Evaluation of the Effectiveness of a Biological Product Based on Saprophytic Bacteria and Natural Sorbents for the Rehabilitation of Oil-Contaminated Soils

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ABSTRACT

Currently, oil pollution of the soil with oil products is one of the serious environmental problems in Russia. Oil pollution adversely affects the biological, physical and chemical properties of soils, agrochemical soil indicators are also deteriorating [1]. Under the influence of petroleum product components, the quantitative and qualitative composition of beneficial soil microorganisms changes, which play an important role in the circulation of substances, providing the soil with important macro- and microelements, and take part in self-cleaning of soils. Therefore, the problem of restoration of oil-contaminated territories is very relevant. Often used methods for the rehabilitation of oil-contaminated soils require significant material costs, withdrawal from the economic turnover of vast land territories. Therefore, to optimize this process, an integrated approach is required involving modern biotechnological approaches, in particular, using hydrocarbon-oxidizing microorganisms. The aim of the study was to develop a biological product based on the *Bacillus pumilus* biodestructor strain and the natural glauconite sorbent for the rehabilitation of oil-contaminated soils.

Materials and methods. The work was carried out on the basis of SEC "Industrial Ecology" SSTU named after Gagarin Yu. A. Samples of oil-contaminated soils obtained from the territory of the Saratov Oil Refinery were used as the object of study. Samples of the studied soils were placed in an evaporation porcelain cup and dried in a muffle furnace at a temperature of 500ºC and ground in a mortar using a pestle. A sample weighing 1 g was taken from the sample, placed in a 100 cm^3 flask with a ground glass stopper, and 10 cm^3 of carbon tetrachloride were poured. The resulting sample was placed for 40 minutes in a laboratory shaker. The resulting extract was filtered and poured into a glass flask with a ground lid. The extraction followed by filtration was repeated 2 more times with new portions of carbon tetrachloride of 10 cm^3 each. All extracts were combined into a graduated cylinder and the total volume was recorded.

To determine the mass concentration of oil products in the samples of the studied soil samples, the KN-2 concentrator, No. 17664-98, was used. For this e lyuat poured into a clean cuvette was set in Kontsentratomer and measured. The result of determining the content of petroleum products in soil $X_{from\ m}$ (mg/kg) is calculated by the formula:

$$X_{meas} = C_{meas} * V * V_2 * V_{eluate} / M * V_1 * V_{al} \quad (1)$$

where, C_{ISM} - readings of the device, mg / dm^3 M is the mass of the sample for analysis, kg; V is the total volume of the extract, dm^3 ; V1 is the volume of extract taken for dilution, dm^3; V2 is the volume of extract obtained after dilution, dm^3; V_{al} is the volume of an aliquot of the extract introduced into the chromatographic column, dm^3; V_{eluate} is the volume of the eluate obtained after passing the extract through the column, dm^3.

To assess the effectiveness of biodegradation of oil products contained in soil samples, we used a bacterial strain *Bacillus pumilus*, a natural sorbent - glauconite, as well as their combinations, the optimal concentrations

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of which were selected experimentally. Previously has been shown that a strain of *B. pumilus* is avirulent, has a high enzymatic and adhesive ability, which made it possible to use it as a component of a biological product, as well as to create its immobilized form on glauconite granules [2]. We used an overnight culture of bacteria grown on meat-peptone ohm broth e (BCH) at 28° C. Glauconite is a clay mineral of variable composition with a high content of ferrous and trivalent iron, calcium, magnesium, potassium, phosphorus, as well as a number of trace elements [3]. All of them are in easily removable form of exchangeable cations, which are replaced by elements in excess in the environment. Due to these properties and the layered structure, glauconites are characterized by high sorption ability, including oil products.

For research, the following samples were obtained: K - control sample of soil containing oil products; P1A - experimental sample containing 84 g of glauconite sorbent; P2B - an experimental sample containing 84 g of the sorbent of glauconite and 1 ml of the broth culture of bacteria *B. pumilus*; P3A is an experimental sample containing 168 g of glauconite sorbent; P4B - an experimental sample containing 168 g of the sorbent of glauconite and 1 ml of the broth culture of bacteria *B. pumilus*; P5 is an experimental sample containing 1 ml of a broth culture of *B. pumilus* bacteria.

Results and discussion. Bytes ylo found that the concentration of oil in the initial sample of the soil was 2217 ± 10,4 mg / kg. The introduction of 84 g of the natural sorbent glauconite into the test sample led to a slight decrease in the concentration of oil products in the test soil sample. A two-fold increase in the mass of glauconite introduced contributed to a significant decrease in the mass concentration of oil products in the studied soil sample by 10.1 times in comparison with the control. The results obtained indicate the possible adsorption of petroleum products on the surface of granules of a natural sorbent. For the possible disposal of petroleum products contained in soil samples, bacteria *B. pumilus* were introduced into their composition . It was found that the exposure of soil samples with microorganisms within 30 days led to a decrease in the mass concentration of oil products by 10.3 times compared with the control. The highest efficiency was shown for the soil sample P4B. A decrease in the mass concentration of petroleum products by 14.9 times in comparison with the control was revealed. It is likely that the high intensity of a decrease in the concentration of petroleum products when *B. pumilus is* introduced into the samples both independently and in combination with glauconite granules indicates the hydrocarbon-oxidizing ability of this microorganism.

Thus, it was found that the introduction into the soil samples contaminated with oil products of a strain of saprophytic bacteria *B. pumilus*, the natural sorbent of glauconite, as well as their combinations, contributed to a decrease in the mass concentration of pollutant. A direct dependence of the intensity of the decrease in the mass concentration of oil products on the mass of introduced glauconite has been established. The results obtained allow us to consider the combination of *B. pumilus* and glauconite as effective components of a biological product for the rehabilitation of soils contaminated with oil products.

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