ISSN 2349-0292
Impact Factor 2.365

Global Journal of Advanced Engineering Technologies and Sciences

BIOREMEDIATION FEATURES OILCONTAMINATED LOAMY GRAY SOILS OF SOUTHERN KAZAKHSTAN

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Abstract

It found that the spread of microorganisms in the soil horizons depends on different types of oil-contaminated loamy grey soils and correlated with a molecular mass of oil products. For carrying out bioremediation was necessary allocation of new microbial cultures with high metabolic activity. In this regard, from a variety of oil-contaminated areas were isolated 255 microbial cultures. Because of the ability of isolates to active growth showed 3 strain - DP-304 B7, B1Ag8G, N 313-C14. The use of a biological product "Peroyl" created on the basis of strain Micrococcus luteus and Rhodococcus erythropolis B1Ag8G DP 304 B-7 enables up to 30 days to degrade petroleum hydrocarbons 44.4-97.2%, while the titer of soil microorganisms increased to 28.2-93.5%. Augmentation of active strains of microorganisms in oil contaminated soils promotes biodegradation bicycloaromatic, monocycloaromatic compounds, toluene resins and asphaltenes. The association of bioremediation operations beginning in early autumn contributes, on the one hand, the adaptation of microorganisms to low temperature followed by earlier timing of microbiological processes, on the other hand, reduces the water consumption.

Keywords: oil contaminated soils, microorganisms, bioremediation.

Introduction

The problem of environmental pollution by oil and oil products, at present, has become one of the large-scale environmental problems of oil-producing states. Oil pollution affects the whole range of morphological, physical, physico-chemical and biological soil properties that determine its fertility and ecological functions. Changing the properties of the soil oil pollution, as well as the processes of its migration, accumulation and metabolism depend on the physical and chemical composition and amount of oil spilled, the soil and climatic and landscape conditions, soil type, channel migration and diffusion in the soil profile [1-3] . It is known that the presence of oil and oil products in the soil affects the number of soil microorganisms. The most deficient aspect of negative impact of oil on the soil components is to suppress the activity of soil biota. Changing aeration conditions lead to a change in the ratio of aerobic and anaerobic microflora that causes an imbalance of enzyme activity soil [4]. In this complex physicochemical and biochemical environment begins the transformation of hydrocarbon compounds of oil. There are three most common stage of transformation of oil in the soil: 1- physico-chemical and microbiological decomposition of partially aliphatic hydrocarbons; 2 - microbial decomposition of low-molecular structures of different classes; 3 - the transformation of high-molecular compounds - resins, asphaltenes, acyclic hydrocarbons [5, 6].

Various methods for the rehabilitation of oil-contaminated ecosystems are known. One of the most promising ways to restore damaged ecosystems is bioremediation using different groups of microorganisms. Allocation of new microbial cultures with high metabolic activity, assessment of their redox abilities - one of the necessary measures for bioremediation. [7-14]. Bioremediation event traditionally includes the step of loosening the soil for the purpose of aeration, augmentation hydrocarbon oxidizing microorganisms or stimulation of spontaneous microflora of soil, making nutrients in the form of various compounds of nitrogen, phosphorus and potassium, followed by loosening the soil moisture and 1 times in 7-10 days [15-17]. However, in the arsenal of biological products developed largely on the basis of narrow groups of microorganisms and are confined to specific soil and climatic conditions. At the same time, a variety of physical and chemical characteristics of soil and oil, and especially climatic conditions dictate the need to develop new approaches to bioremediation of oil-contaminated ecosystems.

Materials And Methods

Soil.
In laboratory experiments, the soil selected in the industrial area of Limited Liability Partnership "PetroKazakhstan Oil Products" ("PKOP") and is a typical medium-loamy gray soils with different content of oil products. Selected oil-polluted soil and control of the basic parameters do not differ among themselves. The content of humus in these soils is 1.6-1.8%, total nitrogen N (Kjeldahl) 0.146% P_2O_5 rolling 38 mg / kg soil.

Soil samples are selected with the help of layers of soil knife, without gaps, the entire thickness of the soil profile. To each soil horizon it has been sufficiently characterized, selected samples of the upper and lower part. The mass of each sample should be about 0.5-0.7 kg. Selected soil samples were placed in plastic bags [18, 19].

Oil and oil products.
The object of our research were: oil, diesel fuel, fuel oil and sludge.

- Oil is as follows: pour point of 100°C, the content of silica gel resins 19.2%; carbo-karboides 5.82%; 5.4% asphaltenes; wax 7.5%; 0.064% sulfur. At a temperature of 200^0C has a density - 0.850 g/cm^3.
- diesel fuel fractions are low-sulfur, acidity of less than 3%. At a temperature of 200^0C has a density - 0.804 g/cm^3. The high content of n-alkanes (24%) can be regarded as a promising raw material for the production of liquid paraffins.
- Fuel oil is characterized by the actual freezing point between 25-42^0C. At a temperature of 20^0C has a density - 0,890-0,899 g/cm^3. The viscosity at 80^0C is not more than 16, a sulfur content not exceeding 0.5%, solids of not more than 1%.

Microorganisms.
Microbiological examination of oil-contaminated sites was performed according to generally accepted methods in the microbiological practice [20].

Isolation of heterotrophic bacteria was carried out on media IPA, hydrocarbon-oxidizing bacteria was carried out in the Voroshilova-Dianova medium, where carbon nutrition of microorganisms used in various fractions of oil products. Oil products of different fractions have been used as for carbon nutrition of microorganisms. Bacteria species have been identified, according to Bergey's method[8]. The analysis of microorganism pathogenicity and non-allergenicity for humans and homoiothermal animals has been carried out in the lab of "Nutritest" LLP.

The preparation of "Peroil" has included the following stages: growing of microorganisms biomass; sublimation drying, using protective media, with final content of microorganisms up 10^8-10^{10} cells per gram of dry substance in the form of powder. Finished biopreparation is milk-white friable powder, where dry milk has been used as a filler.

Determination of oil products.
Determining the number of oil products in the soil was performed by gravimetric method, extraction of oil and oil products with chloroform [21].

Determination of hydrocarbon oil composition was carried out by adsorption column chromatography [22], filled with aluminum oxide. Purity of the samples was monitored by thin layer chromatography plates «Sylufol» of silica-gel, as a selective solvent used was hexane, for the isolation of paraffinic compounds, hexane + benzene in a ratio of (9: 1), to isolate the monocycloaromatic compounds and hexane + benzene in the ratio (8 : 2) to isolate bicycloaromatic compounds, benzene and alcohol-benzene mixture at a ratio of (1: 1), to isolate asphaltene and toluene resins.

Bioremediation.
Bioremediation works were carried out under real production conditions in the industrial zone LLP «PKOP." Methods of reclaiming contaminated soils included the following main activities: loosening (regular plowing), introducing biomass hydrocarbon-oxidizing bacteria, watered with the introduction of nutrients -1% ammophos, in the future - irrigation water. Repeated loosening of the soil carried out at least once a month, irrigation takes place every 5-7 days. Cleaned soil moisture was maintained at 50-60% of full capacity. Sawdust used for the immobilization of bacteria was inoculated in an amount of 2-10 t/ha, depending on the type and extent of oil pollution.
During the bioremediation of contaminated soils with climatic conditions of farming practices began in the fall (October, beginning of November), when the microorganisms immobilized on sawdust were introduced in soil. After introducing a biological product into the soil and its moisture, works stopped before the end of February to early

March. This soil moisture is maintained by natural moisture (rain, snow). Deadline for remediation work after this period for each subject area determined by the result of analysis on the oil content in the treated soil.

Statistical analysis of the results.
Experiments were carried out five times in repetition, calculate the standard deviation at 0.95> P> 0.80. Statistical processing was performed using the statistical software package Microsoft Excel on a PC «Pentium-IV». By the number of measurements and in general diagnostic group determined the arithmetic mean [23].

Results And Discussion

It was shown that the reaction of the soil microflora to oil pollution is ambiguous and depends on the keys for microorganisms and physico-chemical characteristics of oil. It was found that the largest number of hydrocarbon oxidizing and heterotrophic bacteria concentrated in the upper soil layer 0-10 cm, which can be explained by environmental factors favoring of life of this group of microorganisms: the oxygen supply needed moisture, air-gas mode.

Several conflicting research data presented in **Table 1**, for example, the number of hydrocarbon-oxidizing bacteria in the horizon of 10-20 cm may be due to stratified alternating unpolluted / contaminated layers of soil. Studied oil products by the degree of bacteriostatic effects can be arranged as follows: oil - diesel fuel and heating oil. At the same time, despite the toxicity of low concentrations (20 ml/kg soil) increase in titer diesel hydrocarbon oxidizing microorganisms marked by 60 hours. In all likelihood, 60 days - a period when there is a change in the soil 3 adaptive zones of resistance, stress and homeostasis during which many soil microbial processes are stabilized.

Table 1. Dynamics of number of heterotrophic and hydrocarbon-oxidizing bacteria in the soil of local areas LLP "PetroKazakhstan Oil Products"

Character contamination , sampling depth, cm	Oil content,%	Heterotrophic bacteria, CFU / g	Hydrocarbon-oxidizing bacteria, CFU / g
control,			
0-10	0	$(3.21\pm0.30)\times10^{6}$	$(9.32\pm0.10)\times10^{4}$
10-20	0	$(5.71\pm0.50)\times10^{5}$	$(3.45\pm0.35)\times10^{4}$
20-30	0	$(2.49\pm0.25)\times10^{3}$	$(1.25\pm0.01)\times10^{3}$
30-40	0	$(8.84\pm0.81)\times10^{2}$	$(7.31\pm0.65)\times10^{2}$
Oil,			
0-10	1.26±0.11	$(2.31\pm0.23)\times10^{8}$	$(5.43\pm0.56)\times10^{4}$
10-20	3.95±0.38	$(2.52\pm0.29)\times10^{7}$	$(6.09\pm0.60)\times10^{5}$
20-30	4.96±0.45	$(2.13\pm0.22)\times10^{5}$	$(5.81\pm0.60)\times10^{5}$
30-40	2.23±0.20	$(1.89\pm0.19)\times10^{5}$	$(5.22\pm0.53)\times10^{3}$
Diesel fuel,			
0-10	4.68±0.45	$(2.73\pm0.25)\times10^{9}$	$(5.85\pm0.55)\text{x}10^{6}$
10-20	2.36±0.22	$(2.49\pm0.23)\times10^{8}$	$(5.04\pm0.50)\times10^{5}$
20-30	3.35±0.33	$(1.97\pm0.16)\times10^{6}$	$(3.39\pm0.33)\times10^{4}$
30-40	2.84±0.26	$(1.96\pm0.16)\times10^{6}$	$(2.97\pm0.28)\times10^{3}$
Masut,			
0-10	2.29±0.21	$(1.72\pm0.16)\times10^{9}$	$(5.04\pm0.49)\times10^{5}$
10-20	3.59±0.34	$(1.68\pm0.15)\times10^{8}$	$(4.62\pm0.45)\times10^{6}$
20-30	4.12±0.40	$(1.19\pm0.11)\times10^{8}$	$(4.26\pm0.40)\times10^{6}$
30-40	5.23±0.50	$(1.13\pm0.10)\times10^{6}$	$(2.78\pm0.25)\times10^{4}$
Sludge			
0-10	4.79±0.45	$(1.95\pm0.19)\times10^{8}$	$(5.16\pm0.50)\times10^{3}$
10-20	5.76±0.55	$(1.68\pm0.15)\times10^{7}$	$(5.04\pm0.50)\times10^{4}$
20-30	2.17±0.21	$(9.91\pm0.85)\times10^{5}$	$(3.57\pm0.34)\times10^{4}$
30-40	1.13±0.10	$(1.13\pm0.10)\times10^{5}$	$(3.39\pm0.30)\times10^{2}$

In our studies of the various oil-contaminated soil has been allocated 255 microorganism cultures, from which, after screening to a variety of oil products were selected strains DP-304 B7, B1Ag8G, N 313-C14, which have shown the ability to grow on nutrient media hexadecane, benzene, naphthalene, diesel fuel oil, fuel oil included as a sole carbon source. After examining the antagonistic characteristics when co-cultured and isolated strains of the taxonomic

ISSN 2349-0292
Impact Factor 2.365

analysis revealed that the strains *Rhodococcus erythropolis* DP-304 B7 and *Micrococcus luteus* B1Ag8G possess high oxidation ability with respect to the most limiting and aromatic oil hydrocarbons (**Figure1**).

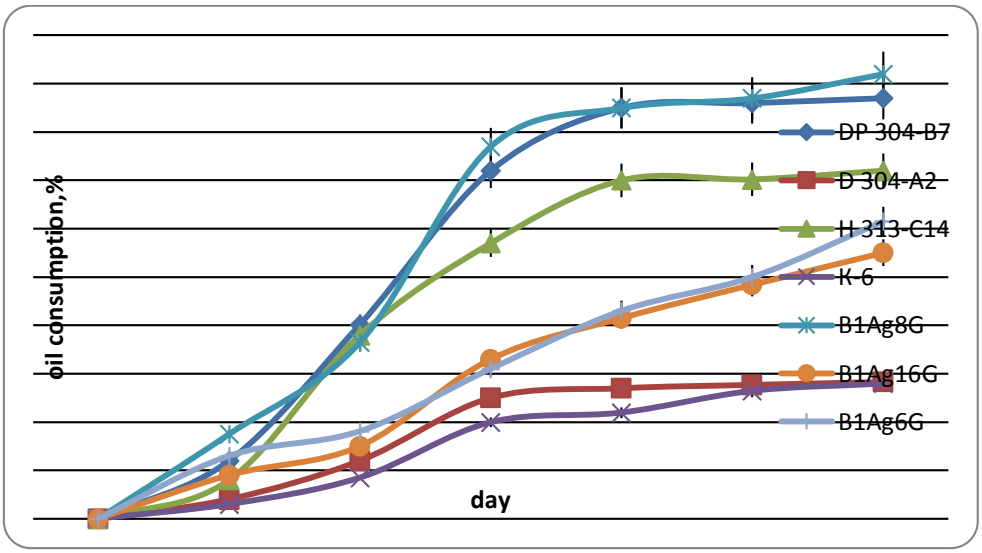

Figure 1. The dynamics of oil consumption microorganisms

It was found that augmentation of microorganisms reduce the oil content in the soil at 44.4-97.2%. At this titer of microorganisms in the soil is increased to 28.2- 93.5% depending on the oil-contaminated site. From the available soil bicycloaromatic, monocycloaromatic, polycyclonaphthenic compounds, toluene resins and asphaltenes in the soil are only polycyclonaphthenic compounds, bio-oxidation which was slower.It is necessary to point out that not only physical-chemical characteristics of oil contamination, but also the weather, climate and landscape in work area are to be taken into consideration, when performing bioremediation activities.

In the countries with arid climate, for instance in Kazakhstan, bioremediation works in summer time are impossible, as the soil dries up, and microbiological activity fades out in the soil. Accordingly, the development of oil-contaminated soil recultivation technology in arid climate shall include searching of optimal time and conditions for hydrocarbon-oxidizing microorganisms' activity in the soil. In South Kazakhstan the autumn may be an appropriate period, when it starts raining, as well as the spring, when the soil is humid and warming up. One of by no means unimportant factors, influencing on the quality of soil bioremediation process, is the temperature, which affects not merely physical nature, i.e. the snow and the rain. Agrotechnical measures (soil loosening) are applied only in dry weather without additional wetting required in accordance with process regulations. Depending on degree of soil wetting, biogenic feeding has been used in the form of 1% solution or solid matter of ammophos. As a result of observations, it has been revealed that the intensity of process of biological cleaning of mazut-polluted soil decreases, when temperature declines to $0+5^0$C. However, after rising of air and soil temperature the process of soil cleaning has been more intensive in the areas, where immobilized biomass of hydrocarbon-oxidizing microorganisms has been applied in autumn, in comparison to territories, where the works have begun in spring. Soil loosening made in early spring has performed the function of water holding in addition to aeration. In consequence of activities completed, the water has been used for bioremediation purposes in the initial phase only, when the biopreparation has been implemented. During remained period the soil has been moistened naturally with atmospheric precipitation. Depending on the nature of oil contamination, overall level of soil cleaning is 68-92%. In addition, quantitative composition of components in samples of oil-contaminated and cleaned soils is the following: before the treatment with preparation a concentration of polycyclonaphtene compounds is 10%, monocycloaromatic compounds – 20%, bicycloaromatic compounds- 60%, toluene resins – 5%, asphaltenes – 5%; after treatment – the soil has been polluted with polycyclonaphtene compounds only. By comparison, better cleaning results are achieved in the areas, where hydrocarbon-oxidizing microorganisms have been used in autumn. Among them: earlier start of activation of hydrocarbon-oxidizing microorganisms, high degree of oil hydrocarbons biodegradations. Probably, it can be explained by an adaptation of hydrocarbon-oxidizing microorganisms for new living conditions. Hydrocarbon-oxidizing microorganisms brought from outside and spontaneous ones take up starting position, so that to begin a

propagation and hydrocarbon nutrition after warming of weather. Therefore, intentional coinciding of start of biorecutivation works with autumn term enables to achieve significant rising of the effectiveness of soil cleaning from oil products, and reduce water resources consumption in arid conditions of the region.

The time of cleaning depends on the level of soil pollution. If the concentration of oil products is up to 3%, the duration of cleaning process can be 2-3 months. Cleaning of oil-contaminated soil with 5% concentration of oil products endures 5-6 months (March-August). For cleaning of soil with high concentration of oil products (7-10%) two-stage treatment with adherence to time limits is recommended. The first stage includes the following activities: loosening of soil 30-40cm depthward (once per 7-10 days), introduction of active strains of hydrocarbon-oxidizing microorganisms, using of nitrogen-phosphate fertilizer (1% ammophos), maintaining of 60% humidity level. This stage is recommended to be completed within the period from March to November. It is reasonable to start the second stage at the end of February or in the beginning of March, when wetted soil is laid down with plant seeds. Soil will be cleaned up to 92-97% by September-October on condition that its natural or artificial humidity is 60%.

Conclusion

The studies found that the spread of microorganisms in the soil horizons depends on different types of oil-contaminated loamy grey soils and correlated with a molecular mass of oil products. Augmentation of active strains of microorganisms in oil contaminated soils promotes biodegradation bicycloaromatic, monocycloaromatic compounds, toluene resins and asphaltenes.

At the beginning of confinement bioremediation works in October, revealed the following positive aspects:

- Adaptation of the hydrocarbon-oxidizing bacteria to the new conditions of existence - augmented spontaneous hydrocarbon oxidizing bacteria occupy the starting position to the beginning of the warming begin to multiply and consume hydrocarbons;
- Water can be used only when augmentation consortium of bacteria at the initial stage (in the case in October precipitation low). This significantly reduces the level of water consumption in the arid climate of the south of Kazakhstan.

Acknowledgments

The authors are grateful to Eugene Mechetner (California, USA) for advice in preparation of this article, Emberdievu Amanali for the opportunity to conduct experiments on the LLP "PetroKazakhstan Oil Products".

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