 37-55°C. Strain C450R, identified by the authors as *P. aeruginosa*, during oil growth (2%) produced glycolipid biosurfactants that reduced the surface tension of the culture medium from 68 to 35 mN/m. Rajaei S. et al. [45] isolated from the oat rhizosphere in Khuzestan (Iran) thermotolerant oil-oxidizing bacterial strains of the genera *Acinetobacter*, *Pseudomonas*, *Enterobacter*, *Stenotrophomonas*, *Bacillus*, *Achromobacter*, *Ochrobacterium*, *Paenibacillus*, *Microbacterium*, *Curtobacterium* and *Sphingobacterium*. The catabolic genes responsible for the destruction of alkanes and aromatic compounds were identified in these bacteria. It was shown in the work that oat rhizospheric bacteria are capable of utilizing oil in the soil by 40.5% within 10 days of cultivation at an initial oil concentration of 2.5%. In the work of Y. A. Delean [46] the ability to utilize hydrocarbons at temperatures up to 50°C in 18 thermotolerant cultures has been analyzed. Strains were identified as representatives of the genera *Gordonia*, *Rhodococcus*, *Paenibacillus* and *Deinococcus*. The most effective thermotolerant destructors are *Gordonia* sp. 1D, *Gordonia* sp. 1G, *Rhodococcus erythropolis* Par7, *R. pyridinivorans* L5A-BSU, *Rhodococcus* sp. Par6, *Deinococcus* sp. A2-6, and utilized oil both at 24°C and at 45°C, in the presence of oil up to 10% and salt up to 7%.

In addition to bacteria, there are also a number of reports on the isolation of natural samples of fungi with oil-oxidizing ability. So, Obuekwe C.O. et al. [47] reported the isolation of *Fusarium lateritium*, *Drechslera* sp. and *Papulaspora* sp. from the saline soils of the desert in Kuwait. The strains of these fungi were able to utilize oil as a carbon source. The authors showed that two strains (*Fusarium lateritium*, *Drechslera* sp.) grew in the presence of 10% salt, *Papulaspora* sp. - in the presence of 5% salt. Salt as a stress factor did not significantly affect the degradation of oil by fungi, but the degree of degradation depended on the presence of nutrients in the medium. Teerapatsakul C. et al. [48] studied the biodegradation of phenanthrene, fluorene, and pyrene by the thermotolerant fungal strain RYNF13, isolated from decayed acacia wood (Rayong Province, Thailand). Strain RYNF13 has been identified as *Trametes polyzona*. The fungus showed a high ability for biodegradation of PAHs at a concentration of 100

mg/L. Complete degradation of phenanthrene was observed within 18 days after incubation at 30°C. 90% of fluorene and 52% of pyrene were degraded under the same conditions. At a temperature of 42°C, the RYNF13 strain degraded approximately 68% of phenanthrene, 48% of fluorene, and 30% of pyrene for 32 days. Thus, the strain RYNF13 is a potential fungus for PAH bioremediation, especially in a tropical environment where the temperature can be above 40°C. Strain RYNF13 isolated three different ligninolytic enzymes, manganese peroxidase, laccase and lignin peroxidase during PAH biodegradation at 30°C. With an increase in temperature from 30°C to 37°C and 42°C, only two ligninolytic enzymes (manganese peroxidase and laccase) were detected. Manganese peroxidase was the main enzyme produced by the fungus.

The choice of recovery method for contaminated soil and water depends to a large extent on the place of production and oil composition. The composition of oil in different countries and, correspondingly, deposits varies greatly. Thus, Venezuela, Kuwait and Iran produce heavy oil with a high sulfur content, and Nigeria, the United Arab Emirates, Angola and Libya are suppliers of high-quality light oil, almost free of sulfur. But light oil is also dangerous because it is enriched with volatile toxic components [49].

The leader in terms of oil production among the CIS countries, whose territories are located in regions with a hot climate, is Kazakhstan. The largest oil fields on the territory of this state are Kashagan, Karashyganak, Uzen, Zhetybai, Tengiz, Zhanazhol and Kumkol. The density of the produced oil mainly ranges from 0.79 to 0.82 g/cm³, the tar content is from 1.14% (Tengiz) to 8.2% (Kumkol), and sulfur is 0.5-1.1%. The Uzen oil has a high density (0.84-0.87 g/cm³), tar content up to 20% and sulfur content up to 2%; Zhetybai oil is medium in density, viscous, and also highly paraffinic, since the temperature of its yield loss is +33°C [50].

There is the following world classification of oil by density: super light - up to 0.780 g/cm³; ultralight - 0.781 - 0.820 g/cm³; light - 0.821 - 0.870 g/cm³; average - 0.871 - 0.920 g/cm³; heavy - 0.921 - 1,000 g/cm³; superheavy - over 1,000 g/cm³ and natural bitumen - whose density is also higher than 1000 g/cm³, viscosity more than 10000 mPa s [51]. In accordance with this classification, oil extracted from various fields of Kazakhstan, in terms of density, relates mainly to light and medium, although dense oil is also found. The higher the density of the produced oil, the less it is subject to biodegradation, since it contains a large amount of high molecular weight components such as resins, asphaltenes, bitumen.

In compiling a microbial consortium, which is planned to be used to clean oil from territories in a hot climate, data are also needed on the ability of bacteria to utilize hydrocarbons in the presence of salt in the medium, the maximum possible concentration of pollutant and the minimum allowable soil moisture. This information will allow to estimate the cost of irrigation of the plot treated by the preparation, as well as to reduce the concentration of oil and salt using available methods.

Microorganisms adapted to the climatic conditions of their habitat regions are capable of efficiently degrading oil even under seasonal temperature differences, low soil moisture, and soil salinity [52]. The specific climatic conditions of the regions under consideration for a long time have been a breeding factor contributing to the survival of indigenous microorganisms with certain properties: the ability to withstand high (average up to 50°C) temperatures, salinity up to 7% and lack of moisture in the soil. It is the bacteria with such properties that are capable of efficiently utilizing oil in territories with a hot arid climate. Long-term studies conducted in various geographical regions show that microorganisms belonging to different taxonomic groups and significantly differing in physiological and metabolic characteristics are capable of degrading hydrocarbon pollutants.

The taxonomic diversity of thermotolerant bacteria allows them to be used in remediation technologies not only as monocultures, but also to form microbial associations in order to accelerate and increase the overall efficiency of the degradation process.

The enzymes used by thermotolerant bacteria for the destruction of hydrocarbons operate at higher temperatures than the enzymes of common mesophilic destructors, which makes thermotolerant strains promising remediation agents for oil-contaminated soils and water at high ambient temperatures.

In the framework of the project “Thermotolerant microorganisms for bioremediation of oil-contaminated soils of Western Kazakhstan” (state registration number 0118PK00176), as a result of the screening of collection and newly isolated cultures of oil-oxidizing microorganisms, we selected 38 cultures that showed good growth on oil at 35°C, 16 cultures - at 40°C and 19 cultures - at 50°C. Further studies showed that the destruction of oil by the studied cultures was 23.7–70.5% [53]. The most active strains of oil-oxidizing microorganisms were identified by Sanger sequencing and assigned to the genera *Pseudomonas*, *Bacillus*, *Achromobacter*, *Rhodococcus*, *Brevibacillus*, *Gordonia* [54]. For further work, we will select the most active thermotolerant strains of microorganisms and compose consortia.

CONCLUSION

To solve the problem of oil pollution in conditions of elevated climatic temperatures, an effective approach has not yet been proposed. When developing microbial preparations, as well as the technology for cleaning soil and water from oil in hot arid climates, it should be taken into account that the climate of such regions is characterized by large daily temperature differences, salinity, and low soil moisture. Most of the known microorganisms-oil destructors are not adapted to such conditions, which leads to a decrease in the efficiency of the destruction of pollutant. For the destruction of oil in a hot climate, it is advisable to use thermotolerant microorganisms for which such conditions are not stressful. The study of the biology of such microorganisms is important, from the point of view of their use as a part of biological products for the bioremediation of oil-contaminated soils. The development of biological products based on consortia of thermotolerant bacteria and their use for cleaning soils and water at high temperatures can be a promising solution to the problem of oil pollution in Kazakhstan.

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МҰНАЙМЕН ЛАСТАНҒАН ТОПЫРАҚТЫ ҚАЛПЫНА КЕЛТІРУ ҮШІН ТЕРМОТӨЗІМДІ МИКРОАҒЗАЛАРДЫ ҚОЛДАНУДЫҢ МҰМКІНДІКТЕРІ

**Айткельдиева С.А., Файзулина Э.Р., Татаркина Л.Г., Алимбетова А.В.,
Ауэзова О.Н., Спанкулова Г.А.**

*Микробиология және вирусология ғылыми-өндірістік орталығы,
Бөгенбай батыр көш., 105 Алматы қ., 050010, Қазақстан
sa.kz@list.ru, ecomicrolab@gmail.com*

ТҮЙІН

Әлемдегі мұнай кен орындарының көпшілігі жоғары температуралық режимі бар географиялық аймақтарда шоғырланған. Ыстық климат жағдайында аумақтарды қалпына келтірудің басты мәселерінің бірі, жоғары температура мұнайдың тұтқырлығын төмендетеді және оның жерге терең диффузиясын тездетеді. Бұл әдеттегі әдістермен топырақ жамылғысын қалпына келтіру процестерін едәуір қиындатады. Балама ретінде жоғары

температураға және төмен ылғалдылыққа төзімді мұнай тотықтырғыш микроағзаларды қолдана отырып биоремедиация бола алады. Бұл әр түрлі елдердің ғалымдарының термотолерантты микроағзаларды мұнай мен мұнай өнімдерінен ластанған топырақты қалпына келтіру кезінде қолдану мақсатында жүргізген зерттеулерін талдауға арналған шолу. Температура, оттегі, рН, тұздылық, аэрация, қоректік заттардың болуы және т.б. сияқты қоршаған ортаның негізгі экологиялық факторларының әсері, термотөзімді көмірсутек тотықтырғыш микроағзаларының өміршеңдігі мен тіршілік белсенділігі туралы мәліметтер келтірілген. Термотөзімді микроағзалар үшін биохимиялық және молекулярлық-генетикалық сипаттамалардың мысалдары келтірілген. Термотөзімді көмірсутек тотықтырғыш микроағзалардың таксономиялық әртүрлілігі көрсетілген, сонымен қатар белсенді штамдармен мұнайдың жеке компоненттерінің ыдырауының мысалдары келтірілген. Қазақстанның негізгі кен орындарындағы мұнайдың физикалық-химиялық көрсеткіштері туралы мәліметтерді ұсынылған.

Негізгі сөздер: термотөзімді микроағзалар, биоремедиация, мұнай, мұнай ыдыратқыштар, температура

ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ ТЕРМОТОЛЕРАНТНЫХ МИКРООРГАНИЗМОВ ДЛЯ РЕМЕДИАЦИИ НЕФТЕЗАГРЯЗНЕННЫХ ПОЧВ

Айткельдиева С.А., Файзулина Э.Р., Татаркина Л.Г., Алимбетова А.В.,
Ауэзова О.Н., Спанкулова Г.А.

*Научно-производственный центр микробиологии и вирусологии
ул. Богенбай батыра, 105, г. Алматы, 050010, Казахстан
sa.kz@list.ru, ecomicrolab@gmail.com*

АБСТРАКТ

Большинство нефтяных месторождений в мире сконцентрировано в географических зонах с повышенным температурным режимом. Одной из главных проблем ремедиации территорий в условиях жаркого климата является тот факт, что высокие температуры снижают вязкость нефти и, таким образом, ускоряют ее диффузию вглубь грунта. Это значительно усложняет процессы восстановления почвенных покровов обычными методами. Альтернативой может служить биоремедиация с помощью нефтеокисляющих микроорганизмов, устойчивых к высоким температурам и пониженной влажности. Представленный обзор посвящен анализу исследований термотолерантных микроорганизмов учеными разных стран с целью их использования при ремедиации загрязненных почв от нефти и нефтепродуктов. Приведены данные по влиянию основных экологических факторов окружающей среды, таких как: температура, кислород, рН, соленость, аэрация, наличие питательных веществ и др., на жизнеспособность и жизнедеятельность термотолерантных углеводородокисляющих микроорганизмов. Приведены примеры характерных для термотолерантных микроорганизмов биохимических и молекулярно-генетических признаков. Показано таксономическое

разнообразие термотолерантных углеводородокисляющих микроорганизмов, а также приведены примеры деградации отдельных компонентов нефти активными штаммами. Представлены данные по физико-химическим показателям нефти основных месторождений Казахстана.

Ключевые слова: термотолерантные микроорганизмы, биоремедиация, нефть, нефтеструкторы, температура