FEDERAL AGENCY OF SCIENTIFIC ORGANIZATIONS

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"APPROVED"
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(signature) A. A. Leontievskiy
(Official Seal affixed)

R&D Conclusion
"Study of effectiveness of soil cleaning from petroleum hydrocarbon by application of lignin and biopreparation in the laboratory conditions"

R&D objective: assess the efficiency of removal of petroleum hydrocarbon from soil due to application of sorbent (dry lignin) and biopreparation in the laboratory conditions.

Description of the experiment for study of effectiveness of soil cleaning from petroleum hydrocarbons:

Liquid biopreparation (prepared as water solution of dry concentrated biopreparation with KFU $1x10^{10}$) is applied for oil-contaminated soil (oil-content 10%) as $1x10^{6}$ bacteria per 1 kg soil. Then, dry lignin is applied as 1 g lignin per 1 g oil. If necessary, mineral salts (fertilizers) and deoxidizing agents are also applied and optimal moisture by water spraying is provided. For uniform mixing the soil is re-plowed twice or four times a month. Treatment affect is achieved in 2-3 months.

Schematic course of experiment:

Work was provided in plastic containers "Magenta" with a lid. Each container contained 70 g of gray forest oil-contaminated soil (oil content 10%) with moisture 30%. Experiment duration: 1 month.

Experiment cases:

№1 oil-contaminated soil – control of abiotic oil decrease

№2 oil-contaminated soil + lignin - control of abiotic oil decrease and oil sorption

№3 oil-contaminated soil + biopreparation – bio-degradation effectiveness

№4 oil-contaminated soil + lignin + biopreparation – bio-degradation effectiveness in presence of lignin

To determine the **total number of microorganisms** the 0.5 g soil samples are taken, microorganisms are washed out by physiological solution and after serial tenfold dilution the seeding is provided onto the cups with rich agar medium 5/5 for counting the colonies.

Residual hydrocarbon concentration was measured by IR- spectroscopy using oil-product analyzer AH-2 (MYK 4.1.1956-05).

Oil-product (oil) concentration in a soil sample was calculated by the following formula:

$$C_i = \frac{D \times (K \times C_{H_k} - C_{H_o}) \times V_o}{P}, \quad \text{[mg/kg soil], where}$$

D - proportionality coefficient obtained as a result of processing of a calibration curve for real and measured oil concentration in the soil, specified for a specific kind of soil;

K - coefficient of dilution of eluate of carbon tetrachloride, cm^3/cm^3;

C_{Hk} – oil concentration in the diluted eluate specified using a calibration curve, mg/dm^3;

C_{Ho} – concentration of non-specific components of humus in the eluate, as well as concentration of residual oil products which can be in a soil sample taken as a control one, mg/dm^3;

V_o – volume of initial carbon tetrachloride taken for extraction of oil from the soil sample, dm^3;

P – soil lot, kg

Table 1. Residual oil content and degree of oil decrease in model systems for 30 days

№	System	Residual concentration, g/kg	Degree of oil decrease, %
1.	oil-contaminated soil	74,12±2,14	-

2.	oil-contaminated soil + lignin	29,88±2,59	59,68±3,18
3.	oil-contaminated soil + biopreparation	46,58±4,23	37,16±5,70
4.	oil-contaminated soil + lignin + biopreparation	14,68±0,66	80,19±0,81

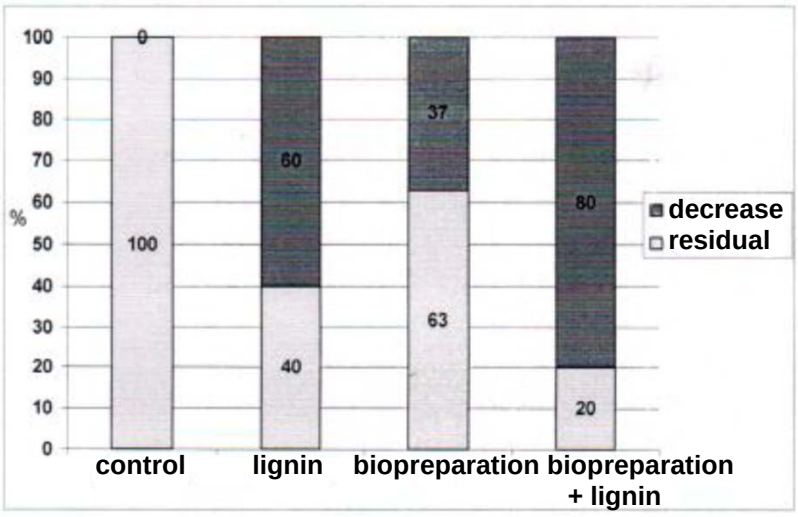

Fig. 1. Residual oil content and degree of oil decrease in model systems for 30 days

The maximal decrease (80%) of oil is noted in case “biopreparation + lignin” (Table 1, Fig. 1). When adding lignin the oil decrease in soil was 60% which was provided probably by oil sorption by lignin. When adding only bio-preparation (without lignin) oil decrease (bio-degradation) was 37%.

Thus, the most effective technology is, obviously, the oil-contaminated soil cleaning by introduction of bio-preparation with the following application of dry lignin assuming that 1 g lignin can sorb up to 3 g oil.

Table 2. Total number of microorganisms in model systems with oil

№	System	**Number of microorganisms of KFU/g soil**			
		0 days	**8 days**	**15 days**	**30 days**
1.	oil-contaminated soil	$3,0x10^5$	$8,5x10^6$	$3,3x10^7$	$2,1x10^7$
2.	oil-contaminated soil + lignin	$4,5x10^5$	$1,2x10^7$	$6,0x10^7$	$3,0x10^7$
3.	oil-contaminated soil + biopreparation	$1,3x10^6$	$4,0x10^7$	$3,2x10^8$	$5,3x10^7$
4.	oil-contaminated soil + lignin + biopreparation	$1,1x10^6$	$4,7x10^7$	$1,5x10^8$	$3,1x10^7$

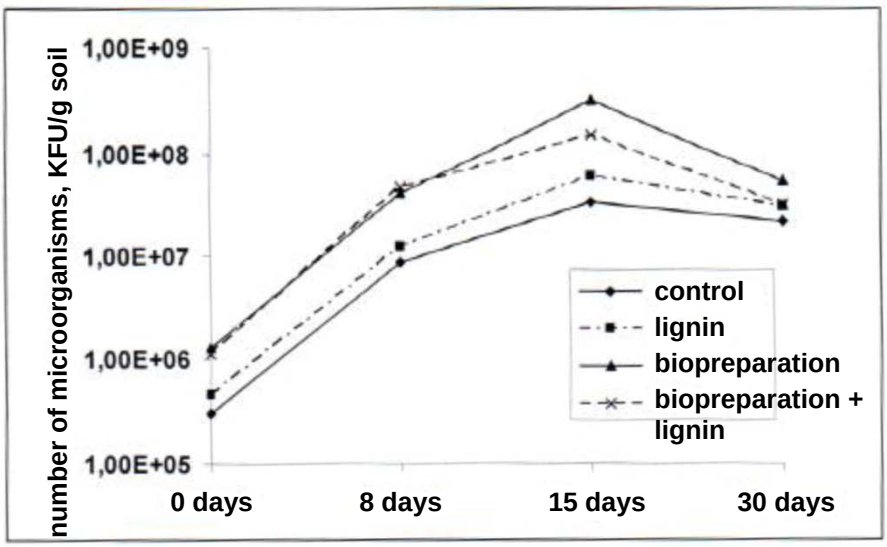

Fig. 2. Population dynamics of microorganism in model systems with oil

Population dynamics of microorganisms in all experiment cases had similar character (Table 2, Fig. 2). For the first two weeks of the experiment the number of microorganisms in soil increased, then slight decrease was observed. In cases of "bio-preparation" and "bio-preparation + lignin" the regular increase of the number of microorganisms was observed due to introduction of microorganisms as a part of bio-preparation. It appears that introduction of lignin stimulated some increase in number of native soil microorganisms **which serves as an additional argument in favor of application of lignin in the oil-contaminated soil cleaning technology**.

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Signature of Filonov A. E. is certified