

The Problem of Oil-Contaminated Soil and Microbial Preparations

Stankevich D.S., Emtsev V.T.

Timiriázev Agricultural Academy, Moscow, Russia

Abstract: Actually the methods of the recultivation of oil-contaminated soil based on use of such microorganisms-destroyers are developing. From oil contaminated soil there were selected two strains of bacteria *Pseudomonas* for bioremediation such soils. There was made a new microbial preparation "Pseudomine" with those strains. It was established that the bacterium *Pseudomonas* applying with the preparation to the soil were well multiply there. That fact indicated to possibility of their prolonged exposure to residual amount of oil in the soil. Later it was test with different microbial biopreparation developed by several organisations. The aim of these research was to estimate capacity of the offered preparations to precipitation of bioremediation oil-contaminated soil process in laboratory conditions. The results exceeded all expectations and "Pseudomine" got a high appreciation. Our microbial biopreparation "Pseudomine" is a promising means for bioremediation of the oil-contaminated soil and can be recommended for industrial conditions.

Keywords: bioremediation, microorganisms, soil, petroleum

Introduction

At present oil and other oil products have unfavorable effect upon the biosphere of our planet, they are recognized as priority pollutants of the environment (2). Natural self-purification of soil, water and other objects from the oil is very prolonged (3). It is effected by complex processes in biocenosis containing associations of microorganisms that are able to use hydrocarbons of oil for food. Actually the methods of the recultivation of oil-contaminated soil

based on use of such microorganisms-destroyers are developing (1), (4). Because of directed selection microorganisms used in the biopreparations have a higher efficiency than natural forms.

Material and Methods

It was the purpose of our research:

- to obtain the promising strains of microorganisms-destroyers of oil by selection methods;
- to develop the technology of peaty preparation based on these active strains;
- to test the preparation to effect of oil-contaminated soil biodegradation.

Obtaining of the microbe strains for the oil destruction.

For isolation of microbe strains were able to decompose of petroleum and other oil-products had chosen more than 200 samples of oil-contaminated soil. With a view to obtain pure culture were used the following nutrient medium: $(\text{NH}_4)_2\text{HPO}_4$ -3,0 g; KH_2PO_4 -2,0 g; NaCl -0,5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -0,3 g; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ -0,1 g; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ -0,01 g; agar-20,0 g; distillate water-1000 ml. There was oil added to that medium by way of the carbon source.

New microbial preparation.

The technique of the new microbial biopreparation "Pseudomine" was developed from active strains *Pseudomonas putida* st. 91-96 and st. 9:IV. The preparation was made up from sterile peat by inoculation of daily culture liquid. There was made a party of the preparation for testing in industrial conditions on the oil-contaminated soil.

Field tests the new microbial preparation were run on the experimental area in Surgut University, Tumen region, Russia. Soils of area was acid podzolic, initial pH was 4.8. Before the experiment pH was brought to 6.8 by appending of lime. Before application of the biopreparation there was spilled petroleum (60% heavy and 40% light fraction). Brome smooth (*Bromus inermis*) was used as a bioassay and as a possible active agent of recultivation of the contaminated area.

The comparative laboratory tests of different microbial biopreparation.

In Surgut University (Tumen region, Russia) were carried out of investigations of efficiency of the biopreparations for destruction of oil carbohydrates in the soil. 14 biopreparations patterned by different science organizations took part in that test. The "Pseudomine" developed of our laboratory also participate in it. The aim of that research was to estimate capacity of the offered preparations to precipitation of bioremediation oil-contaminated soil process under laboratory conditions.

The investigation was concerned with pollutant soil have taken from the site of oil wreckage flood on the West-Surgut oilfield. This soil was processed by following biopreparations:

- | | |
|---------------------|------------------------|
| 1. Devoroil 1 | 8. Degradojlas Nj9 |
| 2. Devoroil 2 | 9. Remediast |
| 3. Devoroil (paste) | 10. Biopreparation 999 |
| 4. Inipol | 11. Biopreparation 670 |
| 5. Faerzaim | 12. Naftoks |
| 6. Bioprine | 13. Naftoks (1) |
| 7. Degradojlas 81 | 14. Pseudomine |

For tests of each preparation there were used by two boxes with oil-contaminated soil. The treatment of soil samples by biopreparations were conducted according to arrangement of this preparation employment. The temperature in studies of activity was 18-23°C.

Quantitative estimation of residual oil was conducted by IR-spectral analysis. Initial concentration of the oil in the soil was 254-371 mg/kg. Design formula for definition of the biopreparation efficiency was:

$$Eff, \% = \frac{(Cin. - Cfin.) \times 100}{Cin.}$$

Cin. – initial oil concentration in the soil

Cfin. – final oil concentration in the soil

The grade of the preparations efficiency was considered by following factors:

Adjusting of 50%-efficiency of biopreparations time, i.e. to choose from the preparations ones were able to fast transformation of the oil.

Estimate of the biopreparations efficiency after 11 weeks of experiment.

Results and discussion

There were obtained the perspective strains of microorganisms-desructors of oil by selection methods. Repeated platting of soil serial dilution and artificial selection allowed to obtain a number of active bacteria strains. Two of them were defined as bacteria of genus *Pseudomonas*-*Ps.putida* st. 91-96 *Ps.putida*. st. 9:IV. The strains were able to use alkanes as the only source of carbon and energy. That materials are the toxic agent for the most other microorganisms. This fact suggested that present strains can be use for recultivation of oil-contaminated soil.

On the base of that strains it was made the microbial biopreparation "Pseudomine" which was probed in field on the area of the oil-contaminated soil in Surgut University, tumen region, Russia. In that experiment before and after the petroleum applying there were defined contents of organic matter and separately oil in the soil by chromatography. The contents of organic matter were 1569 mg/kg of the soil before applying and 5907 mg/kg of the soil after applying. The contents of petroleum were 1408 mg/kgg and 4259 mg/kg respectively.

The results of the field tests are reflected in the table 1. There are present different variations of preparation applying with various supplements.

Table 1. An Influence of microbial preparation "Pseudomine" to the oil-contaminated soil

Characteristics	Pseudomine + Brome smooth		
	Pseudomine	N ₁₂₀ P ₁₈₀ K ₉₀ + Pseudomine	N ₁₂₀ P ₁₈₀ + Pseudomine+peat
Total amount of soil organic matter mg/kg of soil	138	164	232
Residual amount of the oil mg/kg of soil	82	128	109
Percentage of degraded oil from the total amount	98.1	97.0	9979.4
Amount of plants, piece/1m ²	-	72	34
Mass of plants, g/1m ²	-	100	51

Applying of "Pseudomine" in the soil were leaded to almost entire biodegradation of the oil in the soil. It was transformed on 97.0-98.1% in 60 days because of high enzymatic activity of microorganisms-destructors of petroleum. It was established that the bacterium *Pseudomonas* applying with the preparation to the soil were well multiply there. That fact indicated to possibility of their prolonged exposure to residual amount of oil in the soil. Such kind of results were obtained in subsequent experiments.

In the table 2 are represent the results of laboratory tests of the biopreparation efficiency for destruction of oil carbohydrates in the soil. There are results of the beginning period (5 weeks).

Table 2. Efficiency of the biopreparations for destruction of oil carbohydrates, %

Biopreparation	Period of action, weeks				
	1	2	3	4	5
Devoroil 1	8.22	36.54	40.47	49.41	54.46
Devoroil 2	8.20	61.49	63.03	67.22	68.37
Devoroil (paste)	15.54	40.51	42.28	50.25	53.74
Bioprime	8.22	31.29	35.08	49.42	51.10
Inipol	15.22	58.94	63.98	67.56	68.85
Faerzaim	27.71	58.32	61.45	63.80	66.75
Degradoilas 81	29.60	29.99	46.05	52.72	53.82
Degradoilas Nj9	8.73	29.69	36.72	48.82	59.06
Remediast	20.00	65.32	65.67	72.37	73.07
Biopreparation 999	5.52	63.36	66.28	72.34	72.84
Biopreparation 670	15.82	67.37	68.54	69.5	74.54
Pseudomine ("low")	11.26	26.98	41.68	42.44	52.59
Pseudomine ("high")	16.85	25.36	27.90	30.98	34.60
Naftoks ("low")	32.02	41.38	44.09	46.31	50.49
Naftoks ("high")	12.50	60.10	61.13	64.21	65.58
Naftoks (1) ("low")	-8.62	1.97	24.63	41.87	46.80
Naftoks (1) ("high")	10.53	15.60	21.24	28.57	32.10

"low"-concentration of the oil about 20% in the soil

"high"-concentration of the oil about 60% in the soil

It is easily seen that "Pseudomine" didn't have high rate of the oil decomposition, especially on the high concentration. But the total efficiency of this preparation is very large (see Fig. 1). In this diagram there are represented values of efficiency for biopreparations which validate their results.

It was noted that the action of testing biopreparations was more effective with adding of mineral fertilizers. By these the efficiency of "Pseudomine" increased for 41,9% (see Fig. 2). The results exceeded all expectations and "Pseudomine" got a high appreciation. Especially good results were obtained on the middle degree of pollutant (about 20%). Then its effective action were 75.7% for 11 weeks.

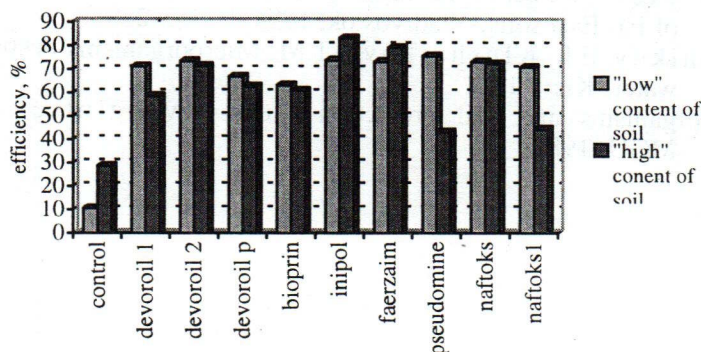


Fig. 1

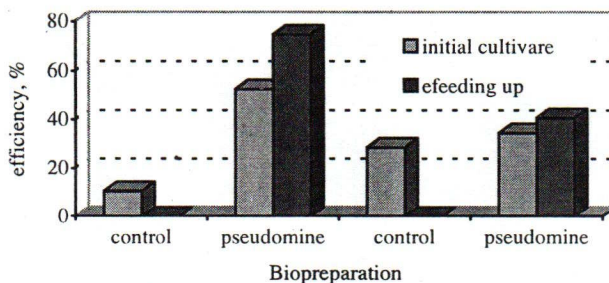


Fig. 2

Conclusion

Thus, our microbial biopreparation "Pseudomine" based on bacteria *Pseudomonas* is a promising means for bioremediation of the oil-contaminated soil. On the basis of experimental dates it can draw conclusion that optimal bioremediation of oil-contaminated soil is in absence of plants, under pH 6.5-7.5 and good aeration. According to field experiment information the application of

mineral fertilizers are not necessary, then in laboratory tests are established considerable increase (41%) of biopreparation effective with fertilizers.

Our preparation "Pseudomine" can be recommended for industrial conditions.

References

- Anderson R.K., Bagautdinov F.Y., Boyko T.F. Using of microbiological method for the purification of oil-contaminated soil // Microbial introduction in the environment. Abstr. Conf.-Moscow, 1994.-P. 10.
- Golodyaev G.P. Biodegradation of petroleum in soils // Genesis of the south of Far East soils.-Vladivostok, 1994.-P. 253-268.
- Kvasnikov E.I., Klushnikova T.M. Microorganisms-destructors of oil in water.-Kiev.-1981.
- Microorganisms and soil protection / edit. Zvyagintsev D.G.-Moscow, MGU.-1989.

PROBLEMI U VEZI SA ZEMLJIŠTIMA ZAGAĐENIM NAFTOM I MIKROBIOLOŠKE PRIPREME

-originalni naučni rad-

Stankevich D.S., Emtsev, V.T.

Moskovska Poljoprivredna Akademija, Timirjazev, Rusija

Rezime

Danas se uveliko razvijaju metode rekultivacije zemljišta zagađenog naftom. Metode se zasnivaju na primeni mikroorganizama koji vrše razgradnju nafte. Iz zemljišta, kontaminiranog naftom, izolovana su dva soja bakterija roda *Pseudomonas*. Od njih je proizveden novi mikrobiološki preparat "Pseudomin" koji se primenjuje za biološku melioraciju zemljišta zagađenih naftom. Utvrđeno je da se *Pseudomonas* dobro razvija u ovakvim zemljištima i da se odlikuje produženim delovanjem na rezidue nafte. Ovo su potvrdila istraživanja sa različitim preparatima iste namene.

Istraživanja su imala za cilj da ocene nivo biomeliorativne sposobnosti ponuđenih preparata u laboratorijskim uslovima. Rezultati su prevazišli sva naša očekivanja. Naš mikrobiološki preparat "Pseudomin" je perspektivno sredstvo za biomelioraciju zemljišta, koja su zagađena naftom i može se preporučiti za primenu u industrijskim postrojenjima.