ACTIVE CONSORTIA OF MICROORGANISMS FOR OIL DEGRADATION IN A WIDE RANGE OF POSITIVE TEMPERATURES

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Abstract

Soil pollution with petroleum hydrocarbons has become a global problem and was a consequence of the intensification of human industrial activity. Remediation of contaminated sites using a microbiological process (bioremediation) has proven to be effective and reliable due to its environmental performance. For bioremediation of oil-contaminated ecosystems, both individual strains of microorganisms and their associations are used. It was noted that the use of consortia of hydrocarbon-oxidizing microorganisms leads to a more complete destruction of hydrocarbons. One of the main factors for the oxidation of oil hydrocarbons is the temperature regime. In this study, the selection of consortia of oil-oxidizing microorganisms capable of effectively degrading oil in a wide range of positive temperatures was carried out. Experiments were carried out on a mineral medium with oil at 10℃, 30℃ and 50℃. It is shown that the most active transformation of oil occurred at 30℃. 3 consortia were selected that utilized oil from the Zhanatalap and Dossor fields at all temperatures under study. The use of such consortia will contribute to the effective cleanup of contaminated areas throughout the growing season. This is very important in an arid climate, which is characterized by sharp seasonal and daily temperature fluctuations.

Keywords: *oil pollution, bioremediation, consortia of oil-oxidizing microorganisms, oil biodestruction, temperature*

1. INTRODUCTION

Environmental pollution by hydrocarbons of natural and anthropogenic origin is a serious environmental problem. These compounds, which have different chemical nature, enter the environment as a result of accidental spills of oil and oil products, during the combustion of various types of fuel, emissions from coke, gas and petrochemical industries, as well as with vehicle exhaust gases. This problem is especially acute for countries producing, transporting and processing oil [1].

Oil is a complex mixture of various hydrocarbons, including aliphatic (linear or branched), cycloalkanes, mono- and polyaromatic, asphaltenes, and resins, and most of these compounds are stable, toxic, and carcinogenic [2, 3].

Recently, various strategies have been intensively used to protect the environment by preventing the spread of toxic pollutants in it. For example, physicochemical methods such as electrochemical treatment, excavation, ion exchange, precipitation, reverse osmosis, evaporation, and sorption have been developed to remove toxic substances [4, 5, 6]. However, many of these methods cannot be widely used due to significant drawbacks, such as high cost and damage to the environment as a result of secondary pollution [7].

Currently, much attention is paid to the development of environmentally friendly technologies for the rehabilitation of natural environments contaminated with crude oil and oil products. Microbial bioremediation is attracting much attention as a promising technology that can overcome the shortcomings of the physical and chemical methods used. It is a multifunctional technology with high stability, cost-effectiveness, environmental friendliness, no interference with the ecology of the ecosystem, and wider public acceptance. Bioremediation has been approved by the US Environmental Protection Agency (USEPA) as an effective environmentally friendly method of restoring a polluted environment and promoting sustainable development [8, 9].

Bioremediation is the use of microorganisms to remove various pollutants from the environment. It often occurs naturally, without direct intervention, however, this process can be very lengthy. In such cases, for the complete removal of contaminants in a short time, biostimulation (adding nutrients or adjusting conditions) and bioaugmentation (introducing microorganisms capable of bioremediation) are applicable [10]. Bioremediation technology uses microorganisms to decompose toxic pollutants into harmless products such as CO_2, H_2O, and other inorganic compounds [2].

The effectiveness of the bioremediation strategy for ecosystems polluted with oil depends on biotic and abiotic factors such as temperature, pH, oxygen, and salinity [11]. Thus, J. Aislabie et al. [12] reported that with an increase in temperature, the solubility of hydrophobic pollutants increases, viscosity decreases, and diffusion and transfer of long-chain n-alkanes from the solid phase improves. At low temperatures, the viscosity of oil increases, the volatilization of toxic short chain alkanes decreases, and their solubility in water decreases, which delays the onset of biodegradation [13].

For oil-oxidizing microorganisms, the temperature regime is one of the main factors for the oxidation of oil hydrocarbons. Microorganisms adapted to the climatic conditions of their habitats are capable of efficient oil degradation even under conditions of seasonal temperature fluctuations, low soil moisture and soil salinity [14].

The aim of this study is to create consortia of oil-oxidizing microorganisms capable of effectively degrading oil at different temperatures.

2. MATERIALS AND METHODS

2.1 Materials

Nutrient media. Mineral medium of the following composition, g/l: NH_4NO_3 - 1.0, K_2HPO_4 - 1.0, KH_2PO_4 - 1.0, $MgSO_4$ - 0.2, $CaCl_2 \times 6H_2O$ - 0.02, $FeCl_3$ - traces, NaCl - 10.0 , pH=7.0-7.2.

Oil. Oil was provided from the Zhanatalap and Dossor fields in the Atyrau region of the Republic of Kazakhstan.

Zhanatalap oil: density - 0.87 g/cm^3, content of paraffins - 1.51%, sulfur - 0.12%, asphaltenes - 0.02%, resins - 6%.

Dossor oil: density - 0.88 g/cm^3, paraffin content - 2.07%, sulfur - 0.22%, resins - 7%.

2.2 Methods

The antagonistic activity of strains of oil-oxidizing microorganisms was determined by the method of perpendicular strokes [14].

To create a consortium, strains were first cultured individually in nutrient broth at 30°C for 24 h. Cells were harvested by centrifugation (10,000 g for 10 min) and resuspended in sterile 0.9% NaCl. Then, the strains were mixed in a ratio of 1:1:1. 5 ml of suspension were added in flasks with 100 ml of mineral medium. Oil was added in an amount of 5% v/v and 10% v/v. The consortia were cultured at 10°C, 30°C and 50°C in thermostatically controlled shakers at 180 rpm for 14 days.

Residual oil in the medium was determined by the gravimetric method. Oil extraction was carried out with chloroform.

All experiments were performed in triplicate. Statistical processing of the research results was carried out according to the generally accepted criteria of variance-statistical analysis with the calculation of average values (M), arithmetic mean error (m) using the Microsoft Excel, 2010 software package.

3. RESULTS AND DISCUSSION

3.1. Antagonistic activity of oil-oxidizing strains

Since oil is a complex substrate, it is promising to use consortia of microorganisms consisting of several active strains for the most effective purification of contaminated soil. There are currently no clear criteria for compiling microbial consortia. When developing such consortia, most researchers rely on the compatibility of strains and their catabolic activity, and the main parameter on the basis of which an association is formed is the degree of total oil destruction [16]. We previously isolated and selected active strains of oil-oxidizing microorganisms that utilize oil at different temperatures. The presence or absence of antagonism between them was studied by the method of perpendicular strokes. The results of the study showed that there were no zones of growth inhibition and lysis (Fig. 1). Thus, all selected strains of hydrocarbon-oxidizing microorganisms were mutually tolerant to each other.

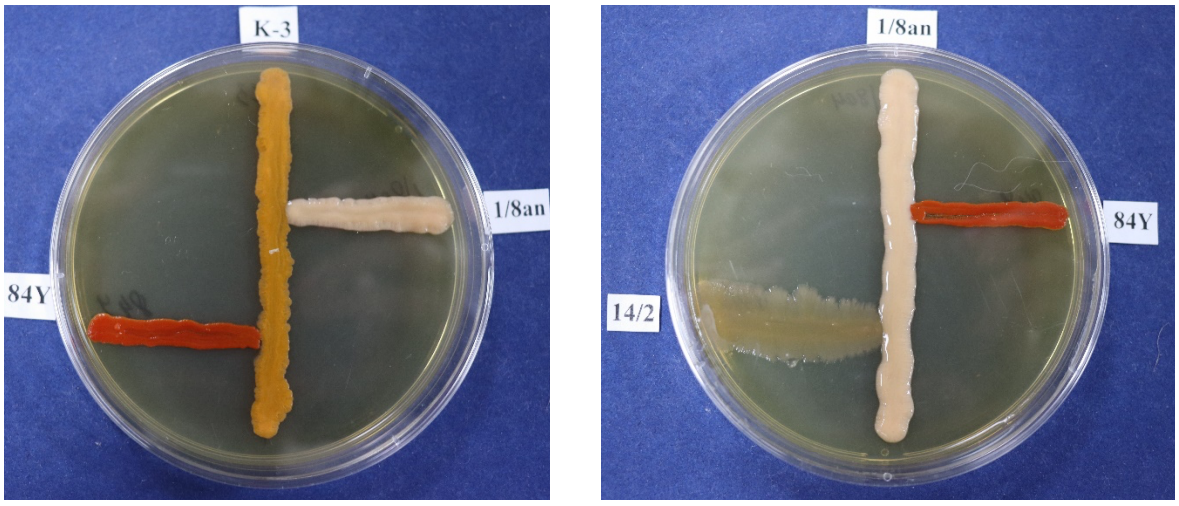

Fig. 1. Determination of antagonism between strains by the method of perpendicular strokes

3.2. Selection of effective consortia of oil-oxidizing strains

From active strains of oil-oxidizing microorganisms, 15 consortia were composed, consisting of representatives of the genera *Rhodococcus, Dietzia, Pseudomonas, Pusillimonas, Rhizobium*. Each consortium consisted of three strains. Their ability to degrade Zhanatalap oil (5% v/v) was studied. The consortia were cultivated in a liquid mineral medium at temperatures of 10°C, 30°C and 50°C. The results showed that the created consortia at 30°C utilized 53.2-73.6% of oil in 14 days (Table 1). At the same time, the natural loss of oil amounted to 16.7%. At 10°C, oil biodegradation was 23.6-64.4%, at 50°C - 27.2-72.3%.

Temperature is one of the factors that influence oil biodegradation by affecting its physical and chemical composition. At low temperatures, the degradation rate is generally observed to decrease, which is thought to be a result of reduced enzymatic activity rates [17]. The rate of hydrocarbon metabolism reaches the maximum level in temperatures ranging from 30 to 40°C [11].

All consortia showed high oxidation potential at 30°C. At 10°C, the destructive ability of most consortia decreased. Only four consortia (5, 6, 7, 10) remained active. These consortia included strain *Pusillimonas thiosulfatoxidans*. Members of the genus *Pusillimonas* are known to be resistant to low temperatures, and certain species can biodegrade persistent pollutants such as diesel fuel and nicotine [18, 19]. At 50°C, seven consortia (1, 6, 7, 10, 12, 14, 15) had the best ability to degrade oil. Consortium 5 showed high activity at 10°C and 30°C, consortia 1, 12, 14 and 15 - at 30°C and 50°C. Three consortia (6, 7, 10) effectively degraded oil at all three studied temperatures. They included the following strains: consortium 6 – *Rhodococcus sp.* K3, *Dietzia sp.* 84Y, *Pusillimonas thiosulfatoxidans* 1/8an, consortium

7 – *Dietzia sp.* 84Y, *Pusillimonas thiosulfatoxidans* 1/8an, *Pseudomonas sp.* 14/2, consortium 10 - *Pusillimonas thiosulfatoxidans* 1/8an, *Rhodococcus sp.* 14/3, *Rhizobium nepotum* PAH-K-5.

Table 1. Destruction of Zhanatalap oil by consortia of oil-oxidizing microorganisms at different temperatures

Consortium	Degree of oil destruction, %		
	10°C	30°C	50°C
1	33.1±4.3	73.4±2.7	72.3±4.9
2	37.4±1.7	73.6±1.1	37.3±3.2
3	23.6±2.8	61.9±5.3	31.9±1.8
4	30.1±0.8	65.4±5.9	37.2±0.1
5	62.2±4.2	60.6±2.6	28.1±1.7
6	53.2±0.2	53.2±2.2	51.1±2.5
7	64.4±2.5	60.8±2.0	45.2±4.4
8	30.7±1.5	64.8±2.8	30.7±1.3
9	40.2±2.1	70.8±4.9	29.6±1.4
10	59.6±1.5	57.5±2.4	53.7±5.0
11	33.6±3.5	66.7±4.6	27.2±3.6
12	25.3±0.7	58.5±3.1	64.8±3.2
13	27.3±1.5	63.4±4.1	38.6±0.0
14	27.3±0.9	69.4±2.7	64.3±5.5
15	30.6±2.0	61.5±1.4	67.3±0.1
Control	14.8±0.3	16.7±1.1	16.4±0.1

3.3. Growth of active consortia on Dossor oil

The ability of consortia 6, 7, 10 to utilize oil from the Dossor field at concentrations of 5% v/v and 10% v/v was studied. Figures 2-4 show the biodegradation of Dossor oil at different temperatures. Consortium 6 was the most active at 30°C. At 5% oil content in the medium, destruction was 56.7%, at 10% - 53.5%. Consortia 7 and 10 utilized 5% of oil at the same level - 51.9-53.2%. Consortium 10 showed a greater degree of destruction on a medium with 10% oil.

At 10°C, the oil-oxidizing activity of consortium 6 increased slightly. The degree of oil destruction was 60.1% and 56.4% at 5% and 10% content, respectively. The activity of consortia 7 and 10 remained almost at the same level as at 30°C.

As the temperature increased to 50°C, the rate of oil biodegradation decreased. For 14 days, 41.4-52.1% and 30.7-44.6% of oil were utilized, respectively, at an initial concentration of 5% and 10%. Consortium 6 showed the highest activity, consortium 7 - the least.

Natural oil losses at 5% content amounted to 14.5-16.0%, at 10% - 12.3-13.5%.

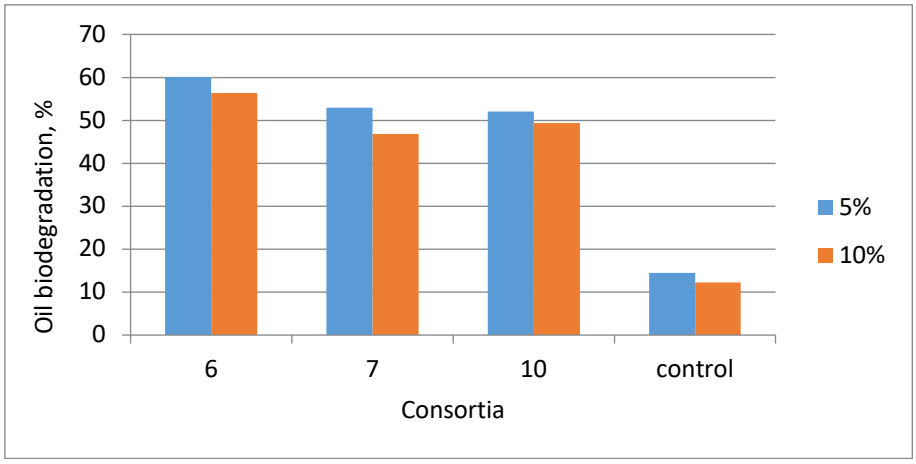

Fig. 2. Destruction of Dossor oil by consortia of oil-oxidizing microorganisms at 10°C after 14 days

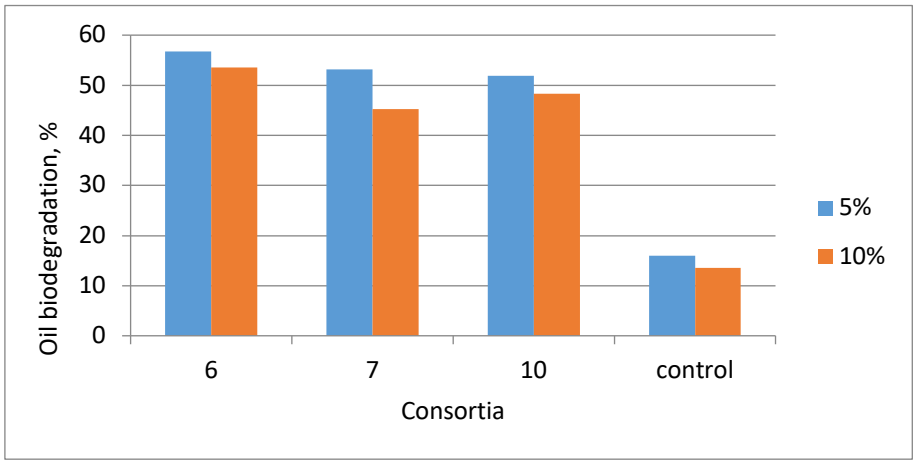

Fig. 3. Destruction of Dossor oil by consortia of oil-oxidizing microorganisms at 30°C after 14 days

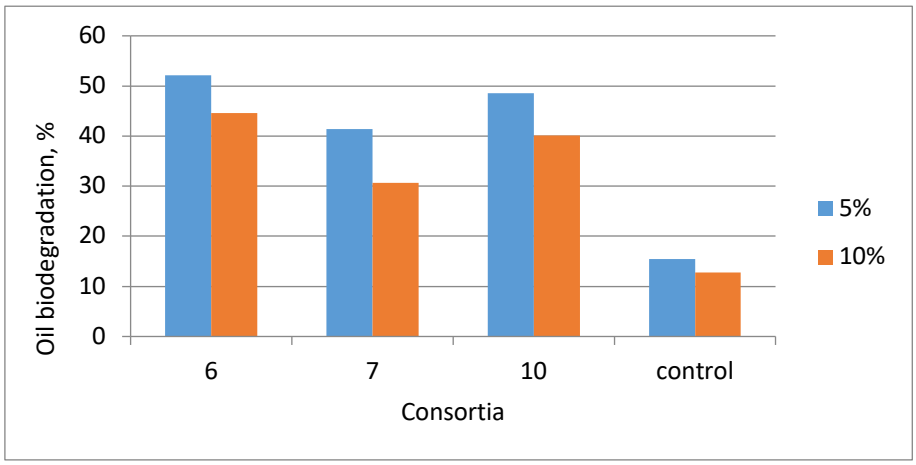

Fig. 4. Destruction of Dossor oil by consortia of oil-oxidizing microorganisms at 50°C after 14 days

4. CONCLUSIONS

15 consortia were formed from the previously isolated and selected active strains of oil-oxidizing microorganisms. The biodegradation of Zhanatalap oil under their influence at different temperatures (10°C, 30°C, 50°C) was studied. All studied consortia showed high degradation potential at 30°C. At low and high temperatures, the activity of most consortia decreased. Seven consortia effectively degraded oil only at 30°C, one consortium - at 10°C and 30°C, four consortia - at 30°C and 50°C. Three consortia showed activity at all temperatures.

Consortia growing on Zhanatalap oil at different temperatures also utilized Dossor oil at different concentrations in the medium (5% v/v and 10% v/v). However, their activity was higher at 10°C and 30°C.

Consortium 6 (*Rhodococcus sp.* K3, *Dietzia sp.* 84U, *Pusillimonas thiosulfatoxidans* 1/8an) showed the highest activity at all temperatures studied. It was noted that this consortium degraded Dossor oil more efficiently than Zhanatalap oil. At the same time the oil-oxidizing activity of the consortia 7 and 10 decreased with growth on Dossor oil.

Thus, consortia of oil-oxidizing microorganisms capable of degrading oil in a wide range of positive temperatures were selected. The use of such consortia will contribute to the effective cleanup of contaminated areas throughout the growing season. This is very important in an arid climate, which is characterized by sharp seasonal and daily temperature fluctuations.

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