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Bioremediation of Oil-Polluted Soil of Western Kazakhstan

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***Abstract*—**15 strains of oil-destructing microorganisms were isolated from oil polluted soil of Western Kazakhstan. Strains 2-A and 41-3 with the highest oil-destructing activities were chosen from them. It was shown that these strains oxidized n-alkanes very well, but isoalkanes, isoparaffin, cycloparaffin and heavy aromatic compounds were destructed very slowly. These both strains were tested as preparations for bioremediation of oil-polluted soil in model and field experiments. The degree of utilizing of soil oil by this preparation was 79-84 % in field experiments.

***Keywords*—**Bioremediation, n-alkanes, oil-polluted soil, oil-oxidizing microorganisms.

I. Introduction

ACCORDING to many scientists, ecosystem in the Western Kazakhstan is characterized as precritical. In case of not taking complex of protective measures the region would threaten with ecological accident with heavy consequences not only for the given area, but also on a global scale [1]. Escalating of an oil recovery and gas, high aggression of taken raw material result in processes of intensive pollution of an atmosphere, superficial and subsoil waters, and pollution of soil and vegetative cover by heavy metals, radionucleids and mineral oil [2].

Nowadays petroleum polluted soil is widely bioremediated by using preparations containing oil-destructor microorganisms. Such works are intensively carried out in Russia [3], [4]. Investigations are directed on intensification of biodestruction process of oil components.

The main task of our work is investigation of oil destruction processes by native microorganisms which were isolated from soil of the Western Kazakhstan polluted with petroleum and mineral oil.

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II. Materials and Methods

To study activity of microorganisms researched strains of microorganisms were sowed in three repeats on mineral media of Voroshilova-Dianova containing 1 % of oil. The control variants did not contain microorganisms. Analyses were carried out on the first day (the beginning of experiment) of cultivation and on the twelfth day (the end of experiment).

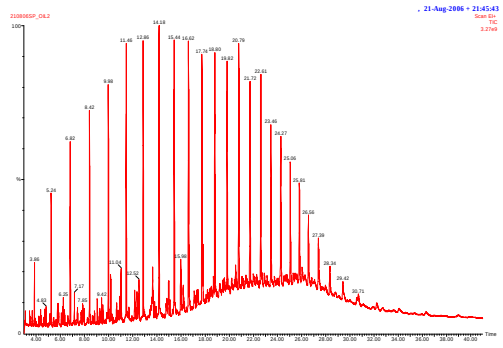
Determination of petroleum hydrocarbons in cultural liquids were carried out by method of extraction of mineral oil from analyzed tests by hexane in dropping funnel. The subsequent division of an extract on aliphatic and aromatic fractions using method of column chromatography on silicagel was conducted. Qualitative and quantitative analysis of petroleum carbon substances was carried out on gas chromatography HP-5890 (Hewlett-Packard, USA) [5] and gravimetric methods [6].

III. Results and Discussions

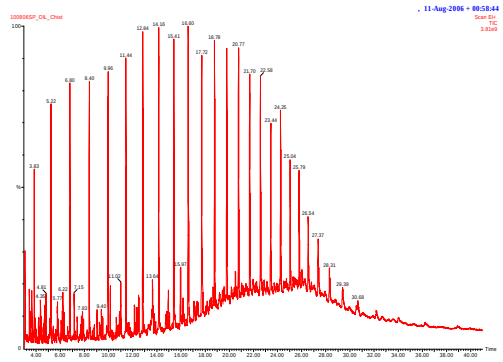
Most of modern investigations are concentrated on the search of active oil-oxidizing microorganisms and its associations [7]. In this way our work was carried out. Fifteen strains of oil-oxidizing microorganisms were isolated from oil-polluted soil of the Western Kazakhstan. Their ability to utilize petroleum was investigated. Results of research have shown, that the most active appeared cultures are *Acinetobacter calcoaceticus* 2-A and *Microbacterium lacticum* 41-3 which were able to destruct 70,92% and 76,07% of oil respectively. In control variant oil was destructed very slowly, in this case destruction within 12 day was equal to 4,95 %.

Also destruction products during cultivation of the most active strains of oil-oxidizing microorganisms were analyzed by gas chromatography with mass selective detector (HP-5890).

Gas chromatographies of oil in control variant at the beginning and at the end of experiment are shown in Fig. 1.



(a)

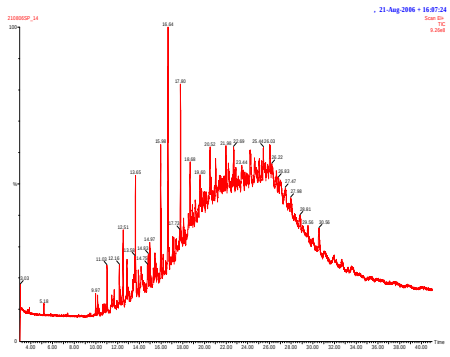


(b)

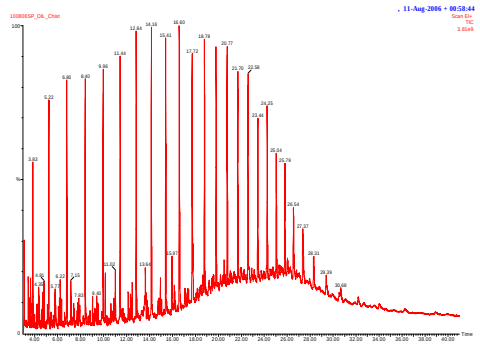
Fig. 1 Gas chromatographies of oil sample (a) and the same sample in control variant (b) after 12 days

The data of figure show that oil sample and the same sample in control variant after 12 days were similar in gas chromatography pattern. In other words, the compositions of carbohydrates were identical. So n-alkanes have some maximas. There were n-alkanes, isoalkanes and cycloalkanes in the composition of oil samples.

Destruction of individual hydrocarbons of petroleum (n-alkanes) by strain 2-A and 41-3 in cultivation process is presented at Fig. 2.



(a)



(b)

Fig. 2 Gas chromatography of carbohydrates after 12 days of cultivation of strains 2-A (a) and 41-3 (b)

As it is shown on Fig. 2, during cultivation of 2-A strain the n-alkanes practically disappeared except carbohydrates C_{17}, C_{19} and peaks of isoalkanes did not change. In the same time substances isoparaffin, cycloparaffin and heavy aromatic fractions were destructed very slowly, in this reason the process of oil destruction slowed down to the end of cultivation.

During cultivation of strain 41-3 degradation of n-alkanes is observed. Whereas isoalkanes almost did not change.

Strain 2-A destructed 77,6-99,3% of individual n-alkanes as it shown on Table I. Decane and Dodecane are totally destructed, whereas Hexatriacontane is destructed worse.

TABLE I
DESTRUCTION OF INDIVIDUAL CARBOHYDRATES OF OIL DURING CULTIVATION BY STRAINS

Carbohydrate	Percent of destruction, %	
	2-A	41-3
Decane	99.3	99.5
Dodecane	96.8	98.2
Tetradecane	91.3	85.8
Hexadecane	94.0	78.7
Octadecane	84.4	75.3
Nonadecane	86.6	85.4
Eicosane	95.3	81.7
Docosane	91.8	79.8
Tetracosane	85.9	75.2
Hexacosane	90.4	73.2
Octacosane	89.0	70.9
Triacontane	95.1	64.5
Hexatriacontane	77.6	0.0

Strain 41-3 also practically destructed Decane and Dodecane. N-alkanes were destructed on 64,5-85,8%, except Hexatriacontane which was not utilized.

Modeling experiments on remediation of oil-contaminated soil by studied strains of bacteria were carried out. The initial content of oil in soil was 35026 mg/kg of soil. After two months of bioremediation of soil by chosen strains loss of oil was 45,4 % and 60,4 % (Table II). Whereas in the control variant 1 it was 4,1 %, in the control 2 (with zeolite) - 13,8 %. The greatest activity was shown by strain 41-3.

TABLE II
CONTENTS OF OIL IN SOIL AFTER TREATMENT BY ACTIVE CULTURES OF OIL-OXIDIZING MICROORGANISMS

Culture	Amount of oil, mg/kg of soil		Percent of oil utilization, %
	At the beginning	After 2 months	
2-A	35026	19145,0	45,4
41-3	35026	13890,0	60,4
Control 1 (polluted soil)	35026	33587,5	4,1
Control 2 (polluted soil with zeolite)	35026	30210,0	13,8

Then field experiments with oil-oxidizing bacteria were carried out on Balgimbayev oil deposit. We investigated polluted brown solonetzic soil. A sowing material was introduced to the soil in ratio 108 cells/gr of soil. Soil was moistured and loosened time to time. The control variant was without introduction of microorganisms. Results of research have shown, that content of oil decreased on in 84,13% after treatment by strain 2-A and on 78,68% when strain 41-3 was introduced (Table III).

TABLE III
PERCENT OF OIL DESTRUCTION IN OIL-POLLUTED SOIL BY STRAIN 2A AND 41-3

Strain	Content of oil in soil, mg/kg		Percent of oil destruction, %
	At the beginning	120 days	
2-A	32675	5186	84,13
41-3	32675	6966	78,68
Control	32675	29244	10,5

Thus, we isolated very active bacterial strains 2-A and 41-3 which have high oil-oxidizing activity, practically totally destroy n-alkanes and can be used for remediation of oil-polluted soil.

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