Cultivation of biocultures for the purpose of their use for cleaning soils from oil pollution

S M Kurbatova, L Yu Aisner, A G Rusakov and A A Rusakov

1Krasnoyarsk State Agrarian University, 90 Mira Ave., Krasnoyarsk, 660049, Russia
2Reshetnev Siberian State University of Science and Technology, 31 Krasnoyarskiy rabochiy Ave., Krasnoyarsk, 660037, Russia

E-mail: sveta_kurbatova@mail.ru

Annotation. The article considers a number of aspects of the use of biocultures for cleaning the soil when it is contaminated with oil. Oil is represented as a multi-component hydrophobic and non-fermentable substrate. Heterotrophic microorganisms, when certain favourable conditions ensuring their activity are created, are able to completely utilize all the components of oil. An effective means of eliminating oil contamination of the soil is the activation of the regenerative ability of native biocenoses, or the introduction of certain selected and adapted cultures of microorganisms.

1. Introduction
Oil and oil products pollution affects the morphological, physical, chemical, biological (which determine its fertility) and ecological properties of the soil, its functions, processes of migration, accumulation and metabolism. The depth of penetration of petroleum products into the soil depends on various reasons, but the main amount is retained in the upper 15 cm layer [1]. Soil respiration reacts to oil pollution, especially in the first period, when the microflora is suppressed by a large amount of hydrocarbons. The complex of soil microorganisms after short-term inhibition responds to oil pollution by increasing the gross number and increasing activity. First of all, it refers to HCOM (hydrocarbon-oxidizing microorganisms), the number of which increases sharply relative to unpolluted soils [2].

Soil contamination with oil and petroleum products causes changes in its humus content, there is a decrease in the content of mobile forms of phosphorus and potassium, changes in soil acidity. Since the main element in the composition of oil is carbon (83-87 %), the content of organic matter per total carbon and humus in polluted soils increases due to the carbon of oil. Alongside the increase in the introduced carbon, there is a process of qualitative change in bituminous substances and the group composition of humus [3].

The impact of oil on the complex of soil microorganisms is ambiguous. On the one hand, oil pollution stimulates the growth of certain species, on the other hand, it inhibits. Any of the modern methods of collecting spilled oil and petroleum products (burning, mechanical collection, chemical and physical-chemical methods) can not do without the stage of biological purification. Thin films of oil remaining after physical and chemical methods can be removed only with the use of sorbents and biosorbents.

2. Cleaning of oil-contaminated soils
To clean oil-contaminated soils, various technologies are used that differ in the method of destruction of petroleum products (mechanical, physical-chemical, biological), implementation, duration and efficiency of the process, and economic costs.

A common technology for cleaning oil-contaminated soils is bioremediation technology, which is characterized by the duration of the process of biodegradation of oil hydrocarbons (several growing seasons) and the dependence of the process efficiency on climatic conditions, so that the intensification of this process is an important environmental and technological task.

One of the main and priority directions in the field of environmental protection and rational use of natural resources is the development and implementation of improved technologies for soil restoration by bioremediation method using biosorbents, which is based on the principle of self-cleaning of oil-polluted territories with the participation of native or introduced microflora.

Bioremediation is a method of cleaning and restoring soils by biological decomposition of pollutants by microorganisms (bacteria, microscopic fungi, actinomycetes), as a result of various biochemical reactions and physical and chemical processes carried out with the participation of biota [4].

Activation of the native microflora is aimed at creating an optimal environment for the development of certain groups of microorganisms that use oil as a food source, which is achieved by applying mineral fertilizers to the soil, maintaining the optimal temperature, humidity and acidity of the environment using agrotechnical techniques [5].

Soil application of various biologically-based products stemming from certain cultures of microorganisms improves the efficiency of the process of cleaning soil from oil and oil products by increasing the number of HCOM [6].

Biological products can be used in the form of aqueous suspensions of microorganisms or dehydrated microbial biomass (immobilized on a solid carrier of microbial cells) [7].

When using biologics, the biological balance in nature is not disturbed, since they effectively decompose pollutants without the formation of toxic degradation products, gradually restoring soil fertility.

More promising is the area of work, in which the cleaning of the soil from oil contamination is carried out on site without removing the soil. Thus, a suspension of microorganisms in the nutrient medium is introduced into the treated soil using a mobile unit.

To prevent the leaching of HCOM cells on oil-contaminated soil, they are immobilized on a porous solid material (polymer sorbent). This process creates a microenvironment for the cells of HCOM, stabilizes their physiological condition, and increases the availability of nutrients, water, and oil.

The polymer sorbent has a number of unique properties: it is an aerating agent and a soil-aggregate stabilizer, it is non-toxic, it sorbs oil, preventing its spread, and it works in a wide pH range of the medium. During the treatment of the oil contamination site with a sorbent, it gradually changes and loses its structure, and does not require collection and disposal. These characteristics allow using it in combination with biomass of HCOM.

The use of such complex biologics allows creating favorable microenvironment conditions for HCOM cells, structuring the soil and ensuring their growth and reproduction due to the process of oxidative enzymatic activity and decomposition of hydrocarbons.

It is possible to stimulate biodegradation with the help of oxygen, using special equipment, or by introducing nutrients into the soil (nitrogen-and phosphorus-containing fertilizers) [8].

The decomposition of oil in the soil under the action of biologics is caused by the enzyme activity of living microorganisms in its composition, the ability of biologics to contribute favourably to the native microbial community of the soil, increasing its ability to utilize oil with the formation of organic compounds of a humus-like nature. The use of biologics together with the seeding of oil-tolerant grasses at all the stages of soil cleaning significantly reduces the cleaning time and contributes to the early restoration of soil fertility.

The development of bioremediation technology for soil contaminated with a chemical substance is a long multi-stage complex process that requires the collaboration of specialists in various fields: microbiologists, analytical chemists, biochemists, biotechnologists and toxicologists. This work allows
Complete degradation of petroleum products in nature is possible only with the use of oxidative HCOM enzymes, and only in cases when the system has a set of conditions for their development. The microbiological synthesis of proteins and vitamins from oil is based on the process of enzymatic oxidation of hydrocarbons and their derivatives [10].

An important factor in the cultivation of microorganisms on hydrocarbon substrates is access to oxygen. Oxygen is required to build biomass, to oxidize carbon, and to meet the energy needs of bacteria.

Microbiological oxidation of petroleum hydrocarbons can be carried out in two directions: to grow microorganisms to be used in the form of protein and vitamin concentrates or to extract valuable products produced by microorganisms from the aquatic environment. The mechanism of oxidation of hydrocarbons by microorganisms, as shown by numerous experiments, differs from the mechanism of chemical oxidation. During the oxidation of hydrocarbons in the liquid phase, the primary product of oxidation is hydroperoxide, which is responsible for the further degenerate branching of complex chain oxidation.

The absorption of hydrocarbons by microorganisms depends on the following conditions: the penetration of hydrocarbons into the cell, the ability of microorganisms to adapt to hydrocarbons, and the presence of enzymes necessary for the primary oxidation of hydrocarbons. Microorganisms that absorb hydrocarbons must adapt to the nutrient medium, which undergoes great changes in natural conditions. Some microorganisms that fail to adapt to the new conditions die. Others, gradually adapting, begin to assimilate new substrates.

Microbiological oxidation of petroleum hydrocarbons occurs in a complex system of several phases. In the water environment, the hydrocarbon is dispersed, and there are also microbial cells. As a result of the growth of microorganisms, surfactants that cause additional emulsification of hydrocarbons are formed. The chemical processes here are influenced by factors related to the life of microorganisms (limiting and inhibiting), as well as the colloidal-chemical nature of the system [11].

One of the solutions to this problem is the use of a polymer sorbent with HCOM. The substrate-specific and activated actinomycete microflora introduced with the sorbent makes a significant contribution to the enzymatic degradation of hydrocarbon and provides a deeper reduction of the pollutant. The modified polymer sorbent significantly accelerates the processes of oxidative degradation of hydrocarbons. [12].

Microorganisms that can absorb oil hydrocarbons can be used to solve environmental problems, so when searching for destructive microorganisms, it is necessary to take into account that the microbial biomass introduced into the soil should not be foreign to the soil microflora. Another important condition is their non-pathogenicity. Destructive microorganisms must be highly resilient, since they can be exposed to adverse environmental factors, such as temperature fluctuations, high and low humidity, changes in the pH of the environment, and lack of nutrients.

To eliminate oil pollution in the soil, a number of biologics containing an active strain-destroyer or community of microorganisms with catabolic activity against petroleum hydrocarbons, and mineral additives have been developed [13].

Pseudomonas bacteria have an extremely diverse metabolism. Some strains of the genus Pseudomonas are used to convert complex organic substances into biodegradable organic compounds. There are studies showing that strains of the genus Pseudomonas living in nature can be used to clean soils contaminated with oil and its products. [14]

Yeast fungi introduced at a dose of 108 KL / ml actively decompose crude oil, aromatic compounds with a concentration of 1% within 180 days, and are also able to utilize kerosene and gas.

The preference for using Pseudomonas bacteria and yeast fungi of the genus Candida and Rhodotorula is due to their lower hazard to humans and the environment [15].
3. Use of biologics to clean the soil from oil pollution

Biological products on the basis of the UVM strains are divided into two main groups: mono- and polybacterial. Polybacterial biologics have wider adaptive and ecological possibilities for use.

As with any technological process, the use of biosorbents in the bioremediation technology of oil-contaminated soil has its advantages and disadvantages. The main advantages of using immobilized cells on the surface of the carrier can include: the possibility of longer operation of cell properties in the immobilized condition; increased productivity as a result of increasing the concentration of microbial biomass per unit of working volume of the carrier; reduced energy consumption, since working environments often contain fewer soluble impurities, which makes it easier to isolate and clean the final products; cell preservation and partial deactivation of microorganisms; resistance of cells to various adverse inactivating external factors (temperature, acidity, concentration of electrolytes, etc.) as a result of immobilization; additional protection of the culture from the effects of pathogenic microflora in cases of the sterility failure of the biotechnological system.

In addition, when using a biosorbent, natural self-purification is activated due to natural mechanisms that are inhibited without the drug by the action of spilled petroleum products. Practical application has shown that the biosorbent simplifies the mechanical collection of oil by 50-60 %, if the hydrological and meteorological conditions are favourable for this. Treatment of an oil slick with a biosorbent prevents its spread (the effect of physical and chemical booms), which allows collecting more than 90 % of oil in favourable hydrological and meteorological conditions.

When applying a biological product to oil-contaminated soil, the number of microorganisms begins to grow, using oil hydrocarbons as food. In natural conditions, the strains of these microorganisms retain their activity and population size for the time of biodegradation (elimination) of hydrocarbons, and when its quantity decreases, their number also decreases. As a result of complete absorption of petroleum products, bacteria lose their nutrient medium and die, creating humus on the ground, and silt in the water. Microorganisms also die if they are located separately from the carrier. Thus, the possibility of such a phenomenon as introduction - microbial contamination of the natural environment is excluded and makes the storage and use of biosorbent safe.

4. Experiments with oil-oxidizing strains

Bacteria of the genus Pseudomonas and yeast fungi of the genus Candida and Rhodotorula are prototrophs, so they are able to accumulate biomass in semi-synthetic environments where the source of carbon and energy is crude oil.

Experiments with oil-oxidizing strains, other things being equal, have shown that microorganisms are able to grow on crude oil and are resistant to it. The studied strains can be used for obtaining biologics, since only microorganisms that have enzymes for oxidative destruction are able to reproduce in an aerobic liquid-phase process.

In liquid-phase tests, the sorbents quickly changed their structure, which indicates the gradual decomposition of sorbents into components and their inclusion in the medium, which makes them an additional source of nutrients for HCOM. Consequently, the influence of the added filler affects the quality of the sorbent.

Oil contamination in the experiment was 1 %, yeast cultures within three weeks reduced the concentration of oil to 90 % of the introduced amount due to their enzymes, food source, oil energy and nutrients contained in the sorbent and in the medium. The speed of oil utilization is related to the properties of sorbents, which convert oil into a film form, increase the availability of oxygen for HCOM, and create a favourable microenvironment, due to which microorganisms perform their physiological functions – nutrition and reproduction.

As HCOM the biomass of new native strains of bacteria and yeast fungi as carriers of immobilized microflora was used. Eight types of modified polymer sorbents were used, with a detailed assessment of their main characteristics. Thus, the introduction of biologics based on UVOM into the oil-contaminated environment provides an environmentally safe, relatively fast and economical method of cleaning, which does not cause additional harm to nature. The type of the sorbent modification
determines its properties that affect the microenvironment of cells during immobilization, which is manifested in the speed of the utilization processes of crude oil hydrocarbons.

The high rate of oil utilization in a liquid medium without additional aeration (which is economically important) is associated with the properties of sorbents, which increase the availability of oxygen for HCOM, oil (in film form), and create a favourable microenvironment, due to which microorganisms perform physiological functions (nutrition and reproduction due to the enzymatic destruction of hydrocarbons) instead of survival.

Modification of sorbents reduces dusting, which is important when using plant waste for the production of sorbents and their utilization (in the soil structure), expands the nutritional base for heterotrophs, which is important given their self-destruction in the soil.

5. Conclusions. Experiments with biologics have proved their high efficiency. Thus, when exposed to biological products, the processes of biodegradation and biotransformation of oil cause effective cleaning of industrially polluted soil from petroleum products and improving its environmental condition. Based on the advantages of biologics, their use for introduction into the oil-contaminated environment is the most promising and economic methods of oil elimination.

The mixed culture of yeast fungi, immobilized for sorbents, effectively disposed of oil, which allows us to use the selected biologics for cleaning oil contamination of the soil.

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