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Comparative analysis of the effectiveness of oil-contaminated soil remediation by microbial isolates of Pseudomonas aeruginosa and commercial preparation "Devoroil"

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ABSTRACT

We have conducted a comparative analysis of the destructive activity of the strains of bacteria isolated from the gray forest soil contaminated by stock tank oil produced in the territory of the Republic of Tatarstan, Russia, and the commercial preparation "Devoroil". We have isolated hydrocarbon destructor strains, identified as Pseudomonas aeruginosa, from the gray forest soil contaminated by oil (114 g/kg) produced at Bastryk oil treatment plant (Republic of Tatarstan, Russia). Treatment of oil-contaminated soil with isolated strains allowed reducing the oil content by 62-63% for 3.5 months of remediation under laboratory conditions. Introduction of the commercial preparation "Devoroil" resulted in reducing petroleum products content by 48%. Higher efficiency of the strains isolated compared with that of commercial preparation "Devoroil" allows us to recommend these strains as biological products for soil reclamation in this region.

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INTRODUCTION

Oil contamination of the soil is one of modern critical environmental issues. The normal functioning of ecosystems requires oil-contaminated soils to be remediated, one of the methods to perform this is bioaugmentation - treatment of soil with hydrocarbons destructing microorganisms. Currently, there are a number of commercial preparations developed, intended for cleaning soils from oil [1, 2]. Recent studies, however, show that it is impossible to create a universal biological product that would work effectively in different climatic zones with different soil types [3]. The efficiency of preparations depends strongly on the composition of the oil having caused pollution, as well as climate, soil type, etc. [4, 5]. Therefore, it is reasonable to use representatives of the indigenous microflora allocated in each case directly from the soil subjected to oil pollution for the remediation of oil pollution of soils [6, 7].

The objective of this research is a comparative analysis of the destructive activity of the strains of bacteria isolated from the gray forest soil contaminated by stock tank oil produced in the territory of the Republic of Tatarstan, Russia, and the commercial preparation “Devoroil” under laboratory conditions.

MATERIALS AND METHODS

The subject of the research were strains of hydrocarbon-oxidizing microorganisms isolated from the gray forest soil contaminated by stock tank oil produced in the territory of the Republic of Tatarstan of the Russian Federation (Bastryk oil treatment plant, Prikamneft oil-and-gas production department of JSC "Tatneft"). Oil characteristic: density - 0.885 g/cm^3, sulfur content - 2.3%, paraffin - 3.3%, water - 10%, mechanical impurities - 0.07%.

Isolation of hydrocarbon oxidizing microorganisms from the oil-contaminated soil was performed on a liquid salt medium of the following composition: (NH4) 2SO4 (1.0 g/l), MgSO4 (0.2 g/l), KH2PO4 (3.0 g/l), Na2HPO4 (4.5 g/l), the investigated stock tank oil in an amount of 2% of total volume was used as a sole carbon source. Cultivation of isolated oil oxidizing strains was performed on a culture medium of the following

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composition: peptone (10 g/l), NaCl (10 g/l), yeast extract (5 g/l) at 25°C and stirring. The number of microorganisms, expressed in CFU/ml, was determined by the limiting dilution method [8].

Commercial preparation "Devoroil" was provided by Prikamneft oil-and-gas production department of JSC "Tatneft".

Residual oil content in the soil samples investigated was measured by IR spectroscopy [9]. Petroleum products were extracted from the soil samples by carbon tetrachloride in three steps (per 10 ml), the total extract was filtered and measured on the instrument AN-2. Measurements were performed at least in three replications. Accuracy of the differences resulted was evaluated using the Student coefficient ($P < 0.05$).

Identification of the strains isolated was performed by PCR amplification of a fragment of 16S rRNA gene using universal prokaryotic primers [10] by standard technique [11]. Primers were synthesized by Syntol company (Russia, Moscow) 16S-8F: 5'-agagtttgatcctggctcag-3', 16S-1492R: 5'–ggttaccttgttacgactt-3'. PCR was performed on a MJ Mini Personal Thermal Cycler unit (Bio-RAD, Singapore) in 35 cycles of the following regimen: preheating 95°C for 2 minutes, denaturation 94°C for 30 sec, annealing 55°C for 30 sec, synthesis 72°C 2 min, the final synthesis 72°C for 7 min.

Electrophoresis of DNA was performed in the horizontally in agarose gel, wherein the gel composition was as follows: 1X TAE (pH 8.1), agarose, ethidium bromide. Separation of DNA was performed at 100 V for gels of 150 ml, respectively, after completing electrophoresis the gel was viewed under UV light on a UV Transilluminator TFP-M \ WL (Vilber lourmat, France) and photographed with Electrophoresis results registration system Gel Imager GI-2 (Helicon, Japan). To analyze the size of the resulting DNA fragments a DNA marker GeneRullerTM DNA Lader Mix (Sigma, USA) was used. DNA sequencing was carried out using a universal prokaryotic primer 16S-8F and 16S-1492R. Sequencing of 5'-end of the fragment was accomplished based on a single sequencing reaction with the primer 16S-8F.

Identification of microorganisms was performed on the basis of analysis of the primary nucleotide sequence of 16S rRNA gene in the GenBank/EMBL/DDBJ database using multiple alignment in BLASTn program [12].

RESULTS AND DISCUSSIONS

At the first stage, we isolated microbial consortium from the contaminated soil (oil content of 114 g/kg) using a liquid salt medium and oil as the sole carbon source. When separating the individual strains of the consortium on a solid culture medium (SCM) 12 isolates of microorganisms were isolated.

Each of the strains of microorganisms was tested for the ability to oxidize oil in the liquid medium. Oil oxidizing ability of the culture was assessed by the emulsification of oil and the disappearance of the oil slick. A sterile medium with the addition of oil served as a control. It has been established that the emulsion of oil and oil film degradation was observed on day 10 in the presence of the four strains (strains 4.1, 4.2, 5.1, 5.2). These strains were used in the further experiments.

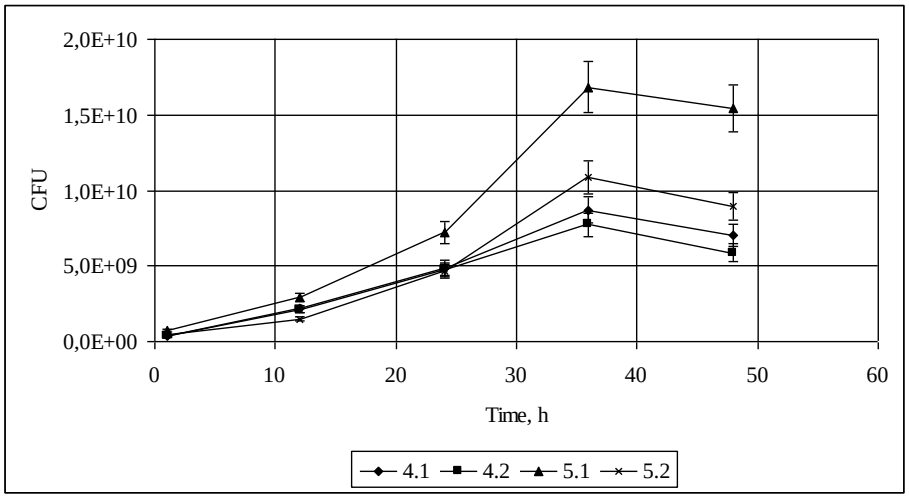

Fig. 1: Number of microorganisms of the enrichment culture, CFU.

At the next stage, we compared the effectiveness of the strains with the same of the commercial preparation. Since it is known that the maximum metabolic activity is appropriate to microorganisms being in the late exponential and early stationary phase of growth, at the next stage we obtained growth characteristics of strains and defined the number of cells in a growth culture. The results obtained are shown in Fig. 1. As can be seen from data obtained, the greatest number of microorganisms in culture were fixed 36 hours after starting the

incubation. Cultures of this age were used in the further experiment. The number of cells was used to calculate the volume of culture of microorganisms used for the soil treatment.

In order to compare the efficiency of oil recovery by microbial isolates with the commercial preparation, we added culture of isolated microorganisms to the contaminated soil in an amount equivalent to the number of cells per unit of working solution of preparation "Devoroil" prepared according to instructions [13]. The amount of working solution, added per 10 kg of soil, is shown in Table 1.

Table 1: Characteristics of the commercial preparation "Devoroil" and isolated strains of microorganisms, used for soil remediation.

Characteristic	Preparation				
	D	4.1	4.2	5.1	5.2
Number of microorganisms in the preparation, CFU/ml	$5.36*10^8$	$8.70*10^9$	$7.74*10^9$	$1.68*10^{10}$	$1.09*10^{10}$
The volume of culture working solution, ml/10 kg of soil	100	6.2	6.9	3.2	4.9

Figure 2 shows the results of changes in petroleum product content in soil samples contaminated with oil, under the influence of microorganisms isolated and commercial preparation. Investigations were carried out in the model samples, artificially contaminated with oil in an amount of 100 g/kg. As can be seen from the data presented, the maximum reduction in oil content was noted in the first 3 days, which is traditional and is associated with the evaporation of volatile hydrocarbon fraction. Further changes in the content of petroleum products in the soil had undulating nature, which is associated with the activity of hydrocarbon-oxidizing microorganisms [14, 15]. As a result of biodegradation of high molecular weight hydrocarbons there is a release of new portions of oligomers [16], which explains the fluctuations in the fixed oil content of soil samples. On the 24th day of the experiment there was a decrease in oil content up to 26.5-36.1 g/kg, the lowest value was obtained for the variant 4.2, further an increase in oil content up to 39.3- 42.7 g/kg was observed in all experimental variants from day 30 through 45. A similar change in the content of petroleum products in contaminated soil subjected to bioaugmentation was observed by Karamalidis *et al*. [16]. By the end of the experiment the variants using isolated strains of microorganism (4.1, 4.2), showed the highest efficiency, 62 and 63%, respectively. Petroleum products content in soils treated with the commercial preparation "Devoroil" reduced by 48%.

Thus, the isolates showed greater oil degradation capacity compared with the commercial preparation "Devoroil". In addition, it should be noted that the use of indigenous strains seems more appropriate from an economic point of view.

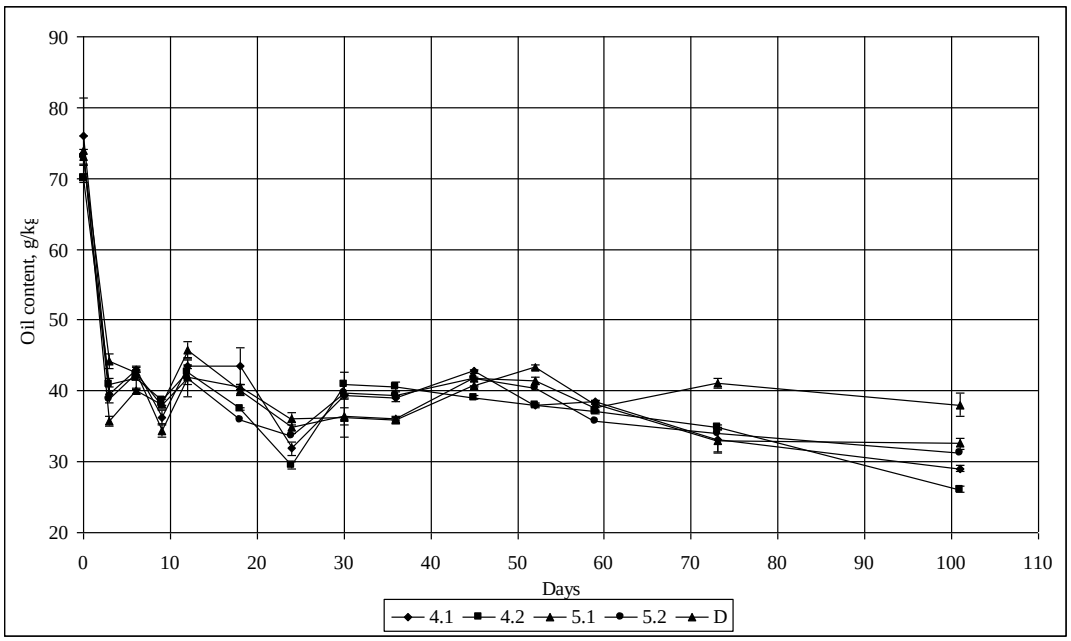

Fig. 2: Petroleum product content in the soil of different remediation variants, g/kg.

At the final stage we identified strains which have shown the highest oil oxidation capacity. For this purpose, genomic DNA samples were isolated and PCR-amplified from two microbial isolates (4.1 and 4.2). Amplification showed the presence of the 16S rRNA gene in both samples. It was established that the length of the amplified nucleotide sequences of 16S rRNA gene fragment is approximately 1500 bp (Fig. 3).

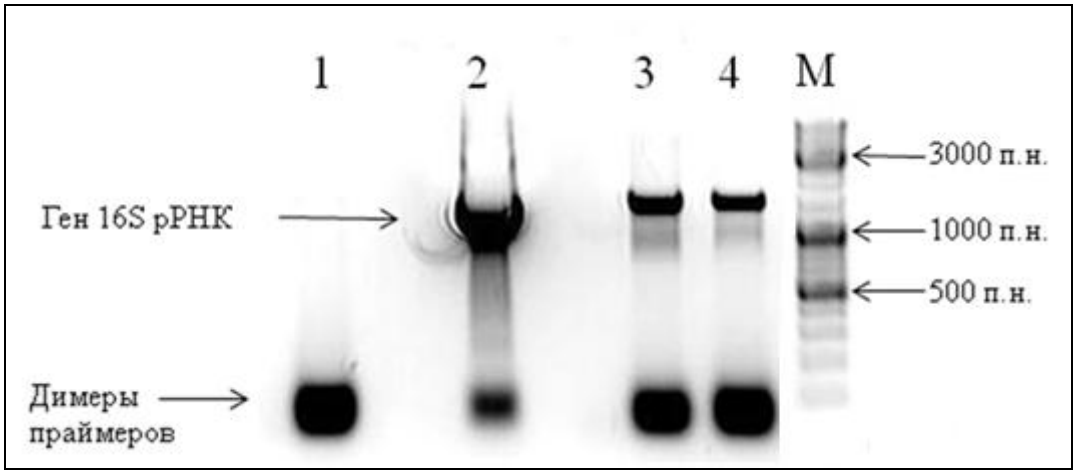

Fig. 3: Analysis of the PCR amplification of the 16S rRNA gene fragment. 1 - negative control, 2 - positive control (16S rRNA gene fragment integrated into pGEM T-easplasmid), 3-4 - samples of microbial isolates, M - DNA marker GeneRuller^{TM} DNA Lader Mix (Sigma, USA).

The nucleotide sequence of the 16S rRNA gene fragment from the 5'-end of the sample 4-1 can be identified as the nucleotide sequence of *Pseudomonas aeruginosa* PAO1 strain (16S rRNA gene, AE004091.2) based on 99% similarity (max identity - 99%, the area of query overlap - 99%, E value - 1e-162).The nucleotide sequence of the 16S rRNA gene fragment from the 5'-end of the sample 4-2 can be identified as the nucleotide sequence of *Pseudomonas aeruginosa* PAO1 strain (16S rRNA gene, AE004091.2) based on 98% similarity (max identity - 98%, the area of query overlap - 100%, E value - 9e-175). Thus, both strains isolated were classified by species as *Ps. Aeuruginosa*.

The fact that strains of *Ps. aeuruginosa* have the ability to biodegrade hydrocarbons is consistent with data presented in the literature [3, 15]. The efficiency of petroleum products degradation is also comparable to the literature data [3, 17].

Summary:

The strains in the amount of 114 g/kg, identified as *Pseudomonas aeruginosa*, were isolated from the gray forest soil contaminated by oil produced at Bastryk oil treatment plant (Republic of Tatarstan, Russia). Treatment of soil with the said strains allowed reducing the oil content by 62-63% for 3.5 months of remediation under laboratory conditions. Introduction of the commercial preparation "Devoroil" resulted in reducing petroleum products content by 48%. The results obtained indicate higher efficiency of the strains, isolated when remediating the oil-contaminated grey forest soil, compared with that of commercial preparation “Devoroil”, which allows us to recommend these strains as biological products for soil reclamation in this region.

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