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USING MICROORGANISMS FOR SOIL PURIFICATION FROM HIGH-PARAFFIN CRUDE OIL

Abstract. This paper presents the research data aimed at resolving the problem of soil purification from high-paraffin crude oil using microorganisms. Samples of oil-contaminated soil were taken from the walls of the oil storage pit at the Uzen oilfield. According to the analysis of the oil-contaminated soil samples, the share of oil was 28 %, the share of mechanical impurities – 9.7 %, and the share of water – 60 %. It has been found that according to the API (American Petroleum Institute) indicators, that oil chemical composition may contribute to its rapid decomposition during bioremediation. Paraffin hydrocarbons are also easily decomposed by bacteria. The bacterial preparation «SHER» was used in laboratory experiments. The method of bacteria immobilization with the «SHER» biological product on the medium made from screenings of shell limestone wastes has been chosen. The conditions for conducting experimental laboratory studies on cleaning oil-contaminated soils by biological remediation are described. According to the results of the research, it was established that the immobilization of bacteria of the biological product "Sher" by a carrier in the form of sifting limestone-shell rock showed a high degree of purification of oil-contaminated soil (88.63%). To study the further activity of bacteria, experiments were conducted on the secondary use of the solution of residual mother water of the biological product "SHER", used in the process of cleaning oil-contaminated soil. The results of experimental studies using solution of residual mother water and immobilization by screening showed the degree of soil purification (45.7%).

Thus, based on the results of studies of the drug "SHER", it can be concluded that this drug can effectively neutralize oil products in the soil.

Keywords: soil, oil, microorganisms, biological product, screenings, immobilization.

Introduction. The main environmental problem arising from various accidents at oilfields where high-paraffin crude oil is developed is the contamination of soil and surface waters. At the same time, the water-and-air conditions of the soil are disrupted, and a sharp reduction of the natural redox reactions occurs.

A widely known and a priority line of efforts in soil purification and remediation of oil-contaminated soil (OCS) is bioremediation [1,2]. It is known that complete decomposition of petroleum hydrocarbons requires exposure to many bacteria of various species, mainly lactic acid bacteria (LAB). The SHER biological product used for soil purification from oil is based on *Lactobacillus* and *Pediococcus*, which form the core of this group of bacteria. It has been found that upon immobilization of carbon-oxidizing microorganisms (COM) with LAB of the *Lactobacillus* genus, exopolysaccharides are stably formed in them. Thus, stable degradation of hydrocarbons is achieved. *Lactobacilli* have a strong potential for synthesizing exopolysaccharides. The possibility of bacterial cells' growth and reproduction in the presence of the medium is also manifested. With that, the degree of the cells' immobilization may achieve 75–85 %. Researchers have found that paraffin hydrocarbons are most easily decomposed by bacteria. A promising line of efforts for soil purification from oil is COM immobilization on various media (peat, vermiculite, etc.). It is known that a neutral medium is perfect for biodegradation [3]. For instance, for

saline (alkaline) soil, the introduction of a medium in the form of shell limestone screenings improves the contact of OCS with the microorganisms. It helps neutralize the medium.

The cells of the product bacteria immobilized on the medium are not subjected to leaching and weathering, and retain viability under direct exposure to solar radiation. In this regard, the use of biological products with several types of microorganisms immobilized by a medium made of local materials is relevant.

The work has been aimed at experimental studying and at assessing the effectiveness of purifying the soil contaminated with such oil using the SHER biological product.

Materials and methods. The physicochemical properties of the oil were studied at the Ken-Sary LLC accredited chemical laboratory. The oil density at 20 °C and 15 °C was determined according to normative document (ND) KR ST 2.153-2008/ST RK. The mass content of water was determined following ND GOST 2477-65. The concentration of chloride salts was determined following ND GOST 21534-76. Chloride salts were extracted from the oil with water, followed by color-indicator titration of the aqueous extract from the oil solution. An oil sample was thoroughly stirred for 10 minutes by shaking in a flask, and filled with water to 2/3 of its capacity. Immediately after shaking, the oil sample was pipetted for analysis. The mass content of solids was determined following ND GOST 6370-83.

Identification of the consortium of microorganisms in the SHER biological product. The data about the total quantitative (in percent) microbial composition of the biological product at the gene level were obtained at the Scientific Production Center for Microbiology and Virology LLP in Almaty, Kazakhstan. The new-generation method of sequencing on a MiSeq Illumina (USA) full-fledged sequencer was used. The data were processed using the MiSeqReporter application by comparing the V3 and V4 regions of the 16S rRNA gene to the corresponding sections of the reference strains in the Greengenes Database international database [4]. The data were obtained directly from the product, without the stage of cultivation on a nutrient media. Twenty-three organic compounds were identified with the probability of at least 90 % using the chromatographic analysis of the hexane extract from a sample of the soil purified from oil. The Agilent MSD ChemStation (version 1701 EA) software was used for controlling the system of gas chromatography and data processing.

The chemical and mineralogical composition of the screenings of shell limestone wastes. The chemical and mineralogical composition of a sample of shell limestone was studied at the Central Laboratory for Certification Testing of Construction Materials LLP (Tselsim LLP) in Almaty, Kazakhstan. The X-ray diffraction analysis (XRD) was performed on a modernized CuK_α radiation DRON-3M diffractometer with appropriate software. The XRD patterns were obtained in the angles interval of 10...70 degrees. The sample was prepared according to the following scheme. Initially, the tested substance was crushed in an agate mortar to powder, the powder was then placed into a Plexiglas cuvette pre-lubricated with vaseline, and pressed a little. Next, in order to remove the texture, the excess of the powder was cut off with a razor blade. The derivatographic analysis (DTA) was performed on an upgraded Derivatograph Q-1500D installation made by the MOM Company. For the analysis, the sample was prepared in the following order. The tested substance was crushed in an agate mortar to powder. A weighed sample of the tested substance was then placed in a crucible. Thermograms were taken up to the temperature of 1,000 °C with the heating rate of 10 °C per minute. Al_2O_3 powder was used as the reference in the thermocouple; the thermograms were taken up to the temperature of 1,000 °C with the heating rate of 10 °C per minute.

Results and discussion. The effectiveness of purification of the soil contaminated with high-paraffin crude oil was studied using the «SHER» biological product developed at «Scientific Industrial Enterprise Altai Agro Farm Ltd». It is known that decomposition of petroleum hydrocarbons with biological products requires studying the soil type in the area. It is also necessary to consider the level of soil contamination with oil.

Samples of oil-contaminated soil (OCS) were taken from the walls of the oil storage pit at the Uzen oilfield. The soil cover at the Uzen oilfield is represented by gray-brown desert soil. This soil is formed on the carbonate and gypsum eluvium of Sarmatian limestones, in the conditions of the sharply nonleaching type. The total share of salts is 1.128 %, including chlorine – 0.06 %, sulfates – 0.72 %, calcium – 0.305 %, and magnesium – 0.05 %. In general, the soil in this region ranges from gray-brown desert soil to saline soil, the same as throughout the entire coastal zone of the Caspian Sea [5,6]. According to the

analysis of the OCS samples, the share of oil was 28 %, the share of mechanical impurities – 9.7 %, and the share of water – 60 %. It is known that oil chemical composition influences the decomposition rate. Lower molecular weight of the product contributes to greater intensity of its decomposition. In the studies of USA scientists in the field of bioremediation, the API (American Petroleum Institute) gravity indicator was used, which reflected these differences in the oil chemical composition. For instance, crude oils with the API index more than 30 and the density less than 876 kg/m³ decomposed faster (within a few weeks and months). However, if the API index of crude oil was less than 20 (density more than 934 kg/m³), the decomposition process extended to three years or more. Table 1 shows some properties of the studied high-paraffin crude oil.

The table shows that according to the API indicators, this oil features the chemical composition that may contribute to its rapid decomposition. For instance, the properties of the high-paraffin crude oil from the Uzen oilfield, which therefore has a high congelation temperature within +30 – 36 °C, allow bioremediation, since the density of this oil in API degrees is more than 30. As to its qualities, this is a unique sweet crude oil, from which high-index (100 and above) motor oils are obtained.

The concentration of asphaltenes in oil (C₄) is much higher than that of polycyclic aromatic hydrocarbons (C). This property of the Uzen oil at the early stages of thermal decomposition, as a result of the asphaltenes' transition to the second phase, ensures formation of a microheterogeneous system. As a result, asphaltenes rapidly turn into carboides (Carbo – coal; Eidos – similar).

Table 1 – Some properties of the sump oil from the Uzen oilfield

Physicochemical and chemical (main components) properties of oil with high congelation temperature	Unit of measurement	Value
Density	API kg/m ³	31.4 – 35.7 856.7 – 874.1
The reaction of the medium	pH units	7.3 ± 0.5
Congelation temperature	°C	+ 30 – + 36 °C
Humidity	%	13.7 ± 2.6
Soil air	mg CO ₂ /(dm ³ ·h)	0.317
Nitrogen by Kjeldahl	% of weight	0.138 – 0.975
Paraffins	% of weight	13.6 – 21.8
Asphaltenes	% of weight	0.7 – 2.7
Silica gel resins	% of weight	16.1 – 23.8

The bioremediation experiment was performed at the «Ecology and chemical engineering» laboratory following the technology that consisted of mixing contaminated soil with the biological product and the medium. One-and-a-half-liter 20 cm high containers were used. During the 10 days of the experiment in the summer, when the temperature reached 35 °C, constant conditions were maintained at the laboratory. The temperature was maintained within 20 ± 2°C, the pH of the medium – within 6.7 – 7.3, and humidity – within 60 – 70 %. Soil humidity was determined by drying a soil sample to the constant weight at 105 ± 5 °C. An important indicator of soil bioactivity, which testified to the live activity of soil and activity of the biota, was soil respiration. The intensity of the microbial biomass respiration was determined following the Rowell's method [5].

The «SHER» biological product is based on the *Lactobacillus* and *Pediococcus* LAB, which form the nucleus of this group of bacteria, as well as the gram-negative spore-forming bacteria (*Citrobacter freundii*), which develop well in contaminated soil. Currently, bacterial strains of the *Lactobacillus* genus are widely used by researchers for developing new probiotics [7]. NGS sequencing of the consortium of microorganisms in the SHER biological product identified a microbiome of the following type: *Firmicutes* (96.42 %), *Proteobacteria* (1.78 %), unclassified (1.4 %), and other phyla (0.4 %). The dominant species in the community were the *Lactobacillus camelliae* bacteria (15.24 %). At the class level, 96.15 % of all the representatives of the consortium were identified as *Bacilli*, of which 95.69 % of the bacteria at the level of the order were identified as *Lactobacillales*. Classification at the level of the family identified the share of the *Lactobacillaceae* bacteria taxonomic unit as 95.28 %.

The SHER biological product was used as the ML. A plastic container (40 liters) was filled with 20 liters of water without chlorine, and 1 kg of the product was placed into it. The water was stirred with a wooden handle until complete dissolution, after which fifteen more liters of chlorine-free water were

added. The mixture was thoroughly stirred and left for 26 hours without closing the container for awakening the microorganisms. The dosages of introducing the SHER biological product as the ML are shown in table 2. The scientific novelty of the experimental studies was that the authors used an adsorbent in the form of limestone waste for achieving the efficacy of soil purification from oil by increasing the activity of COM [8].

For instance, in the experiments, the medium for immobilizing the bacteria in the composition of the «SHER» biological product was the screenings of the shell limestone wastes from the Uzen quarry. The physicochemical composition of the screenings was the following: carbonate calcite CaCO_3 with a small amount of magnesium carbonate $\text{Mg}(\text{CO}_3)$ and dolomite $\text{CaMg}(\text{CO}_3)$. The content of CaO was 53.59 %, and that of CO_2 – 43.71 %. The content of pure calcite was 56 %, and that of CO_2 – 44 %. Immobilization of COM in the SHER biological product on the limestone medium with stirring the solution for 3 – 5 hours contributed to the stable degradation of hydrocarbons only on the second day of the experiment [8,9].

Upon contact of the oil in OCS with the ML, the process of its decomposition by microorganisms started. Paraffin hydrocarbons in the oil were most easily decomposed by bacteria. The oil film on the surface of the solution prevented aeration. During the mixing process, oxygen was absorbed by the ML, which contributed to decomposition of lighter hydrocarbons with the release of a strong odor. Calcite in the screenings contributed to a more intense release of emulsion.

The presence of various bacteria species in the SHER biological product allowed achieving higher degree of oil degradation. The data in the experiments, show that increasing the dosage of the biological product (to 1.2 l) did not improve the effectiveness of soil purification from oil. At this stage, the reason was difficult to explain, since it required additional experiments. Thus, immobilization of bacteria in the SHER biological product on a medium in the form of shell limestone screenings showed a high degree of OCS purification (88.63 %) (figure 1). The appearance of the initial sample of OCS and a photomicrograph of purified soil are shown in figure 2.

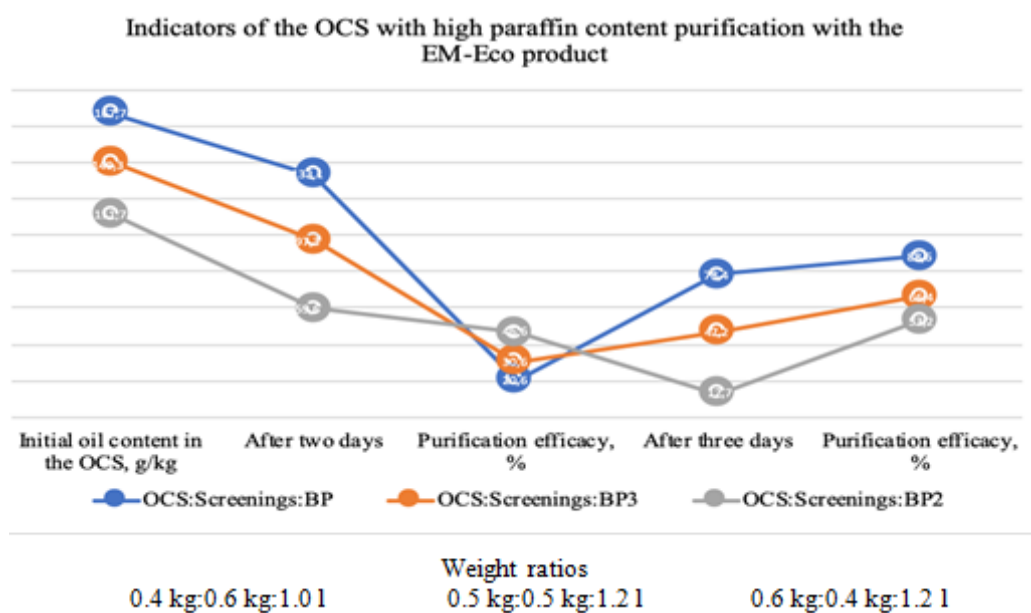


Figure 1 – The degree of OCS purification from oil using the SHER biological product

This result of OCS purification was obtained in a very short time at the laboratory. It is known that the bacteria that show a high degree of soil purification from oil at a laboratory are not always effective in real conditions. In this regard, in the future, in order to substantiate the effectiveness of the «SHER» biological product, studies will be performed directly in the area of DOW accumulation.

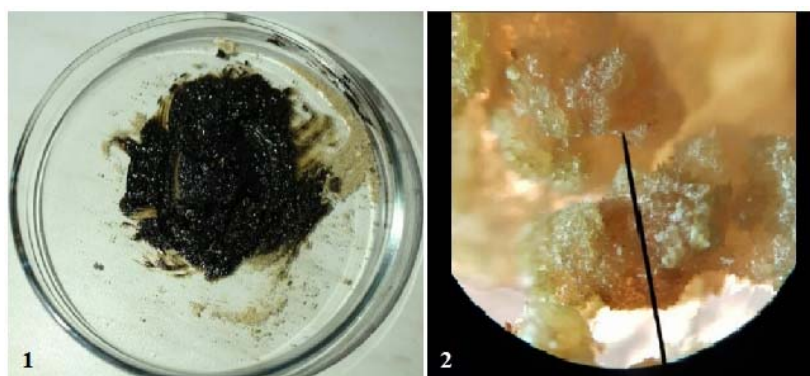


Figure 2 – The appearance of the initial OCS (1) and a photomicrograph of the purified soil (2).
Light crystals of paraffin are visible on the photo

To study the further activity of the bacteria, experiments were made on the secondary use of the residual mother liquor (RML) of the SHER biological product used for purification from organic wastes [10]. One-and-a-half kilogram samples of oil sludge (OS) in the form of a hardly separable emulsion were taken from the upper layer of the sludge tank. For long-term storage of OS, sludge tanks had three layers: the upper (hardly separable emulsion), the middle (contaminated water), and the bottom (with the predominance of mechanical impurities). On the surface of the sludge tank, no oil film was present. The surface of the OS samples was cleaned from the layer of dust and sand abrasive.

According to the results of the analysis, the content of the oil sludge in the samples was 28 %, that of mechanical impurities and residues of sand abrasive – 36 %, that of paraffin and asphaltene-resinous impurities – 26 %, and that of water – 17 %. The RML was filtered to remove the emulsion that had formed during the previous soil purification experiments. With that, the RML was used without delay [11]. Upon the secondary use of the RML with immobilization of the bacteria on a limestone medium during the work, same as in the above experiment, the substrate based on OS and screenings of shell limestone was stirred with the addition of the RML and water (table 2). Stirring was performed for two days 6 – 8 hours a day.

Table 2 – The results of the secondary use of the SHER ML

Indicator	Variants (No.) of the experimental studies in OCS purification		
	No. 1	No. 2	No. 3
Formulation	OS:Screenings:RML:H ₂ O	OS:Screenings:RML:H ₂ O	OS:Screenings:RML:H ₂ O
Weight ratios	50 g:70 g:20 ml:80 ml	50 g:60 g:50 ml:50ml	100 g:135 g:200 ml:20 ml
Initial oil content in OS, g/kg	13.8 ± 3.7	13.8 ± 5.14	27.6 ± 4.9
Residual oil content in the OCS after two days	9.8 ± 4.1	11.4 ± 3.8	21.0 ± 2.9
Purification efficacy, %	28.9	17.4	23.9
Residual oil content in the OCS after three days	7.5 ± 2.9	9.1 ± 4.7	19.8 ± 4.7
Purification efficacy, %	45.7	34.1	28.3

The highest effectiveness of cleaning soil using the RML and immobilization by the screenings was reached in variant No. 1 (45.7 %) after three days, with the ratios (OS:Screenings:RML:N₂O) equal to (50 g:70 g:20 ml:80 ml).

Conclusion. In the experimental studies, determined that paraffin hydrocarbons are most easily decomposed by the Lactobacillus and Pediococcus LAB, on which the «SHER» biological product is based.

Immobilization of the bacteria of the biological product on a medium made from local material in the form of screenings of shell limestone wastes has contributed to stable oil degradation.

The presented data indicate the undoubted relevance of this direction, which is associated with the pollution of the natural environment with high-paraffin oil, experimental studies of the solubility of paraffin and the use of advanced bioremediation methods for soil purification.

Resolving this problem requires a detailed study of the COM ecology and the mechanisms of bacterial communities functioning in OCS. With that, the main task is identifying the main biological factors and the climatic conditions that determine the effectiveness of oil degradation in the soil. Consideration of the biological factors and the climatic conditions is very important in developing biological products.

For instance, the demanded bacterial products offered by companies in Europe, the USA, Japan, and Russia have been developed for specific climatic regions. The use of these biological products in other countries with sharply different climatic, and, therefore, different biological conditions, is sometimes ineffective. The knowledge gained during the research can contribute to further development and improvement of the methods of OCS biological purification.

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ТОПЫРАҚТЫ ЖОҒАРЫ ПАРАФИНДІ МҰНАЙДАН МИКРООРГАНИЗМДЕРМЕН ТАЗАРТУ

Аннотация. Жұмыста микроорганизмдерді қолдану арқылы топырақты жоғары парафинді мұнайдан тазарту мәселесін шешуге бағытталған зерттеу нәтижелері келтірілген. Мұнай ластаған топырақ сынамалары өзен мұнай өндіру кен орнындағы жинағыш-қамбаның ернеуінен алынды. Мұнай ластаған топырақты талдау нәтижелері бойынша құрамында мұнай 28%, механикалық қоспа 9,7% және су 60% құрады. API көрсеткіштеріне сәйкес (American Petroleum Institute) мұнайдың химиялық құрамы биоремедиация кезінде оның жылдам ыдырауына ықпал ететіні анықталды. Сондай-ақ, парафинді көмірсутектер бактериямен оңай ыдырайтындығы байқалды. Топырақты мұнайдан тазарту үшін зертханалық зерттеулерде «SHER» бактериялық препараты пайдаланылды. «SHER» биопрепаратының негізі – сүт қышқылды микроорганизм концорциумдары *Lactobacillus* және *Pediosoccus* болып саналады, олар бактерияның осы топтарын, сонымен қатар ластанған топырақта жақсы дамиды (*Citrobacterfrendii*) дау тудырмайтын терім грамды бактерияларды қалыптастырады. «SHER» биопрепаратының бактериясын иммобилизациялаушы ретінде тасымалдағыш әктас-ұлутас қалдықтарының ұнтағы таңдалды. Биологиялық ремедиация әдісі арқылы мұнай ластаған топырақты тазартудың эксперименттік зертханалық зерттеу жүргізу шарттары сипатталған. Зерттеу нәтижелері бойынша «SHER» биопрепараты бактерияларын әктас-ұлутасты ұнтақ түріндегі тасымалдаушымен иммобилизациялау мұнай ластаған топырақты тазартудың жоғары дәрежесін көрсетті (88,63%). Бактерия белсенділігін одан әрі зерттеу үшін мұнай ластаған топырақты тазарту үдерісінде пайдаланылған «SHER» биопрепаратының қалдық күнгірт ерітіндісін екінші рет пайдалану бойынша эксперименттер жүргізілді. Күнгірт қалдық ерітіндіні пайдалану және әктас-ұлутас ұнтағымен иммобилизациялау арқылы жүргізілген эксперименттік зерттеу нәтижелері топырақты тазарту дәрежесі (45,7%) екендігін көрсетті.

Сонымен, «SHER» препаратын зерттеу нәтижелері бойынша бұл препарат топырақтағы мұнай өнімдерін тиімді бейтараптандыра алады деген қорытынды жасауға болады.

Түйін сөздер: топырақ, мұнай, микроорганизмдер, биопрепарат, ұнтақ, иммобилизация.

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ОЧИСТКА ПОЧВ ОТ НЕФТИ С ВЫСОКИМ СОДЕРЖАНИЕМ ПАРАФИНА МИКРООРГАНИЗМАМИ

Аннотация. В данной работе представлены результаты исследований, направленные на решение проблемы очистки почв от нефти с высоким содержанием парафина микроорганизмами. Пробы нефтезагрязненной почвы были отобраны с бортов амбара-накопителя на месторождении нефтедобычи Узень. По данным анализа нефтезагрязненной почвы, содержание в ней нефти составляло 28%, механических примесей – 9,7% и 60% воды. Установлено, что, согласно показателей API (American Petroleum Institute), химический состав нефти может способствовать быстрому ее разложению при биоремедиации. Также парафиновые

углеводороды легко разлагаются бактериями. Для очистки почв от нефти в лабораторных экспериментах использовался бактериальный препарат «SHER». Основа биопрепарата «SHER» – консорциумов молочнокислых микроорганизмов *Lactobacillus* и *Pediococcus*, которые формируют ядро этой группы бактерий, а также грамтрицательные спорообразующие бактерии (*Citrobacter freundii*), хорошо развивающиеся в загрязненных почвах. Выбран способ иммобилизации бактерий биопрепарата «SHER» на носителе в виде отсева из отходов известняка-ракушечника. Описаны условия проведения экспериментальных лабораторных исследований по очистке нефтезагрязненной почвы методом биологической ремедиации. На основании результатов исследований установлено, что иммобилизация бактерий биопрепарата «SHER» носителем в виде отсева известняка-ракушечника показала высокую степень очистки нефтезагрязненной почвы (88,63%).

Для исследования дальнейшей активности бактерий были проведены эксперименты по вторичному использованию остаточного маточного раствора биопрепарата «SHER», использованного в процессе очистки нефтезагрязненной почвы. Результаты экспериментальных исследований с использованием остаточного маточного раствора и иммобилизацией отсева показала степень очистки почвы (45,7%).

Таким образом, по результатам проведенных исследований препарата «SHER» можно сделать вывод о том, что данный препарат может эффективно нейтрализовать нефтепродукты в почве.

Ключевые слова: почва, нефть, микроорганизмы, биопрепарат, отсев, иммобилизация.

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