

Microorganisms in the Elimination of Oil Pollution Consequences (Review)

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Abstract—The data on the effect of oil and oil products on soil, soil microbiocenosis, and plant cover are summarized. Reclamation and its stages and the bioremediation of oil-contaminated soils with the use of biological products are described. The article discusses some techniques to accelerate hydrocarbon biodegradation in the soil environment, such as the use of biosurfactants and their microorganisms; the introduction of poly-functional bacteria that are capable of pollutant destruction and diazotrophy; the introduction of psychrotolerant microorganisms under cold climate conditions; and the use of microbial-plant complexes.

Keywords: oil pollution of soil, biological products, biosurfactants, diazotrophy, psychrotolerant microorganisms, microbial-plant complexes

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INTRODUCTION

The development of modern society and scientific and technical progress is directly related to the management of nature. The oil industry is the largest consumer of natural resources; its operation disturbs ecosystems and negatively affects the environment throughout the production cycle, i.e., from exploration and raw material recovery and transportation to the production, storage, and consumption of oil products. All of these stages are characterized by the formation of a great amount of oil-contaminated waste, the storage of which leads to the withdrawal of huge land plots from turnover and their pollution. Upon entering one of the natural media (air, water, or soil environment), oil hydrocarbons are involved in general material migration and usually disperse in each medium in the course of time. Soil reclamation is the most problematic in this case, since soil accumulates and fixes substances that have a toxic effect on vegetation, soil animals, and many groups of microorganisms, which results in the diminishment or complete loss of its main property, i.e., fertility.

CONSEQUENCES OF SOIL POLLUTION WITH OIL HYDROCARBONS

There is currently a large volume of scientific data on different aspects of the transformation of soil cover due to the effect of hydrocarbon entering the soil. Pollution with oil and oil products influences the full set of soil features determining soil fertility and ecological

functions. The level of these changes depends on the climate, landscape, and topography of the area, as well as on the type and initial state of the soil and the dose and duration of the exposure to the pollutant and its features. Oil is an integrated pollutant, the effect of which is determined by the amount, composition, and properties of its organic and inorganic components (heavy metals and their salts, mercury and sulfur compounds, etc.).

Oil-related changes in the morphology and physico-chemical soil properties. The penetration of oil and oil products darkens the staining of upper horizons and leads to mosaic changes in the morphological structure due to the uneven oil distribution in the soil stratum [1–3]. Pollution transforms the granulometric composition, an essential genetic and agronomic characteristic of soil that influences its fertility. After pollution, soil particles are covered with an oil film, followed by their aggregation. The pore space is filled with oil products, which displace air and disturb aeration. Anaerobic conditions are then established, resulting in an increase in the level of soil remediation and a reduction of the soil oxidation potential, which may lead to the development of gleying processes and even to the surface bogging of soils [3, 4]. The formation of reducing conditions is also influenced by the increase in the content of organic matter (due to soil penetration by oil components); its decay is accompanied by oxygen consumption.

The decrease in the degree of dispersion changes the pattern of the boundaries between the horizons;

some of them can completely degrade. This results in the formation of a bituminous crust in the upper layers, which hinders plant growth and deep-water penetration [2].

The granulometric composition determines all of the physical soil criteria: porosity, water capacity, water permeability, aeration, thermal storage, and heat conductivity. Particle aggregation due to the effect of oil and its filling of the largest pores negatively affects these properties [5–7]. Pollution with oil and oil products causes an increase in the content of organic carbon, as well as changes in the group and fraction composition of humus and amount and ratio of macro- and microelements. The shift in the C : N ratio towards carbon causes the disturbance of the favorable nitrogen status of soils for the normal development of microorganisms and plants [8]. This also leads to changes in the ratio of nitrogen forms and reduces the content of mobile potassium and phosphorus forms [8–10].

The soil sodium–chloride salinization that accompanies oil pollution leads to complex changes in the soil absorption complex (SAC), in which sodium ions begin to displace calcium and magnesium, prevailing in pure soil. This often serves as a trigger mechanism for the development of the soil alkalization process [11]. On the whole, the soil absorption capacity decreases.

It was revealed that oil and oil products differently influence the activity of soil enzymes, which accumulate in the soil as a result of the vital activity of microorganisms, mesofauna, and the plant root system, as well as after their death. The enzyme activity can increase or decrease, depending on the type and amount of the pollutant, soil type, natural conditions, group of soil enzymes, and duration of pollution [4, 6, 9, 12–15].

Heavy metals and organometallic complexes, including those containing uranium, enter soils together with oil, which may lead to an increase in the radioactive background in contaminated areas [16, 17].

Influence of oil pollution on soil microbocenosis. Microorganisms are an essential component of soil ecosystems; their activity largely determines the capability of oil-contaminated soils for self-purification. The oil effect on the complex of soil microorganisms can stimulate growth for some species and inhibit the development of others. Hydrocarbons (in particular, aromatic hydrocarbons) can directly influence microorganisms, i.e., have a toxic effect on them, or indirectly influence microorganisms, i.e., change physicochemical soil properties (a decrease in the availability of mineral nutrition elements, deterioration of the water and air regimes, etc.). Oil penetration leads to changes in the total abundance and structure of the microbial community; in turn, the composition and level of diversity of the microbial community depend on the type, concentration, and duration of the pollut-

ant, as well as on the type of soil and state of microbocenosis prior to pollutant penetration of the microbocenosis [18–23]. On the whole, small doses decrease the abundance of cellulolytic microorganisms and bacteria assimilating mineral nitrogen forms and increase the amount of hydrocarbon oxidizing microorganisms (HOMs); at the same time, stimulation of the development of each component of microbial cenosis is also possible in this case [20, 21]. When the content of the pollutant is high, the species diversity and density of all groups of microorganisms decrease. Most often, the following pattern is observed in the development of the community after oil penetration: microorganism groups susceptible to pollution are suppressed, the HOM activity is intensified, and the previously suppressed microorganisms are then activated with a decrease in the amount of hydrocarbons in the soil. The community of soil microorganisms then gradually recovers to a level close to the initial one with an increase in time since the date of pollution and a decrease in its concentration [18, 20, 21, 24–26].

On the whole, fungal communities have proved to be more resistant to the effect of oil pollution than bacterial ones. However, they are also characterized by the same processes: the elimination of sensitive species and the dominance of hydrocarbon oxidizing groups; inhibition of the growth of fungal complexes and a reduction of their diversity as compared to those on background soils at high oil concentrations and stimulation of their development at a low pollutant concentration [27]. The most widespread species in soils containing oil and oil products are representatives of the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Candida*, *Mucor*, *Rhizopus*, *Alternaria*, and *Trichoderma* [27–34]. There is a trend towards the accumulation of phytopathogenic and potentially human and animal pathogenic micromycete species in oil-contaminated soils [35, 36].

Plant response to oil penetration into the soil. It was found that oil and oil products differently influence plant bodies. This process also depends on the type, concentration, and duration of the effect of the pollutant, as well as the plant species, soil-climate conditions, and agrochemical background. Low concentrations can even stimulate plant growth by increasing the germinating ability, the length of the aboveground and underground part, the biomass, the assimilative surface, and the chlorophyll content in leaves [37–42]. A higher pollutant content reduces the germination rate and seed number, inhibits plant growth, and shifts its development phases [38, 39, 42–46].

The positive influence of oil can be explained by the effect of the plant growth stimulators that it contains, as well as by the improvement in plant nutrition due to the decomposition of oil organic components and the lower competition between them due to grass-thinning after pollutant penetration of the soil [47]. The negative effects of oil can be both direct and indi-

rect. The direct toxic effect of oil is expressed in the rapid destruction of plant tissues and depends on the fractional composition of the plant, especially on the content of aromatic hydrocarbons. Numerous changes in the morphological structure of plants grown on hydrocarbon-contaminated soils have been recorded [45, 46, 48, 49]. Oil can change the plant habitat by diminishing air exchange, hydrophobizing soil particles, increasing soil lumpiness, etc., or by disturbing the functioning of soil biocenosis, thereby negatively affecting the plants. For instance, oil pollution is characterized by growth in the number of soil fungi, which produce toxins that suppress plants and cause their death [50, 51].

Contradictive data on plant responses to oil pollution indicate the higher significance of the indirect influence, since, unlike the direct influence, it is determined by many ecological factors and can significantly vary depending on environmental conditions.

The content and development of plants in the presence of oil and oil products leads to a disturbance of their morphophysiological and genetic stability. This is expressed in all possible remote effects, as well as in different compensatory responses under chronic pollution conditions [48]. The degree of manifestation of disturbances increases under the effect of oil with a high content of aromatic hydrocarbons. The maximum suppressing effect was revealed with allowance for indicators of root system development (change in the fibrous root system for the taproot system, reduction of root fibrils, epidermis thickening, growth in the number of xylem elements, etc.) [38, 49]. Oil also has a damaging effect at the cellular level: plants under oil pollution conditions are characterized by an increase in the content of Schiff bases, a decrease in the concentration of flavonoids and phenol compounds in cells, and a decrease in the number of pigments in assimilating plant organs; the latter leads to a decrease in the activity of photosynthesis processes and, as a consequence, to minimization of the gain in organic matter [45, 46, 52–57].

A negative effect of hydrocarbon contamination on the state of phytocenoses was established; it is expressed by a reduction of the total projective cover and a decrease in the level of the species and genetic diversity, productivity, and phytomass stocks, as well as in the succession of some ecomorph groups by others [55, 56, 58].

The same concentrations of oil and oil products under the same conditions differently influence different plants. Numerous studies have revealed many species, including wild-growing and cultivated species (both herbaceous and wood species), that can be used for the phytoremediation of oil-contaminated soils [59–63].

On the whole, it can be stated that oil and oil products predominantly negatively affect all soil properties. These pollutants lead to worsening of the morpholog-

ical, physicochemical, and chemical characteristics of soil; a reduction of the level of its aeration and drainage and a decline in biological activity and capability for self-purification and self-recovery; a disturbance of the ecological balance in soil biocenosis; the degradation of the plant cover and depression of the functional activity of flora and fauna; and the withdrawal of a large volume of lands from agricultural use due to a reduction or complete loss of their productivity. Disturbance of the soil cover and vegetation results in the intensification of adverse natural processes (erosion, degradation, cryogenesis), which lead to a reduction or complete loss of soil fertility.

RECLAMATION OF OIL-CONTAMINATED SOILS

The natural self-purification of soils from anthropogenic pollution is a long-term process, especially in regions with moderate and cold climates. Therefore, reclamation is used to eliminate the consequences of the oil effect and mobilize the internal resources of the ecosystem in order to recover its initial properties and functions. The term *reclamation* refers to measures to prevent the degradation of lands and (or) to improve their fertility via the recovery of their state to a level suitable for use according to the target purpose. The period and methods of reclamation depend on the extent and nature of pollution, as well as the time since the spill, the type of the contaminated area, the degree of its biological activity, and the state of vegetation at the specific site. As a rule, reclamation measures are carried out in two remediation stages: technical and biological stages.

Technical stage of soil reclamation. After a spill, it is necessary to carry out a set of measures for the maximal inhibition of the spread of contamination, as well as to prepare the relief and landscape of the affected area for works to eliminate the emergency situation and reclaim disturbed soils as promptly as possible. Our review will not focus on this reclamation stage, which is implemented with mechanical and physicochemical methods. A low level of purification is the disadvantage of the former methods, while significant economic costs and energy consumption and complex instrumentation are the disadvantage of the latter. Both groups of methods negatively affect the environment, which is manifested by the destruction of the fertile soil layer and the transformation of some substances into others (which sometimes prove to be even more destructive than the potential damage by oil pollution), as well as in the formation of secondary waste, which, in turn, must be disposed [64].

Biological reclamation stage. Its purpose is to recover the economic and ecological value for oil-contaminated lands by improving their agrophysical, agrochemical, biochemical, and other properties and creating conditions for the further recovery of the species diversity of flora and fauna. This is achieved by a

set of agrotechnical, agrochemical, biotechnological, and phytomelioration measures. Sometimes, it is admissible preliminarily to dilute highly polluted soil with clean soil, sand, sawdust, or straw to improve the quality of purification.

The rates of hydrocarbon biodegradation depend on many factors, and it is necessary to optimize the growth and development conditions for microorganisms and plants in order to increase the efficiency of the process; different agrotechnical and agrochemical techniques are used for this purpose. For instance, mechanical treatment methods, such as loosening and milling, are used to improve the air and water regime of reclaimed soil, to destroy bituminous crusts on the soil surface, and to mill dead trees and shrubs. An important role in the biodecomposition of oil and oil products is played by the acidity of oil-contaminated soils [65, 66], as well as by moisture [67], temperature [64, 68, 69], and the availability of biogenic elements (nitrogen, phosphorus, and potassium) [65, 70–72].

In addition to the aforementioned factors, the process of oil biodestruction in soil is influenced by factors such as the activity of endogenous microbiota, the concentration, chemical structure, the pollutant bioaccessibility, and the age of pollution [64, 69].

PURIFICATION OF OIL-CONTAMINATED SOIL USING MICROORGANISMS

Agrotechnical measures to improve soil properties are accompanied or immediately followed by bioremediation, which is understood to be a complex of treatment methods involving the biochemical potential of biological objects (microorganisms, algae, higher plants, and worms) to detoxify pollutants or reduce their concentration in the environment [73, 74]. The advantage of bioremediation technologies is determined by the ability of living organisms, especially microorganisms, to metabolize a great number of different organic substances, as well as by their safety for ecosystems and the absence of secondary waists and pollutants as a result of their activity [73, 75]. In addition, the cost of bioremediation is much lower than that required for mechanical and physicochemical methods [76]. The disadvantages of biological processes of soil treatment and remediation are the low rate of toxicant biodegradation and dependence on soil and climate conditions.

The key role in the bioremediation process is played by microorganisms that can utilize hydrocarbons during their vital activity [74, 77–81]. This property is associated with their enzyme system, namely, the oxygenase system, which allows them to integrate molecular oxygen directly into hydrocarbon, thereby forming oxygenates. This results in the partial transformation of carbon from oil and oil products into carbon dioxide and methane; part of the carbon is incorporated into the cell biomass, and another part is

transformed into humus and consolidated in soil [82]. The bioremediation of oil-contaminated soils by means of microorganisms uses two main techniques [69, 83, 84]:

(1) biostimulation, i.e., the activation of endogenous HOMs via the introduction of fertilizers (mineral and organic fertilizers) and the use of agrotechnical methods (loosening, irrigation, addition of structures, etc.);

(2) bioaugmentation (biological addition), i.e., the introduction of HOM cultures (generally in the form of biological products). It is reasonable to introduce these cultures when the abundance of native microorganisms is low or when they cannot degrade the entire range (or the majority) of the hydrocarbon-containing substances included in the pollutant or when the rate of its decomposition is low, which is characteristic of regions with cold climate conditions. It is also necessary to introduce microorganisms in the case of accidental spills, i.e., when the local microbiota experiences toxic shock due to massive pollutant impact. This review will focus on this area of bioremediation.

Many biological oil-destroying products have been developed in our country [85, 86]; however, most of them are not applied, some of them have not been patented, and some are limited only by advertising data. Many biological products are recommended not only for soil purification but also for water environments and for the neutralization of oil sludge. Biological products are based on one or several strains of decomposing microorganisms that effectively decontaminate oil hydrocarbons. Biological products can also include sorbents, carriers, stabilizers, preserving agents, enzymes, surfactant substances, and different organic and mineral substances. Biological products consisting of several strains have recently been used increasingly more often, since the HOM monoculture does not have the entire set of enzymes required for the complete decomposition of a complex multicomponent pollutant such as oil. In addition, monobacterial products are characterized by a rather narrow range of temperature, pH, salinity, and pollutant concentration, which is optimal for microorganism activity. Polybacterial strain-containing products from different taxonomic groups and with different growth rates, different consumed substrates, and different metabolic features have higher adaptive and ecological capacities. Synergetic interactions are also possible between members of the association. Therefore, when microbial associations (consortia) are used (including natural and artificially developed ones), oil biodegradation is more complete and takes less time [65, 87–91].

The complex oil composition, which significantly varies due to deposit conditions and differences in the chemical properties of oil and oil products, as well as due to unequal natural and climate and hydrothermal conditions in mining, refining, and storage areas for oil and its derivatives, makes it impossible to develop a

universal oil-decomposing biological product. Therefore, studies on the development of biological products to cleanse the environment of hydrocarbon pollution and technologies for their application will remain relevant.

USE OF MICROORGANISMS WITH PREDETERMINED PROPERTIES FOR SOIL PURIFICATION FROM OIL

Use of biosurfactants and their microorganisms. The main factor hindering the microbiological decomposition of oil and oil products is the hydrophobicity of hydrocarbon molecules, which leads to their sorption on different surfaces and transition to a biologically hard-to-access form, as well as to the failure of effective contact with microbial cells, which usually have a hydrophilic external shell. This barrier can be removed by biosurfactants, i.e., various surface-active substances synthesized by microorganisms. The mechanism of their action is determined by the processes of the desorption of organic pollutants and their transfer into the water phase and, as a consequence, by the increase in their bioaccessibility for microorganisms, as well as by modification of the external surface of bacteria in the form of hydrophobization for better contact with hydrocarbon molecules [92, 93].

The ability to form bioSASs was revealed for a wide range of microorganisms (representatives of the genera *Rhodococcus*, *Acinetobacter*, *Pseudomonas*, *Candida*, *Nocardia*, *Bacillus*, *Torulopsis*, *Ochrobactrum*, *Gordonia*, *Burkholderia*, etc. [94–102].

Unlike synthetic analogs, biosurfactants are less toxic, have a high biodegradability, are rapidly eliminated in the environment, are active at lower concentrations, are synthesized by microorganisms from renewable raw materials (e.g., from food-industry wastes), and do not lose their activity at extreme temperature, salinity, or pH values [94, 103–105]. The value of biosurfactants is very significantly influenced by the necessary stage of treatment of the biotechnological product, which can reach 60% of the total production cost. However, it is quite possible to neglect this high-cost operation for biosurfactants used in ecological biotechnology or in the oil and petrochemical industries [93, 100].

There are examples of the successful use of biosurfactants for the treatment of sand polluted with motor oil [106]; degrading pyrene [102], kerosene, and diesel fuel [107, 108], crude oil in the soil [109] and liquid medium [110], and polynuclear aromatic hydrocarbons [96], in particular, naphthalene and phenanthrene [111]. Many microbial associations, and even a great variety of biological products containing bioSAS-producing microorganisms, were developed to purify soils and grounds from oil pollution [87, 112, 113].

Despite numerous scientific articles on hydrocarbon biodegradation with the use of biosurfactant-syn-

thesizing bacteria, the published data have no references to successful commercial bioremediation based on biosurfactants. Little is known about the production of these substances by microorganisms in situ. Most of the described studies were carried out in laboratory conditions in which a single source of pollution was used. The effective use of these compounds in environmental treatment processes requires additional data on the structure of biosurfactants and their interaction with soil and additional data on pollutants and their effect on native microbiota, as well as the development of methods to monitor their content in soil and new technologies of economic production [95, 114].

Nitrogen-fixing microorganisms and their role in environmental treatment and remediation. The rate of biodestruction of oil and oil products also depends on the availability of microelements for HOMs; the essential element among them is nitrogen. In most cases, its deficit due to oil-penetration of the soil is eliminated by large volumes of mineral nitrogen fertilizers, which is economically unprofitable and even ecologically dangerous [64, 115]. Therefore, it is reasonable to introduce bacterial strains into hydrocarbon-contaminated soil, which can simultaneously degrade xenobiotics and exhibit diazotrophy. They include many representatives of the genus *Azotobacter*, which can assimilate hydrocarbons as the only source of carbon and energy both in the presence of bound nitrogen and nitrogen fixation [116, 117]. It was shown that the use of nitrogen-fixing microorganisms, such as *Azotobacter* sp., *Bacillus polymyxa*, and *Chroococcus* sp., to accelerate the bioremediation of soil contaminated with crude oil was more effective than the use of a combined mineral fertilizer (N : P : K 15 : 15 : 15) [118]. It was demonstrated that, in addition to a high nitrogen-fixing activity, representatives of the genera *Pseudomonas*, *Enterobacter*, *Stenotrophomonas*, *Bacillus*, and *Burkholderia* isolated from petrochemical sludge can also metabolize the main groups of compounds contained in the sludge: aliphatic and polyaromatic hydrocarbons, phenols, and glycols [119]. It was proposed to use nonsymbiotic, nitrogen-fixing bacteria of the genera *Acinetobacter*, *Achromobacter*, *Alcaligenes*, and *Arthrobacter* isolated from different samples of oil-contaminated soils as biofertilizers during bioremediation to increase the nitrogen content and to consider crude oil itself as a source of carbon and energy for the growth of diazotrophic microorganisms [120].

There are some reports indicating that the rhizosphere of plants cultivated in oil-contaminated soil contains the nonsymbiotic, nitrogen-fixing bacteria *Clostridium pasteurianum*, *B. polymyxa*, *Pseudomonas aeruginosa*, *Azotobacter* sp., *Klebsiella pneumoniae*, and *Derris gummusa*, which degrade hydrocarbons [121].

Some data indicate that the coastal and desert soils of Kuwait, especially those with a long-term history of oil pollution, contain large amounts of bacteria (10^7 – 10^8 CFU/g) that have a wide range of properties, such

as the ability to degrade oil, fix nitrogen, and resist mercury. Among them, strains of *Pseudomonas stutzeri*, *P. pseudoalcaligenes*, *P. putida*, *Citrobacter freundii*, *Citrobacter sp.*, and *Exiguobacterium aurantiacum* are of greatest interest; it is proposed to use them for bioremediation of oil-contaminated desert soil with an extremely poor nitrogen content [122].

Laboratory experiments on the bioremediation of soil contaminated with crude oil established the high efficiency of pollutant degradation by the diazotrophic bacteria *Paenibacillus polymyxa*, *P. lautus*, *Bacillus sp.*, and *Brevibacillus agri* [123]. Experiments on the introduction of nonsymbiotic, nitrogen-fixing bacteria isolated from areas that had long been exposed to oil and oil product contamination in the oil-contaminated soil were successfully carried out. It was proposed that these microorganisms be used to treat nitrogen-deficient soils contaminated with hydrocarbons [124].

Psychrotolerant microorganisms for purification from oil pollution under moderate and cold climate conditions. A significant number of oil-production enterprises in Russia are concentrated in the north of the European part of the country and in western Siberia in zones with moderate and cold climates. In these regions, the self-purification of soil-grounds from oil pollution with the use of endogenous hydrocarbon microbiota is limited by unfavorable soil-climate factors, namely, low average annual temperatures, the weak effect of physicochemical factors of decomposition (solar insulation and the intensity of evaporation of volatile hydrocarbon fractions), a low nutrient content, an elevated salt concentration, insufficient aeration, etc. In addition, the characteristic features of northern ecosystems are the presence of permafrost rocks, the low thickness of humus horizons, the low biological activity of soils, and the relatively poor species composition of plants, microorganisms, and soil animals. Under these conditions, the most effective treatment method is to introduce psychrotolerant (psychroactive) microorganisms that can grow at a low positive temperature and adapt to seasonal temperature variations and have a sufficient destructive effect on pollutants. The introduction of such microorganisms will make it possible to extend the period of bioreclamation by several months.

The development of bioremediation methods involving psychrotolerant microorganisms is of great interest in Russia. For example, large-scale screening isolated 220 HOM strains, of which the 15 most effective psychrotolerant strains were sampled; these strains form biological emulgators and can degrade high concentrations of oil and oil products (up to 30%) in the presence of salt at a temperature range from 4 to 42°C and pH values of 4–10. The strains belong to the genera *Rhodococcus*, *Pseudomonas*, *Acinetobacter*, *Micrococcus*, and *Serratia*. Different combinations of these microorganisms served as the basis for the development of the MicroBac biological product and V&O

microbial association for the bioremediation of soil and aquatic ecosystems [112, 113, 125, 126].

Four hundred and twenty-four strains of psychrotolerant and halotolerant (5–10% NaCl) microorganisms, which can decompose oil and oil products at low positive temperatures, were isolated from natural biocenoses of Siberia. Different associations that can be used to develop products for soil and water bioremediation in regions with cold climate were compiled from the strains that most efficiently decompose oil products (*Yarrowia lipolytica*, *Enterobacter sp.*, *Acinetobacter junii*, *A. calcoaceticus*, and *Pseudomonas sp.* strains) [127–129].

The microbial DTA-1 association, which is compiled from psychrotolerant HOMs of the genera *Pseudomonas*, *Enterobacter*, *Bacillus*, and *Acinetobacter*, is recommended as a basis for the creation of an oil-decomposing biological product to decontaminate northern areas [130]. In addition to microbial compositions, individual patented oil-decomposing strains belonging to different genera (*Pseudomonas* [131], *Exiguobacterium* [132], *Bacillus* [133], *Rhodococcus* [134, 135], *Arthrobacter* [136], etc.) are also proposed in ecological biotechnology. The Ufa Institute of Biology (Ufa Federal Research Center, Russian Academy of Sciences) revealed and validated a new species of microorganisms—*Pseudomonas turukhanskensis*. The IB 1.1 type strain of *P. turukhanskensis* is psychrotolerant and serves as the basis for the commercial biological product Lenoil®—NORD, SHP (dry preparative form), which is designed for soil purification from oil and oil products under the conditions of western Siberian [137, 138].

Microbial-plant associations as a prospective area of ecological biotechnology. One method to stimulate oil decomposition in soil is the use of microbial-plant complexes. Their action is based on rhizodegradation, i.e., the decomposition by microorganisms of toxic agents associated with plant roots [139–142]. Plant roots provide surfaces for the fixation of microorganisms and secrete exudates, i.e., extracellular water that contains enzymes, sugars, amino acids, organic acids, growth stimulators, different secondary metabolites, etc. They create optimal conditions for the existence and reproduction of microorganisms, the number of which is much higher in the rhizosphere than in the surrounding soil [49, 143]. In addition, roots prepare nutrient components and other substrates, thereby increasing the efficiency of their assimilation and, when possible, degrading organic substrates contained in the soil into compounds that have a lower molecular weight and are more easily assimilated by microorganisms (roots carry out this process using exudate enzymes). In turn, the latter increase the catabolic activity in the near-root zone and can enhance plant growth by extracting different biologically active substances (phytohormones, vitamins, secondary metabolites, etc.), improving phosphorus and nitrogen

nutrition, and increasing the stress resistance, as well as on the basis of indirect stimulation by antagonism to phytopathogenic agents [144–147]. Representatives of the genera *Pseudomonas*, *Arthrobacter*, *Flavobacterium*, *Bacillus*, *Achromobacter*, and *Rhizobium* are dominant in the rhizosphere of plants that grow in hydrocarbon-contaminated soils. Microbial-plant associations and symbioses with flexible metabolism and unique enzyme systems have major advantages for survival under unfavorable environmental conditions; their advantages are determined not only by their increased tolerance to xenobiotics but also by their ability to actively eliminate toxic agents from the habitat sphere [148–150]. The use of microbial-plant interactions makes it possible to accelerate the treatment and remediation of soils contaminated not only with oil and oil products but also with polycyclic aromatic hydrocarbons, synthetic SASs, chlor-, nitro-, and phosphorus organic compounds, and other organic pollutants [49, 139, 143, 151–154].

Effective bioremediation complexes were developed for soil purification from oil. They consist of *Azospirillum braselense* SR80 microorganisms, which produce indoleacetic acid (IAA), and legume-grass mixtures [155, 156]; *Candida maltosa* 569 and lucerne [157]; the bacteria *P. aeruginosa* AS03 or *P. aeruginosa* NA108 with antifungal action and tea plants [158]; *R. erythropolis* CD106 and ryegrass [159]; *R. erythropolis* VKM As-2017D and lucerne or wheat [160]; *Acinetobacter* sp. S-33 and lucerne [161]. Good results were achieved during the purification of phenathere-ne-contaminated sand with a complex of IAA-synthesizing bacteria *Sinorhizobium meliloti* P221 and legume-grass mixture [162]. The inoculation of plant seeds by the diazotrophic bacterium *P. stutzeri* KOS6, which decomposes hydrocarbons and produces IAA, contributes to an increase in plant root length and shoot growth, as well as to an increase in the total biomass under development conditions on petrochemical sludge containing heavy metals; this also leads to a decrease in the pollutant content in this sludge [163].

Microbial-plant complexes for soil bioremediation can include not only individual strains but also their compositions. A V&O-barley microbial-plant association was developed based on the above-mentioned V&O consortium consisting of plasmid-containing hydrocarbon-oxidizing bacteria (*R. erythropolis* S26, *A. baumannii* 1B, *P. putida* F701, and *A. baumannii* 7) [126, 164]. A microbial-plant association in which lucerne is used as a phytoextractant and the microbial component consists of strains of nitrogen-fixing bacteria (*Sinorhizobium meliloti* S3) and phosphate-mobilizing bacteria (*Serratia plymuthica* 57) was proposed. Both strains also produce IAA [165]. A bioremediation complex that includes microorganisms of the Devoroil biological destructor (representatives of *Rhodococcus*, *Pseudomonas*, and *Candida*) and perennial herbaceous plants (timothy grass or clover) was developed [166]. The efficiency of the use of the association based on

Pseudomonas delhiensis B-11400, nodule bacteria *Rhizobium lotus* RL-5, and bird's foot plants for bioremediation was tested [167].

The possibility of using transgenic plants and their complexes with microorganisms to increase the efficiency of soil purification from oil pollution was studied. The combined use of transgenic lucerne with the *rhlA* gene, which is responsible for the synthesis of a rhamnolipid biosurfactant and *Candida maltosa*, increased the level of pollutant degradation to 86% [168].

As follows from the examples given above, the potential of microbial-plant complexes for soil purification from oil pollution is actively studied at present. Further studies of the mutual influence of the pollutant, native or introduced decomposing microorganisms, and remediating plants will contribute to the creation of reliable and highly efficient technologies for environmental bioremediation.

CONCLUSIONS

The oil pollution of ecosystems is a global ecological problem and will remain so for a very long time. This is due to the fact that the aforementioned substances are the main source of energy on the planet; this is also determined by the ability of hydrocarbons to disperse quite rapidly in all natural environments. There is currently a large volume of data on the effect of such pollutants on different natural objects. It was shown that, on the whole, hydrocarbon contamination negatively affects the entire set of morphological, physicochemical, and biological soil properties that determine soil fertility and ecological functions. Oil penetration causes changes in the population abundance and biocenose structure. In some cases, its use at low concentrations can stimulate the activity of some soil enzymes and enhance the growth and development of certain microorganism and plant species.

Environmental self-purification from oil pollution is a rather long-term process, especially in regions with a cold climate. Therefore, it is necessary to use treatment methods; among them, bioremediation techniques based on the possibility of the use of oil and oil products by living organisms (plants, animals, and microorganisms) in the process of their vital activity are the most environmentally friendly and most economically justifiable techniques. Here, the main role is played by microorganisms that degrade hydrocarbon to final products, i.e., carbon dioxide and water. The use of microorganisms that, in addition to their ability to degrade pollutants, also have other properties, such as the production of biosurfactants that decrease the hydrophobicity of hydrocarbon molecules, as well as the capability for diazotrophy and nitrogen enrichment of reclaimed soil, adaptation to low positive temperatures, and the formation of bioremediation complexes with plants, is a promising area of ecological biotechnology. Despite the large number

of already developed, oil-decomposing biological products, studies in this area are still relevant due to the complexity of the composition of oil and oil products, as well as to the different natural-climate conditions in oil mining and refining areas.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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