Application of New Biotechnologies in the Remediation of Black Soil with Mixed Pollution

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Abstract. During remediation of black soil with mixed pollution we assessed the environmental and economic efficiency of new biotechnologies for the PJSC Tatneft. They included biological preparation «Lenoyl SKhP» based on strains of hydrocarbon-oxidizing microorganisms (development of the Institute of Biology of the Ufa Scientific Center of the Russian Academy of Sciences); complex biological preparation – destructor of oil pollutions «DNZ»; joint introduction of nanosorbents and effective indigenous hydroc arbon-oxidizing microorganisms (development of the Tatar Research Institute of Agrochemistry and Soil Science); and «Gumaks» (development of the LLC «Center Spas») and potassium humate. Experiments were carried out over two field seasons on leached and typical agricultural black soils contaminated due to the spill of Devonian watery and sour oil. Based on the data about dynamics of oil content in soils, results of agrochemical and microbiological monitoring over two seasons we evaluated the efficiency of new biotechnologies and developed recommendations for production.

Keywords: admissible residual oil content; methods of bioremediation of contaminated land; indigenous microorganisms-destructors; humate

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Introduction

Currently there are many new biotechnologies suggested in the literature and Internet resources to remedy the fertility of oil-contaminated soils. But in practice, when depressurising oil pipelines on the fields at the late stage of development with watered oil, oilfield fluid that enters the soil leads to a mixed type of pollution: salt and oil. Therefore, remediation activities should be aimed at the restoration of soil parameters, ensuring their fertility, including their desalinization and bringing the oil etoncentration in the soil to a value of admissible residual TPH in soil (Ibatullin et al., 2006; Shaydullina, 2006; Malykhina et al, 2012;. RD 153-39.0-716-11 ..., 2010).

Earlier we have conducted joint testing of biotechnologies on leached black soils with the release of native strains of hydrocarbon-oxidizing microorganisms in conjunction with nanobentonite (FBGNU Tatar NIIAHP of the Russian Agricultural Academy) and using humic preparation – "Gumaks" (developed by LLC "Center Spas" and LLC "NPP") on the artificially contaminated beds with different types and doses of entering oilfield environment. The traditional method was used as a controlling mean, including manure and planting of soil improver. It has been shown that recommended biotechnologies are economically safe for the adjacent natural environments, as well as less expensive compared to conventional soil remediation option (Shaydullina et al., 2015).

The aim of this work was to identify on promising conditions for the PJSC Tatneft of environmentally friendly and cost-effective methods of black soil biological remediation with a mixed type of contamination on areas with spilled Devonian watered and sour oil. Based on the literature reviewed we selected the following: biotechnology on the basis of biological preparations of hydrocarbon-oxidizing microorganism strains "Lenoyl SKhP", "DNZ"; biotechnology with the release of native strains of hydrocarbon-oxidizing microorganisms in combination with nanobentonite (FBGNU Tatar NIIAHP of the Russian Agricultural Academy) (Yapparov et al, 2011.); biotechnology with humic substances "Gumaks" (proposed by LLC "Center Spas") (TU 2458-001-09265941-2,012.2012) and potassium humate of brand BP 20 (TU 2189-004-54775950-2000). The traditional method of remediation of oil-contaminated soil lies in the manure introduction in the amount of 60 t/ha and phosphogypsum as a soil improver in the amount of 10 t/ha and periodically loosening of the soil.

Materials and methods

The works were carried out for two seasons on the experimental plots after the spill of watered sour oil (the type of soil – leached black soil) and watered Devonian oil (type of soil – typical black oil).

Plots located near the place of laying the field studies with no visible signs of land violations were selected as background.

Plots with spill of watered sour and watered Devonian oil were divided into 6 plots where the following remediation options were investigated: "Native strains + bentonite", "DNZ", "Lenoyl SKhP", "Gumaks", "Potassium humate" in comparison with the traditional method. In addition, control plot with exclusively farming practices was laid in each of the test sites.

Working aqueous suspensions of biological preparations were introduced into the soil by sprinkling.

Working suspensions "Lenoyl" and "DNZ" before introduction were intensified in the nutrient solution for one day. When using the method of FBGNU Tatar NIIAHP on the surface of oil-polluted soil we added agromineral bentonite as nanosorbent and ammonium nitrate at the rate of 0.15 t/ha; further during the growing season we performed twofold treatment of oil-contaminated beds by biologic liquid hydrocarbon-oxidizing microorganisms (titer of inserted community 1.4-1011 CFU/ml), diluted with tap water (1:1) in combination with conventional agronomic activities. In the first season, plowing was carried out 3 times. In the second season, only farming practices were held and sowing of wheat on the field with the Devonian oil spill. In the area of sour oil spill observations were made for the field of the dominant species of vegetation.

When testing the effectiveness of remediation biotechnologies in oil-contaminated soil, an integrated approach was used. Environmental biotechnology efficiency was evaluated by the results of the survey for two field seasons by agrochemical, microbiological characteristics of the averaged samples of tested soil, their phytoproductive and reduction dynamics of petroleum products in the soil; cost-effectiveness was evaluated by calculating the costs of applied methods.

Experimental studies to assess the effectiveness of the considered technologies conducted by the accredited laboratories TatNIPIneft and the Kazan Federal University, as well as FBGNU Tatar NIIAHP.

Field survey, sampling and sample preparation to the analysis were carried out by conventional methods (GOST). Determination of the mass fraction of oil in the soil were determined by spectrometry at the PC device KN-1 (Measurement procedure ..., 1998).

Indicators of biological activity, phytoproductivity of soil, agrochemical and microbiological characteristics of soils were assessed using accepted in the practice monitoring methods (GOST, RD, PNDF et al.). All laboratory experiments were performed at least in triplicate.

The statistical data processing was carried out using Statistica 8.0 software package. The significance of differences was evaluated with the results obtained using the Student factor (P = 95%), the characteristics of a random error component were calculated by the conventional scheme (Korn, Korn, 1978).

Results and discussion

Agrochemical monitoring includes determining the particle size distribution, pH of salt extraction, cation exchange capacity, pH of aqueous extraction, solid residue of the aqueous extract, hydrolytic acidity, chlorides, sulfates, available phosphorus, organic matter, hydrolyzable nitrogen.

Analysis of the results showed that for the area of Devonian oil spill in all cases with the use of microbial technology, the total carbon content was reduced more rapidly than in case with traditional method. For a plot a sour oil spill, the greatest value of total carbon content recorded for the options "traditional" and "control".

According to the complete analysis of soil water extraction, the type of salinity in the studied areas belongs to the "chloride". According to the SNIP 2.06.03-85 "Reclamation constructions and facilities" for chloride type of salinity, the upper limit of the amount of toxic salts has to be 0.10%, sulfate ions – 0.02%, chlorine ions – 0.03%. Maximum allowable concentration of sulfate ions in the soil is 160 mg/kg of soil (SanPiN 42-128-4433-87,1987). In the area of Devonian oil spill by the end of the first season, all biotechnologies, except from the option "Lenoyl-SKhP" were better than the traditional and "control" options as a result of reducing the content of chloride ions in the soils. The traditional method and normal farming activities (option "control") to the beginning of the second season failed to reduce the content of chloride ions to the desired level of SNIP 2.06.03-85. The laboratory results are shown in Table 1.

By the end of the second season in soils contaminated with Devonian oil, a slight excess of sulfate ions was observed only for the plots treated with potassium humate (0.026%), the traditional method (0.025%) and biological preparation "Lenoyl SKhP" (0.026%). For soils contaminated with sour oil, all plots to the beginning of the second season met the requirements of SNIP 2.06.03-85 on the content of chloride ions, but on the content of sulfate ions in the topsoil, plot with the traditional option remained contaminated (0.046%).

For a plot with spill of Devonian oil in the option of 'traditional method' content of dry residue of water extraction in soil samples by the end of the second season was 0.458%, which is above the established limits. For the area of sour oil spill content of dry residue of water extraction has a slight excess for option "Lenoyl SKhP" (0.115%).

For other agrochemical indicators the research results for all options were comparable.

Thus, by agrochemical parameters according to the data obtained for the site with Devonian oil spill, remediation options: "native strains + nanosorbent", "Tumake", "DNZ" are the most preferred. For site with spill of sour oil – all the methods are preferred except the "traditional" method and "Lenoyl SKhP".

Microbiological monitoring consisted in determination of the amount of hydrocarbon and heterotrophic microorganisms, microscopic fungi, total microbial biomass, basal respiration of soil microbial community.

Technology	27.05.13	07.10.13	14.05.14	07.08.14
Native strains + bentonite	0,1960	0,0487	0,013	-
DNZ	0,2230	0,0602	0,012	
Lenoyl SKhP	0,2320	0,1560	0,023	-
Gumaks	0,2010	0,0398	0,019	-
Potassium humate	0,1860	0,0657	0,012	-
Traditional method	0,1560	0,1010	0,052	0,0186
Control	0,1560	0,1028	0,04	0,0159
Background	0,0030	0,003	0,016	-

Table 1. The content of chloride ions in the topsoil 0-15 cm, % (experimental site with Devonian oil spill).

The number of hydrocarbon-oxidizing (HOM) and heterotrophic microorganisms in contaminated soil is an important diagnostic parameter, because the soil microflora is actively involved in the natural process of hydrocarbons degradation. For a plot with Devonian oil spill for the first season HOM were most actively developed in the first selection (after the spill) in the options "native strains + nanosorbent", "Gumaks", "DNZ", "traditional method", "Lenoyl-SKhP" (0.25 million/g). However, these figures were much lower than in soils of background areas (0,025 million/g). The second soil sampling was conducted in 1.5 months. It showed for all options a sharp decrease in the relevant indicators (0.025-0.00025 million/g). For the third selection carried out in the end of the season (after 2 months), the amount of HOM increased by about an order of magnitude (0.25-0.025 million/g). In all options, except for "Lenoyl SKhP" (0.025 million/g), the amount of HOM was comparable with the value for the background regions.

The number of heterotrophs in all experimental options during the first selection, except "Lenoyl SKhP" (16.0 million/g) was comparable to background plots typical to the soil (10.8 million/g). After 1.5 months an increase was revealed in their numbers by 3.8-6.3 times. Values were similar in all experimental options. By the end of the season their number was minimal (8.8-10.3 million/g).

The investigation of the presence in the soil of micromycetes is of considerable interest. They are a group of microorganisms, universal in their significance for the formation of soil fertility. In addition to the background soil, small number for a site with Devonian oil spill was observed only in three experimental options (1.0-5.0 million/g). In the second selection all experimental options showed an increase in their number (40.0-80.0 thousand/g). In the third selection, the number of fungi decreased in 3.2-5.2 times and amounted to 10.0-20.0 thousand/ha.

The microbial biomass and respiratory activity are integral indicators of soil. During the second selection, the maximum values of microbial biomass for the area of Devonian oil spill were reported in versions "native strains +nanosorbent" and "Gumaks" (50.6-56.0 mg Cmic/r), slightly lower figures have been experienced in other variants (22.8-41.4 mg Cmic/r). At the end of the season, this figure was lower (9.8-21.4), while in the background the soil it was 34.1 mg Cmic/r.

Basal respiration during the first selection was the lowest in option "potassium humate" (2.8 mg CO_2/(100g*24 hours) and the highest – in the option "DNZ" (17.8 CO_2/(100g*24 hours). In the second selection in all experimental variants respiratory activity was 22.3-31.4 mg CO_2/(100g*24 hours) and decreased to 12.0-18.1 mg CO_2/(100g*24 hours) to the end of the season.

From the combination of all parameters studied to remedy soil fertility in option with Devonian oil, the best picture is marked for the first season in options "native strains + nanosorbent" and "Gumaks".

In the second year of studies microbiological parameters were determined by the end of the season. In two options – "native strains + bentonite" and "DNZ" – the number of HOM was not changed in comparison with the characteristic of the first year. In "Lenoyl SKhP" the number of HOM decreased by 5.2 times.

Heterotrophic microorganisms actively grew in the options "DNZ", "potassium humate" and "control". In these samples their number increased almost twice compared with the number in the first year. The best indicators were registered in variants "native strains + bentonite" (31.5 million/g) and "Lenoyl SKhP" (29.0 million/g of soil). Only in two samples – "Gumaks" and "traditional" – the number of these organisms remained at the level of the first year.

In all the options we observed an increase in the growth of microscopic fungi. The highest rates were recorded for the option "traditional" (55.0 thousand/g). Composition of micromycete species was studied. In the option "potassium humate" we found phytopathogenic soil fungi (the genus Altemaria). It should be noted that the micromycetes of genus Trichoderma were detected only in "native strains + bentonite" and "control". This is a positive fact, as members of this genus have a high antagonistic activity to phytopathogenic fungi. During the second season on the site with Devonian oil the microbiocenosis condition improved markedly. Proof of this are the indicators of microbial biomass and respiratory activity, which in all experimental variants have increased several times.

For plot with sour oil spill at the first sampling, the maximum number of HOM (2.5 million/g) was observed in the variant "native strains + nanosorbent". The second selection showed a decrease in their numbers for all experimental options, but by the end of the season it slightly increased or remained at the level of the second selection.

Number of heterotrophs in the experimental variants was also not significantly different in the selection of a particular date. However, we should highlight options "Lenoyl SKhP", "DNZ", "potassium humate", "control", in which a few big figures obtained over time.

It must be noted that after the contamination, sour oil had a depressing effect on microscopic fungi. Only at the end of the first season the number was almost the same as in the soil of background portion.

Dynamics of microbial biomass is as follows: the first and third selections have shown comparable results, only rates of the second selection were slightly higher (45.1-66.8 mg Cmic/r).

In the study of basal respiration, rates in the first and third selection were 27.1-47.5 and 18.3-42.4 mg CO_2/(100g*24 hours), respectively. The exceptions were options "traditional", "DNZ", "native strains + nanosorbent", "Gumaks" during the second selection, in which the respiratory activity decreased (15.5-29.1mg CO_2/(100g*24 hours).

In the second season, the number of hydrocarbon-oxidizing microorganisms increased in the variants "native strains + bentonite", "Gumaks" and "control". The decline of this indicator is noted in options "Lenoyl SKhP" and "traditional". In options "DNZ" and "potassium humate" their number is compared with the first-season data.

Similarly, the number of heterotrophic organisms changed in the second season. Only in the "traditional" version number of this group remained at the level of the first season, and in the "control" version fell slightly.

Number of microscopic fungi increased in most options. The maximum rate is obtained in the "control" (130.0 thousand/g). The value remained almost unchanged compared to the first season in the version "Gumaks". Thus, the inhibitory effect of sour oil on micromycete leveled. Micromycete species composition was also studied. In the two variants – the "traditional" and "control" we found phytopathogenic soil fungi (of the genera Fusaiium and Altemaria).

The microbial biomass and respiratory activity in most of the investigated variants increased. In two versions – "potassium humate" and "control" – with an increase in microbial biomass indicators of basal respiration remained at the level of the first season.

Thus, for the samples contaminated both with sour and Devonian oil, according to microbiological parameters studied for the first season in the majority of variants comparable data were obtained. For the second season in most experimental options microbial cenosis condition improved significantly. In options using alternative biotechnologies "native strains + bentonite" and "Gumaks" we revealed a higher level of hydrocarbon degradation processes.

By changing the content of petroleum products in the soil for the first season we could not highlight the advantage of any method, the monitoring required for a second season. The visual condition of the soil at the end of the season did not differ from the soil state to the background area and had no odor of oil, which is due to volatilization and degradation of light, but the most toxic for plants hydrocarbons.

In the second season, compared with the results obtained at the end of the first season, more active reduction of petroleum products in the area of Devonian oil spill (soil type – the typical black soil) was recorded for the options "native strains -1-bentonite" (by 56%), "Gumaks" (by 54%). The lowest value of the petroleum products content was identified for option "native strains + bentonite" (5700 ± 1425 mg/kg), which exceeds the norm of admissible residual TPH-in-soil for typical black soil (3100±310 mg/kg) (On approval of regional standards..., 2012).

According to the change dynamics of oil content in soils contaminated with sour oil (soil type – leached black soil), for the second season there was a noticeable decline in the oil concentration for variants "traditional method" (by 71%), "Gumaks" (by 72%), "Native strains + bentonite" (by 56%). By value, close to the norm of admissible residual TPH-in-soil for leached black soil of Tatarstan (2900 ± 290 mg/kg), oil content was achieved for versions "native strains + bentonite" (3341 ± 835 mg/kg), "Gumaks" (4071±1018 mg/kg).

Phytoproductivity is a complex indicator, revealing chronic phytotoxicity, which was proved by our previous studies at the development of standards of admissible residual TPH-in-soil (Ibatullin et al., 2006; Malyhina et al, 2012; RD 153-39.0-716-11 ..., 2010).

Determination of phytoproductivity of contaminated soil after the remediation measures for Devonian oil spill was conducted in the first season for wheat germ (Triticum L.), grown at the experimental plots after harvest taken from uncontaminated area. From each plot 15 plants were investigated. We have studied their appearance, average height and dry phytomass per plant. The results revealed the lowest result of development and growth of plants in options "control" (11.7 cm) and "traditional method" (13.6 cm), the highest in option – "indigenous strains + nanosorbent" (19 cm).

By the magnitude of phytoproductive the plot "indigenous strains+nanosorbent" (0.0471 g) was the best, the worst – "control" (0.0229 g), "Lenoyl-SKhP" (0.0224 g), "traditional" (0.0280 g). On the unspoiled area (background) indicators of average height of wheat plants reached 23.7 cm, and a biomass – 0.1325 g.

Furthermore, to determine phytoproductivity in laboratory conditions we set an experiment in vegetative pots (volume 500 cm3) with soil samples from experimental plots, the exposure time 3 weeks. As test objects we used two types of plants: monocotyledon – spring wheat (Triticum vulgare L.) of varieties Ekada-97 and duocotyledon plant – pea (Pisum sativum L.), of varieties Varne. The choice of these species was due to the high economic importance for the Republic of Tatarstan.

Data analysis by vegetative pot experiments in the laboratory for areas with spill of Devonian oil showed the worst development of plants for the test object peas – in a version "control" (0.0384 g), wheat – in the version "traditional method" (0.0091 g). The best development of plants on the peas was in the variant "DNZ" (0.0663 g), wheat – "potassium humate" (0.0130 g). The value of phyto-mass in the pots with background soil for peas was 0.0591 g, for wheat – 0.0107 g.

The results of pot experiment conducted in the laboratory for areas with spill of sour oil were comparable for all variants. On both test objects, options "Lenoyl SKhP", "traditional", "DNZ", "native strains + nanosorbent", "Gumaks", "potassium humate".

Thus, based on the above stated for the first season, we may conclude of the effectiveness of all biotechnologies. The process of reclamation using the agrotechnical measures covers the lower horizons of the contaminated soil, where non-volatile heavy hydrocarbons remained. In many ways, if you prefer full-scale field study, methods "native strains + nanosorbent", "Gumaks" have some advantage.

Parameters of plant development in the second season for the area of Devonian oil spill were evaluated on appearance, color, height, weight of fresh-cut plants, and ears of wheat (Table 2). On average phytomass of a single plant, a single ear, height, color and plant growth parameters the best reclamation was shown by technologies "native strains + bentonite" and "Gumaks", the traditional method is inferior. The lowest values, as we would expect, were obtained for option "control".

For the plot with sour oil spill, the best phytoproductive performance on the test object Tripleurospermum inodorum (L.), comparable to phytoproductivity in the background section, were obtained for the variants "native strains + bentonite", "Gumaks" (Table 3). For the test object Lotus comiculatus L. the best phytoproductive figures were recorded for the options "native strains + bentonite", "Gumaks", "potassium humate" (Table 3).

The options "native strains + nanosorbent" (4070 rub./Ha), "Gumaks" (from 4200 to 5400 rub./Ha), "potassium humate" (5900 rub./ Ha) are the most effective from an economic point of view. The rest of the discussed technologies are much more expensive than the traditional method (31000 rub./Ha). Since agricultural activities cost is about the same, in the calculation we took into account

Technology	Color (11.07.14)	Average height of 1 plant, sm (11.07.14)	Average weight of 1 plant, g (11.07.14)	Weight of 1 ear, g (11.07.14)	Weight of 1 ear, g (14.08.14)
Native strains + bentonite	green	94	17,6	2,0	4,86
DNZ	green with yellowish patch	72	5,4	1,0	2,34
Lenoyl SKhP	yellow-green	66	6,2	1,1	2,36
Gumaks	green	78	17,3	2,0	3,5
Potassium humate	green	67	8,6	1,1	2,28
Traditional method	yellow-green	62	3,6	1,0	1,82
Control	yellow-green	52	3,2	0,9	1,64
Background	green	95	27,1	2,4	4,9

Table 2. The results of the phytoproductivity study on the test object wheat (Triticum L.), (section with Devonian oil spill, 2nd season).

Technology	Color	Average height of 1 plant, sm Tripleurospermum inodorum (L.)	Average weight of 1 plant, g Tripleurospermum inodorum (L.)	Average height of 1 plant, sm Lotus comiculatus L.	Average weight of 1 plant, g Lotus comiculatus L.
Native strains + bentonite	bright-green	73	230,0	30	8,6
DNZ	yellow-green	36	70,0	21	4,4
Lenoyl SKhP	yellow	60	77	17	2,23
Gumaks	bright-green	73	180,0	33	11,3
Potassium	green	60	50,0	31	16,1
Traditional method	yellow	39	23,33	20	5,7
Control	yellow	43	30,0	16	2,3
Background	bright-green	73	180	30	8,5

Table 3. Research results of phytoproductivity (section with spill of sour oil) for test objects Tripleurospermum inodorum (L.) and Lotus comiculatus (L.) (11.07.2014).

only the costs associated with the application of fertilizers, soil improver and drugs.

Thus, within the framework of these studies, the recommended technologies to remedy the black soil contaminated by the oilfield fluid are methods "Native strains + nanosorbent" and "Gumaks".

Recommendations for the production

To remedy the black soil of mixed type of pollution in the Republic of Tatarstan we propose to use:

1) Method using "Gumaks" proposed by LLC "Center Spas", environmentally and cost-effective method, easy to use;

2) Method developed by FBGNU Tatar NIIAHP with the release of native strains of hydrocarbon-oxidizing microorganisms in combination with bentonite.

When using the traditional method, rotted manure (humus) should be used to accelerate the recovery of soils.

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