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Biogeochemical technologies for the reclamation of polluted and disturbed soils in gas-oil industry impacted areas

Biogeohemijske tehnologije za rekultivaciju zemljišta zagađenog ili oštećenog prilikom dobijanja nafte i gasa

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Abstract: It has been established that biogeochemical technologies for the reclamation of pyrogenic and hydrocarbon-contaminated soils, as well as the neutralization of hydrocarbon sludge, tested in vitro and in situ at gas industry facilities in various soil and climatic conditions of Russia (Moscow Region and Stavropol Territory), restore the biogeochemical cycle in its microbial link and the fertility of disturbed and contaminated soils in impact ecosystems.

Keywords: biogeochemical technologies, bioremediation, pyrogenic soils, hydrocarbon pollution

Sažetak: Utvrđeno je da biogeohemijske tehnologije za rekultivaciju pirogenog i ugljovodonički kontaminiranog zemljišta, kao i neutralizaciju ugljovodoničnog mulja, testirane in vitro i in situ na objektima gasne industrije u različitim zemljišnim i klimatskim uslovima Rusije (Moskovska oblast i Stavropoljska pokrajina), dovode do obnavljanja biogeohemijskih ciklusa u njihovoj mikrobnoj vezi i kao plodnosti poremećenog i kontaminiranog zemljišta pogođenih ekosistema.

Ključne riječi: biogeohemijske tehnologije, bioremedijacija, pirogeno zemljište, zagađenje ugljovodonicima.

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INTRODUCTION

Biogeochemical technologies for the reclamation of pyrogenic and hydrocarbon-contaminated soils, and the neutralization of hydrocarbon slurries mean technologies that include approaches to restoring the biogeochemical structure and fertility of pyrogenic soils, cleaning soils from oil, gas condensate and neutralization of hydrocarbon slurries (Galiulin et al., 2017; Galishev, 2017). These technologies have been successfully tested in situ and in vitro in disturbed and polluted ecosystems in various soil and climatic conditions of the country – the Moscow region (55°37' N, 37°44' E) and the Stavropol Territory (45°03' N, 43°16' E) (Afanasyeva et al., 1979; Kaurichev et al., 1982) in order to restore the soils of impacted ecosystems.

1. BIOGEOCHEMICAL TECHNOLOGY OF PYROGENIC SOIL RECLAMATION IN SITU IN THE STAVROPOL TERRITORY

Biogeochemical reclamation technology was tested *in situ* to restore pyrogenic dark chestnut soil of the barn berm, i.e. the strip adjacent to the circular contour of this gas industry facility (Stavropol Territory) (Bashkin, Galiulin, 2010; Bashkin et al., 2011). The cause of accidental ignition of the studied dark chestnut soil of the barn berm, together with the vegetation growing on it, could be its saturation with hydrocarbons of gas condensate entering as part of liquid waste generated during cleaning of the cavity of gas pipelines and reservoirs, which gave high combustibility and led to pyrogenic formation of terracotta color (Fig. 1).

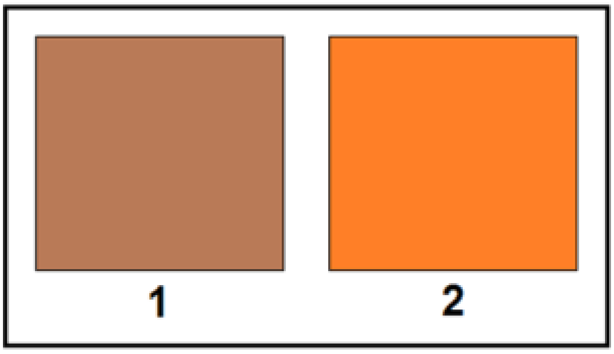

Fig. 1. Morphological (color) signs of dark chestnut soil from: 1 - background soil of dark chestnut color and 2 - pyrogenic soil of terracotta color, Stavropol Territory (45 -03' N, 43-16' E)

It should be noted that soil ignition can occur at concentrations of hydrocarbons 4-8 times lower than concentrations at which the combustible substance is able to separate into a separate phase and form liquid accumulations on the soil surface (Galishev, 2017). As can be seen from Table 1, pyrogenic soil differs significantly from background soil in physico-chemical and biochemical characteristics.

Studies have also shown that the activity of catalase and dehydrogenase of pyrogenic soil was very low, respectively, 23.8 and 4.7% of the activity of the background soil. The minimal activity of these enzymes is due to the fact that during pyrogenation of the soil, only a small pool of thermotolerant forms of microorganisms survives, which remain in an adsorbed state on charred materials and in their inner part, unaffected by fire (Ten Hak Moon et al., 2003; Zaidelman, Romanov, 2007).

Meanwhile, the recultivation of pyrogenic soil was carried out as follows: plots (0.25 m^2) were previously arranged on the barn berm, into which, after loosening the soil (layer 0-15 cm), the "Piksa" biocompost was sealed in doses of 6.5 and 13 kg/m^2; then a mixture of perennial grasses was sown: meadow bluegrass (*Poa pratensis*), red fescue (*Festuca rubra*), pasture grass (*Lolium perenne*), common comb (*Cynosurus cristatus*), white vole (*Agrostis alba*) and common vole (*Agrostis vulgaris*). The control variant was a plot without adding biocompost, but with sowing and growing herbs. Soil and plant samples were taken for analysis on 42 days. The efficiency of pyrogenic soil reclamation was evaluated by determining the activity of catalase and dehydrogenase enzymes, as well as the biomass of a mixture of perennial grasses (Bashkin et al., 2011; Bashkin et al., 2010; Galiulin et al., 2010; Galiulin et al., 2017).

As can be seen from Table 2, the introduction of "Piksa" biocompost into pyrogenic soil increased the activity of catalase and dehydrogenase enzymes, depending on the dose, by 1.4 and 1.6, and 2.5 and 3.0 times, respectively, relative to the control variant.

Table 1. Physico-chemical and biochemical characteristics of soils (Stavropol Territory)

Soil	$pH_{salt.}$	Content, %			Enzyme activity, %	
		silt, <0,001 mm	physical clay, <0,01 mm	physical sand, >0,01 мм	catalase	dehydro-genase
background	5,7	26,0	54,4	45,6	100	100
pyrogenic	3,6	4,3	32,2	67,8	23,8	4,7

Table 2. Enzyme activity and biomass of a mixture of perennial grasses during the recultivation of pyrogenic soil using the "Piksa" biocompost (Stavropol Territory)

Variant	Catalase activity, ml O_2/(min·g)	Dehydrogenase activity, mg 2,3,5-triphenylformazane ($C_{19}H_{16}N_4$) /(g·day)	Biomass of a mixture of perennial grasses, g/m^2
Without biocompost (control)	2,7	0,2	146
Biocompost, 6,5 kg/m^2	3,9	0,5	257
Biocompost, 13 kg/m^2	4,4	0,6	343

As for the biomass of a mixture of perennial grasses, it increased with an increase in the dose of biocompost applied to the soil, respectively, by 1.8 and 2.3 times relative to the control variant.

Thus, the approbation of biogeochemical technology of pyrogenic soil recultivation in situ in the Stavropol Territory showed that an increase in the activity of the studied enzymes and the fact of good growth of a mixture of perennial grasses on the barn berm is sufficient evidence of the restoration of the microbial link of the biogeochemical cycle and, accordingly, its fertility as a result of the introduction of the "Piksa" biocompost. This technology may be in demand when restoring lands that have been exposed to fire due to various emergency situations, for example, in the gas industry.

2. BIOCGEOHEMICAL TECHNOLOGY FOR RECULTIVATION OF SOIL CONTAMINATED WITH HYDROCARBONS *IN VITRO* IN THE MOSCOW REGION

The biogeochemical technology of recultivation of soil contaminated with hydrocarbons (gas condensate and oil) was tested in vitro using an average representative sample of disturbed gray forest soil (Moscow region), i.e. with the absence of an upper organogenic layer and representing an illuvial horizon (50-90 cm layer), and by granulometric composition - heavy loam with a pH of 6.2, fig. 2 (Bashkin, 2017; Galiulin et al., 2017).

The soil sample was treated with gas condensate and oil in doses of 50 and 100 g/kg. In the first series of experiments, “Bioros” biopreparation (0.1 and 0.5 g/kg) was introduced into the soil contaminated with gas condensate and oil in the form of a suspension obtained by homogenizing it in an ammophoska solution (6 g/l), carried out by bubbling air for 3 hours. This biopreparation was developed on the basis of strains (pure cultures) of two physiological groups of microorganisms - bacteria *Rhodococcus sp*. and yeast Candida sp. with a total of 1010 cells/g (Galiulin et al., 2010).

In the second series of experiments, "Piksa" biocompost (50 and 100 g/kg), obtained by accelerated fermentation of peat-manure mixture and enriched with soil microorganisms numbering 106 cells/g and nutrients, was introduced into the soil contaminated with oil (Sementsov, 2006, Sementsov et al., 2006). The soil samples treated with gas condensate, oil and biological agents were incubated in plastic containers (volume 250 ml) at a constant humidity of 70% of the total moisture capacity: the first 20 days at a temperature of 8° C, the next 20 days at a temperature of 18° C. Such a temperature regime was associated with the simulation of the annual course of temperature in the studied soil layer in the period May-July of this region.

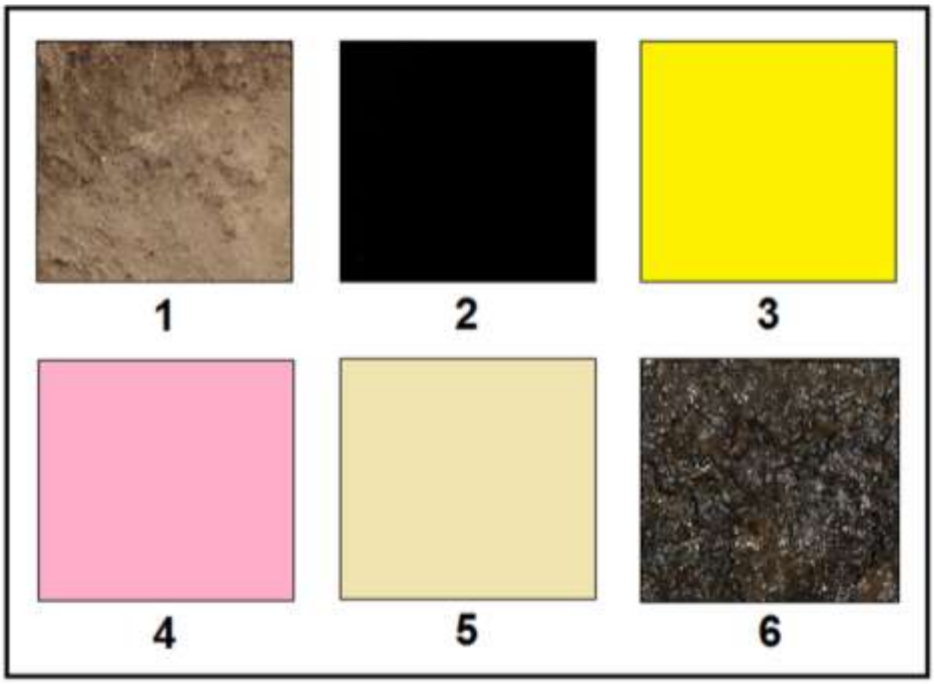

Fig. 2. Morphological (color) signs of disturbed gray forest soil and substances used in the experiment for its purification from hydrocarbons in vitro: 1 - the illuvial horizon of the soil is brownish, 2 - oil, black, 3 - gas condensate, bright yellow, 4 - colonies of microorganisms Rhodococcus sp. pink color, 5 colonies of Candida sp. microorganisms. pale yellow and 6 - biocompost "Piksa", dark brown with inclusions, Moscow region (55°37' N, 37°44' E)

On the 10th and 40th days, the content of hydrocarbons of gas condensate and oil in the soil was analyzed by infrared spectrometry on the IKN-025 concentrator. For this purpose, 1 g of soil sample was extracted with 50 ml of carbon tetrachloride (CCl_4) for 5 min in the Ekros-8000 extractor. After settling (10 min), the extract was passed through a chromatographic column with aluminum oxide (Al_2O_3) and the hydrocarbon content of gas condensate and oil was analyzed on a concentrator.

To confirm the microbiological nature of decomposition, in particular, of petroleum hydrocarbons, on the 40th day, the activity of catalase and dehydrogenase enzymes was determined by appropriate methods (Bashkin et al., 2010b; Bashkin et al., 2010c). It should be noted that according to (Khaziev, Fathiev, 1981) catalase and dehydrogenase are the most important and widespread catalysts of the oil decomposition process in soil microorganisms.

The data of the analysis of the content of hydrocarbons of gas condensate and oil in the soil were used to calculate the time of their almost complete decomposition, i.e. by 99% (T_{99}) exponentially:

$y = e^{-kt}$,

where y is the residual hydrocarbon content for time t, related to the initial y_0; e is the base of the natural logarithm; k is the rate constant of decomposition of hydrocarbons (Galiulin et al., 1984). The corresponding formula for the calculation looks like this:

$T_{99} = ln\ 100/k$.

Studies have shown that the decomposition of hydrocarbons of gas condensate and oil at the same dose accelerated with an increase in the amount of “Bioros” from 0.1 to 0.5 g/kg, respectively, by 2.2 and 2.8 times, Table 3.

As can be seen, the decomposition of oil in the soil was slower compared to gas condensate and, depending on the dose of the applied bioproduct, by 1.4 and 1.1 times, which is due to the high content of arenes in the oil. It is known that alkanes and cyclanes are more readily available for microbiological decomposition compared to arenes, as the three main groups of hydrocarbons in oil and gas condensate (Efremova, Ovchinnikova, 2008).

Studies on the decomposition of petroleum hydrocarbons under the action of “Piksa” biocompost showed that when its doses in the amount of 50 and 100 g/kg were introduced into the soil, the time of almost complete decomposition of hydrocarbons was reduced relative to the control variant at an oil concentration of 50 and 100 g/ kg, respectively, by 1.8 and 4.8 times and 1.7 and 4.0 times, Table 4.

Table 3. The time of almost complete decomposition (T99) of hydrocarbons of gas condensate and oil in the soil under the action of “Bioros” (Moscow region)

Variant	T_{99}, days	Variant	T_{99}, days
Gas condensate (50 g/kg) + “Bioros” (0.1 g/kg)	125	Oil (50 g/kg) + “Bioros” (0.1 g/kg)	171
Gas condensate (50 g/kg) + “Bioros” (0.5 g/kg)	56	Oil (50 g/kg) + “Bioros” (0.5 g/kg)	61

Table 4. Time of almost complete decomposition of petroleum hydrocarbons in the soil under the action of the "Piksa" biocompost (Moscow region)

Variant	T_{99}, days	Variant	T_{99}, days
Oil (50 g/kg), control	329	Oil (100 g/kg), control	1150
Oil (50 g/kg) + “Piksa” biocompost (50 g/kg)	184	Oil (100 g/kg) + “Piksa” biocompost (50 g/kg)	658
Oil (50 g/kg) + “Piksa” biocompost (100 g/kg)	69	Oil (100 g/kg) + “Piksa” biocompost (100 g/kg)	288

As for the mechanism of microbiological decomposition of hydrocarbons of gas condensate and oil, carried out, in particular, by bacteria of the genus *Rhodococcus*, which are part of the “Bioros” biopreparation, it consists in the absorption of substances by hydrophobization of the cell wall, realized through the biosynthesis of specific compounds (lipophilic glyco-, peptido- and peptidoglycolipids) (Russian Gas Encyclopedia, 2004). With direct contact of bacteria with a film of hydrocarbons, the latter penetrate into the cell by passive diffusion, gradually impregnating the cell wall, and reach the location of enzymes on the membranes. Along with the molecular diffuse passage of hydrocarbons through the surface of the entire cell wall, their entry is also possible through special ultramicroscopic pores. Such pores filled with an electron-dense (granular) substance were first discovered in yeast: one of the strains of this physiological group of microorganisms *Candida sp.*, it is included in the “Bioros” as an active component.

Diagnostics of the decomposition of petroleum hydrocarbons by analyzing the activity of catalase and dehydrogenase enzymes showed that the control variants were characterized by the lowest values of these biochemical parameters, Table 5.

Table 5. Activity of catalase and dehydrogenase enzymes during the introduction of "Piksa" biocompost into oil-contaminated soil at doses of 50 and 100 g/kg (Moscow region)

Variant	Catalase activity, ml O_2/(min·g)	Dehydrogenase activity, mg 2,3,5-triphenylformazane ($C_{19}H_{16}N_4$) /(g·day)
Oil (50 g/kg), control	0,1	0,13
Oil (50 g/kg) + “Piksa” biocompost (50 g/kg)	1,5	0,74
Oil (50 g/kg) + “Piksa” biocompost (100 g/kg)	2,7	1,38
Oil (100 g/kg), control	0,1	0,15
Oil (100 g/kg) + “Piksa” biocompost (50 g/kg)	0,9	0,71
Oil (100 g/kg) + “Piksa” biocompost (100 g/kg)	2,2	2,34

However, when applying biocompost at doses of 50 and 100 g/kg, catalase activity in soil contaminated with oil in the amount of 50 g/kg increased by 15 and 27 times, and contaminated with oil in the amount of 100 g/kg - by 9 and 22 times relative to the control variant. When applying biocompost at a dose of 50 and 100 g/kg, the dehydrogenase activity in the soil contaminated with oil in the amount of 50 g/ kg increased by 5.7 and 10.6 times, and contaminated with oil in the amount of 100 g/ kg - by 4.7 and 15.6 times relative to the control variant.

An increase in the activity of these enzymes during the introduction of biocompost confirms the microbiological nature of the decomposition of petroleum hydrocarbons. Studies (Coronelli, 1996) have established that in *Candida tropicalis* yeast grown on hydrocarbons, there is a direct relationship between an increase in the number of cellular peroxisomes (organelles in the cytoplasm of the cell) and an increase in catalase activity in which this enzyme is localized.

Thus, the approbation of biogeochemical technology for recultivation of soil contaminated with gas condensate and oil in vitro in the Moscow region has shown that there is a real possibility of using biological agents in the form of “Bioros” bioproduct and “Piksa” biocompost for cardinal purification of soil from hydrocarbons entering it in emergency situations in the oil and gas industry. At the same time, the microbial link of the biogeochemical cycle is restored.

3. BIOGEOCHEMICAL TECHNOLOGY FOR RECULTIVATION OF SOIL CONTAMINATED WITH GAS CONDENSATE IN SITU IN THE STAVROPOL TERRITORY

Biogeochemical technology of recultivation of soil contaminated with gas condensate in situ was tested on the territory of a booster compressor station (Stavropol Territory) with chernozem heavy loamy soil (pH_{salt} 7,1-7,3) (Bashkin, 2017). There was a chronic contamination of this soil as a result of the intake of gas condensate from the "purge candle" (vertical pipe with a head), which was expressed in the constant feeling of a specific smell of gasoline and kerosene components of this substance and in the complete absence of vegetation in a certain area around the "purge candle", Fig. 3.

In order to recultivate the soil contaminated with gas condensate, plots (0.25 m^2) were arranged on the desired site, in which, after loosening the soil (a layer of 0-6 cm), the "Piksa" biocompost was sealed in doses of 4 and 8 kg/m^2. Then, to create a dense herbage and dense turf that protects the soil from erosion, a mixture of perennial grasses (30 g of seeds per 1 m^2) was sown and grown: meadow bluegrass (*Poa pratensis*), red fescue (*Festuca rubra*), white vole (*Agrostis alba*), etc.

The control variant was a plot without adding biocompost, but with sowing and growing herbs. Soil and plant samples were taken for analysis on 42 days. The efficiency of recultivation of soil contaminated with gas condensate was assessed by determining the activity of catalase and dehydrogenase enzymes, as well as the biomass of a mixture of perennial grasses.

The analysis of the hydrocarbon content of gas condensate in the surface layer of the soil (0-6 cm), carried out by infrared spectrometry (Galiulin et al., 2010), showed an uneven nature of chronic contamination of the area around the "purge candle", i.e. the amount of the substance fluctuated in the range of 1.8-5.4 g/kg on different plots. It is interesting to note that these quantities turned out to be of the same order with the hydrocarbon content observed in soils around a number of gas stations (1.9-8.2 g/kg), which is determined by the state of dynamic equilibrium of evaporation and sorption of substances, depending on hydrothermal conditions (Coronelli, 1996). In general, this indicates the identity of the anthropogenic load on the environment of two different sources of high-boiling fractions of hydrocarbons (gas condensate, gasoline and diesel fuel).

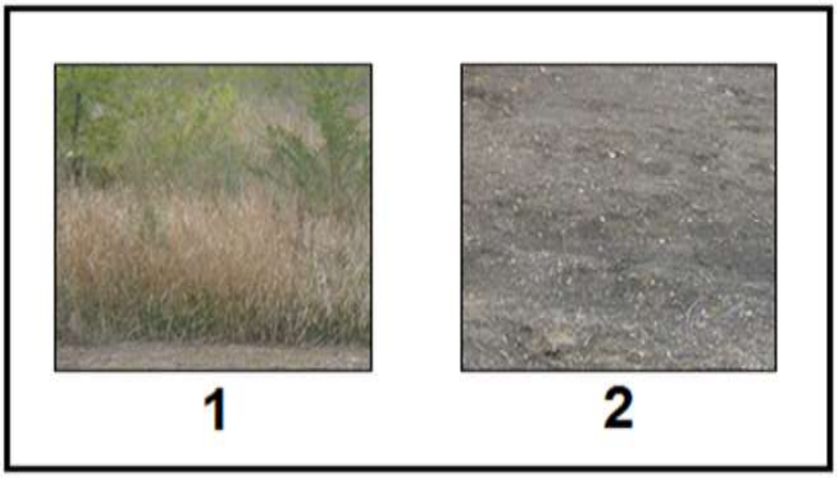

Fig. 3. Chronic contamination of chernozem soil with gas condensate from a "purge candle" on the territory of a booster compressor station: 1 - a background area with vegetation; 2 - a site contaminated with gas condensate and devoid of vegetation, Stavropol Territory (45°03' N, 43°16' E)

Meanwhile, when introducing biocompost into the soil, the activity of catalase and dehydrogenase enzymes increased by 1.9 and 2.4, respectively, and 5.8 and 8.9 times compared to the control variant, Table 6.

Table 6 Enzyme activity and biomass of a mixture of perennial grasses during reclamation of soil contaminated with gas condensate using the "Piksa" biocompost (Stavropol Territory)

Variant	Catalase activity, ml O_2/(min·g)	Dehydrogenase activity, mg 2,3,5-triphenylformazane ($C_{19}H_{16}N_4$) /(g·day)	Biomass of a mixture of perennial grasses, g/m^2
Without biocompost (control)	1,4	53	55
Biocompost, 4 kg/m^2	2,7	306	473
Biocompost, 8 kg/m^2	3,4	470	932

An increase in the activity of catalase and dehydrogenase during the introduction of biocompost indicates the effectiveness of recultivation of soil contaminated with gas condensate, in the decomposition of which the studied enzymes are directly involved (Teranishi et al., 1974).

The results of sowing and growing a mixture of perennial grasses showed that the weight of plant biomass when applying biocompost at doses of 4 and 8 kg/m^2 was 8.6 and 16.9 times higher compared to the control. It should be noted that the chronic contamination of the soil with gas condensate in the area around the "purge candle" determines the need for constant monitoring of the state of the herbage, during the thinning of which it is necessary to resume the introduction of biocompost, sowing and growing a mixture of perennial grasses.

Thus, the testing of biogeochemical technology for recultivation of soil contaminated with gas condensate in the Stavropol Territory in situ showed that an increase in the activity of the studied enzymes and the fact of good growth of a mixture of perennial grasses in the area around the "purge candle" booster compressor station is sufficient evidence of the restoration of soil fertility as a result of the introduction of biocompost "Piksa". This technology makes it possible to actively counteract the process of soil erosion, and, consequently, to avoid a clear threat to the geological stability of engineering and technical structures of the gas industry.

4. BIOGEOCHEMICAL TECHNOLOGY FOR NEUTRALIZATION OF GAS CONDENSATE SLUDGE IN SITU IN THE STAVROPOL TERRITORY

Biogeochemical neutralization technology was tested in situ during the neutralization of gas condensate sludge stored in a storage pond in one of the gas industry facilities of the Stavropol Territory. The storage pond is an object of increased risk due to the constant evaporation of toxic substances from the open surface into the atmospheric air and the possibility of their accidental ignition.

In order to test the biogeochemical neutralization technology, samples of gas condensate sludge were taken from the storage pond into plastic vessels (volume 5 liters) in 6-fold repetition, followed by the introduction of the "Piksa" biocompost in a ratio of 1:1 and homogenization of the resulting mixture. The vessels were incubated under a canopy, in order to avoid atmospheric precipitation, with periodic mixing of the contents (every 7 days). Gas condensate sludge was sampled on day 42 to analyze the activity of catalase and dehydrogenase enzymes directly involved in the decomposition of hydrocarbons.

It was found that the addition of the "Piksa" biocompost to the gas condensate sludge, even in a ratio of 1:1, the activity of both catalase and dehydrogenase enzymes immediately increased from zero, respectively, to 3.3 ml O_2/(min•g) and 11.0 mg 2,3,5-triphenylformazane ($C_{19}H_{16}N_4$)/(g•day). Under these conditions of neutralization of the gas condensate sludge, the conjugate nature of the action of enzymes by the type of linear function was clearly manifested, when hydrogen separated by dehydrogenase from the hydrocarbon molecules of the gas condensate is transferred to oxygen in the air with the formation of hydrogen peroxide, the decomposition of which is carried out by catalase (Khaziev, Fathiev, 1981).

Thus, the approbation of the biogeochemical technology of neutralization of gas condensate sludge in situ in the Stavropol Territory showed that the manifestation of the activity of the studied enzymes when introducing the "Piksa" biocompost into waste is sufficient proof of the fact of their neutralization. This technology can contribute to the cardinal solution of the problem of neutralization of gas condensate sludge in storage ponds by conducting a procedure for continuous neutralization of waste accumulated in them.

CONCLUSION

Biogeochemical technologies for the reclamation of pyrogenic and hydrocarbon-contaminated soils, as well as the neutralization of hydrocarbon sludge, based on methods protected by patents of the Russian Federation for inventions, have been successfully tested in vitro and in situ mainly at gas industry facilities in various soil and climatic conditions of the country (Moscow Region and Stavropol Territory). The restoration of the biogeochemical cycle in its microbial link and the fertility of disturbed and polluted soils in impact ecosystems is shown.

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