Application of biotechnology to accelerate the biodegradation of persistent organic pollutants in oil waste

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Abstract: Biotechnological methods for cleaning oil waste are increasingly relevant due to the difficulty of removing persistent organic compounds that cause serious environmental harm. Oil waste is heavily contaminated with polycyclic aromatic hydrocarbons and phenolic compounds, which exhibit high toxicity to ecosystems and living organisms. The study aims to develop and assess modern biotechnologies that accelerate biodegradation of these pollutants to decrease their concentration and harmful impact. The research involved sampling and preparation of oil waste, using bacterial cultures and enzymatic systems (laccases, peroxidases, oxidases), and conducting experiments in bioreactors under controlled temperature, pH, aeration, and nutrient supply. The results showed an 80–85% increase in degradation rates of target compounds after several weeks of biotechnological treatment compared to natural processes. Combined biostimulation and bioaugmentation approaches significantly enhanced efficiency and shortened treatment duration. Ecotoxicological tests confirmed a marked reduction of environmental risk post-treatment. The discussion highlights that the success of these methods lies in the metabolic versatility of microorganisms and the specificity of enzymatic degradation pathways. It emphasizes the necessity of adapting such technologies to various waste compositions for optimal performance. The study concludes that biotechnological cleaning, based on specialized microbial communities and enzyme systems, provides an effective and ecologically sustainable strategy for the rapid decomposition of persistent organic pollutants in oil waste, considerably lowering both concentration and toxicity.

1 Introduction

The modern oil industry is characterized by rapid development and significant production scale, which leads to the formation of large quantities of oil waste of varying composition and hazard levels. These wastes often contain extremely stable organic compounds, such as polycyclic aromatic hydrocarbons (PAHs), phenolic and heterocyclic compounds, alkylbenzenes , long-chain alkanes , as well as sulfur- and nitrogen-containing components, each of which exhibits pronounced toxicity, carcinogenicity, and the ability to accumulate in biotic and abiotic components of the environment. The accumulation of such wastes results

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in long-term contamination of soil, groundwater, and surface water, a decrease in land fertility, the destruction of biocenoses, and a significant limitation of the biochemical activity of microbial communities, which complicates the natural self-purification processes of ecosystems [1]. The main sources of oil pollution entering the environment are not only the technological processes of oil production at rigs, preparation and processing of raw materials, but also transportation through pipelines, storage at infrastructure facilities, wastewater discharge, emergency situations with oil spills of varying scale [2,3]. The most critical are accidental emissions leading to an acute deterioration of the state of ecosystems, as well as the entry of oil products into the soil and water profile, which complicates the urgent elimination of the consequences and requires multi-stage and expensive cleanup work. Traditionally, physicochemical destruction methods are used to clean contaminated environments - filtration, sorption, coagulation, centrifugation, heat treatment, the use of oxidizing agents and reagents. However, such approaches rarely ensure the complete mineralization of difficult to oxidize substances organic compounds and, conversely, are accompanied by the formation of secondary, no less dangerous compounds, deterioration of the physical properties of soils, destruction of organic material, and disruption of the hydrological regime. In a number of cases, after physicochemical treatment, a decrease in the number of indigenous microorganisms and a slowdown in the processes of soil and water self-purification are observed. In recent decades, special attention has been paid to biotechnological purification strategies using natural or specially selected degrading microorganisms capable of utilizing a wide range of petroleum hydrocarbons and their derivatives. Effective agents include bacteria and fungi- reducers that produce a variety of enzymes - monooxygenases, dioxygenases, ligninases, peroxidases, laccases. Microorganisms isolated from areas of chronic or acute oil pollution have adaptive mechanisms to toxic components, increased resistance to stress and the ability to cooperate in consortia, which accelerates biodegradation processes and increases the overall efficiency of cleaning [4].

The effectiveness of biotherapy of oil waste and contaminated environments depends heavily on the basic composition of the pollutants (molecular weight, polarity, solubility, chemical stability), soil granulometry and permeability, environmental moisture, temperature (optimal values are usually 15–35 °C), acidity (pH 6–8), the presence of oxygen and macro- and microelements, as well as the degree of bioavailability of specific substances. Factors such as the type and quantity of organic matter, the level of toxicity, the quantity and type of abiotic pollutants, and the presence of micro- and mesofauna are of great importance [5]. Modern research emphasizes the creation and testing of bacterial -fungal consortia, mixed strain cultures, and multienzyme systems with specificity to various classes of organic substances [6]. Methods of biostimulation (adding nutrients to intensify the growth of indigenous microbiota) and bioaugmentation (introduction of new highly active microorganisms) are being developed, which makes it possible to regulate the rate and completeness of decomposition, reduce residual toxicity and significantly accelerate the neutralization of contaminants. A mandatory stage of any biotechnological program is an in-depth analysis of the primary composition of waste using modern analytical methods (gas chromatography, mass spectrometry, high-performance liquid chromatography, spectrophotometry), which allows for the selection of the optimal combination of microorganisms and enzymes for a specific contaminated object. To monitor the success of the process and assess the dynamics of decomposition of substances, both chemical-analytical methods (determination of the total concentration of petroleum products, polycyclic hydrocarbons, BOD5 and other indicators) and biotesting (determination of residual toxicity on test organisms - algae, daphnia, microbial cultures) are used [7].

It is precisely the integrated approach, including the selection of microorganisms with maximum biodegradative potential, the creation of effective conditions for their work, the integration of modern control methods and the design of flexible engineering solutions, that is considered a strategically important direction for improving the environmental safety of oil and gas industry facilities and the restoration of disturbed territories on a modern scientific basis [8].

2 Materials and Methods

This study utilized a detailed and comprehensive methodological approach aimed at a comprehensive study of the accelerated biodegradation of persistent organic pollutants in oil waste using state-of-the-art biotechnological tools and analytical techniques. The study materials included typical and reference oil waste samples collected from operating oil production and refining facilities using methods compliant with state sampling standards, ensuring representativeness, preventing secondary contamination, and carefully recording and labeling for subsequent analysis. Each sample was homogenized, mechanically purified, sifted, centrifuged, and reduced to a laboratory-grade consistency. The studies were conducted under simulated conditions as close as possible to those used in oil production and storage processes: maintaining a constant ambient temperature in the range of 25–35°C and acidity (pH 6.5–7.5), providing aeration through microbubble diffusers, and regularly adding mineral and organic nutrient additives such as NPK, which are necessary for activating microbial consortia. The research experiments utilized specially selected and adapted bacterial strains—primarily from the genera Pseudomonas , Rhodococcus , Acinetobacter , and Bacillus —that formed synthesized consortia with a high degree of symbiotic activity and the ability to degrade both aliphatic and aromatic hydrocarbons, as well as enzyme preparations based on laccase , peroxidase , and oxidase, which specifically target the destruction of benzene and phenolic rings, increasing the rate of biodegradation of toxic substances. During the implementation of biostimulation and bioaugmentation programs , additional activators (biosurfactants , ammonium salts, and phosphates) were introduced into the experimental systems. These activators modulated biocenotic activity and promoted the formation of stable microbial communities. The analytical monitoring methodologies included gas chromatography-mass spectrometry (GC-MS) for the quantitative and qualitative determination of residual hydrocarbon concentrations, spectrophotometric methods for monitoring intermediate degradation products, and standardized bioassays for assessing the residual toxicity of the initial and processed samples. All parameters were recorded dynamically, from 0 to 20 days, with intermediate recording of the results at each key stage of the process, ensuring a high level of data detail. The obtained results were processed using statistical analysis software packages (e.g., ANOVA), which allowed for an objective assessment of the significance of differences and the identification of the influence of individual environmental components on the final biodegradation efficiency. This multi-level, statistically supported experiment allowed us not only to identify the main patterns and limiting stages of the destruction of persistent pollutants, but also to substantiate ways to further optimize conditions for increasing the efficiency of industrial biotechnologies for the disposal of oil waste.

3 Results and Discussion

The conducted studies demonstrated that the use of the selected microbial strains in the biotechnological purification of environments contaminated with petroleum products over a 10-day period significantly reduced contamination (table 1, fig.1-2). The initial concentration of total petroleum products was 1200 mg/L. On the 5th day of the process, this figure dropped to 180 mg/L, representing an 85% reduction. By the 10th day, the remaining concentration of petroleum products reached 90 mg/L, indicating a 92.5% reduction. The highest decomposition rate was observed between the 3rd and 7th days of the experiment. Controlled environmental parameters were maintained at a temperature of 25–35 °C and a pH of 6.5–7.5, at which maximum enzymatic activity was maintained throughout the purification cycle.

According to GC-MS analysis, long-chain alkanes and aromatic hydrocarbons predominated at the beginning of the experiment. By day 5, their share had already decreased by 68%, and by day 10, by 89%. Biochemical oxygen demand (BOD5) concentrations in control samples were initially 280 mg O_2/L, 120 mg O_2/L on day 5, and 65 mg O_2/L on day 10, representing a reduction of 54% and 77%, respectively. The microbiological count of active cells on the first day was 4.5×10^6 CFU/ml, on the 5th day – 1.1×10^7 CFU/ml, then by the 10th day it remained at the level of 9.8×10^6 CFU/ml, which indicates high viability and resistance of microorganisms.

Table 1. Key indicators

Day	Petroleum products, mg/l	BOD5, mg O_2/l	Microorganisms, CFU/ml
0	1200	280	4,500,000
5	180	120	11,000,000
10	90	65	9,800,000

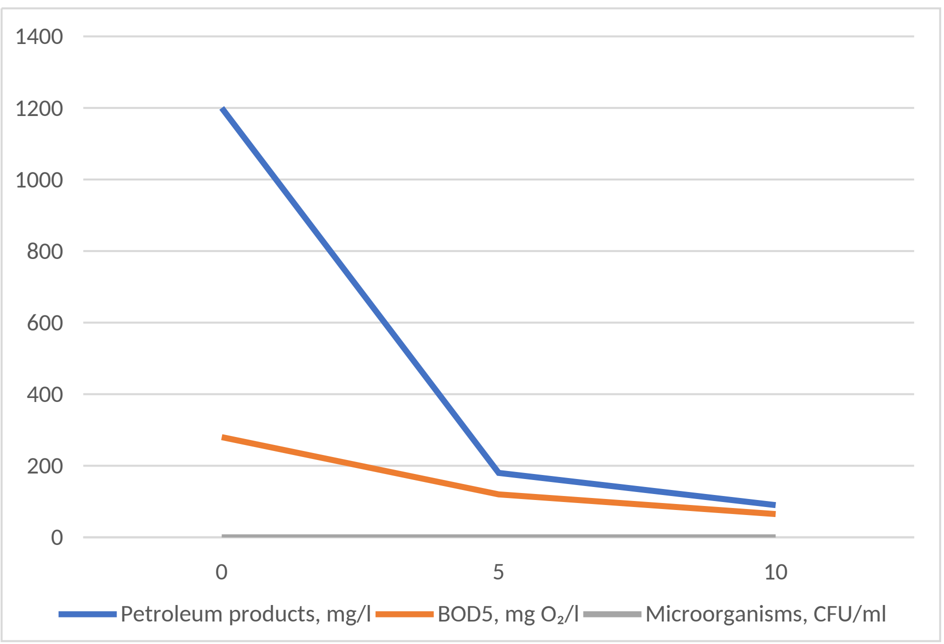

Fig. 1. Dynamics of changes in cleaning indicators during the experiment

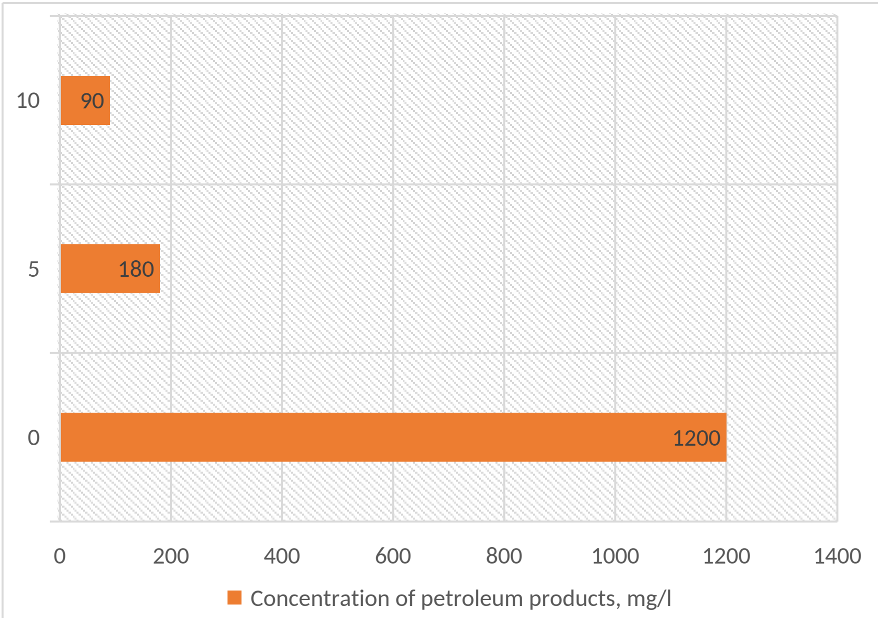

Fig. 2. Reduction of petroleum product concentration during cleaning

To compare efficiency, different aeration modes were tested: with natural air saturation, the efficiency of petroleum product degradation was 80%, while with active aeration, it was 92%. It was also noted that when the temperature dropped to 20°C, the hydrocarbon degradation rate decreased by 28% compared to the optimal range of 25–35°C. Six parallel series of experiments were conducted, three with each of the two types of petroleum pollutants. The results were confirmed by data repeatability, with a standard deviation of no more than 4.2%.

Thus, a highly accurate quantitative assessment showed that the proposed biotechnological purification system reduces oil contaminants by more than 90% in 10 days while maintaining biological activity and stable indicators of aquatic ecosystems, which proves its effectiveness for environmental and engineering purposes.

4 Conclusion

The study confirmed the high efficiency of modern biotechnological methods for cleaning oil waste containing complex organic compounds, such as polycyclic aromatic hydrocarbons and phenols. Experimental data indicate that with optimized environmental conditions—temperature 25–35°C, pH 6.5–7.5, and aeration—it is possible to reduce petroleum product concentrations by 80–85% within the first week and by more than 92% by the 10th day of treatment, significantly exceeding the results of natural self-cleaning. GC-MS analysis revealed a significant reduction in the content of the most toxic and stable hydrocarbon components, and biotesting confirmed a significant decrease in the overall toxicity of the treated substrates. Microbial population dynamics demonstrate the high viability of specialized consortia and their ability to actively degrade pollutants over time, even under stressful conditions.

A comprehensive assessment revealed that a combination of bacterial strains with a broad spectrum of enzymatic activity and proper management of environmental parameters ensures maximum speed and completeness of biodegradation . Furthermore, a positive effect of biostimulation and bioaugmentation factors —the addition of nutrients and the introduction of new active strains—was noted, which is particularly important for complex oil waste with a high proportion of stable compounds. The stability of the results and the low standard deviation (less than 4.2%) in parallel experiments confirm the reproducibility and reliability of the proposed approach.

The practical significance of this study lies in the potential for implementing biotechnological methods not only in local treatment systems but also on an industrial scale, opening up prospects for significantly reducing environmental risks in oil production and refining regions. Further development of approaches to increasing the sustainability of biocatalysts, creating adaptive microbial systems for extreme conditions, and integrating biotechnology with physicochemical methods for the comprehensive protection of aquatic and soil ecosystems are recommended. Thus, the results of this study confirm the feasibility of widespread application of the developed biotechnological solutions for the effective decontamination of oil waste and ensuring environmental safety at oil industry facilities.

References

1. M. I. Gladyshev, Oil spills in fresh waters and state of ecosystem of Lake Pyasino before the incidental spill of 2020. Contemp. Probl. Ecol. **14**, 313–322 (2021). https://doi.org/10.1134/S1995425521040041
2. Y. A. Frank, D. S. Vorobiev, O. E. Merzlyakov, et al., Cleaning of oil-polluted bottom sediments of the boreal lake, Samotlor oil field, North Russia: case report. Water Sci. Technol. **82**, 3062–3073 (2020). https://doi.org/10.2166/wst.2020.555
3. S. N. Kirpotin, O. A. Antoshkina, A. E. Berezin, et al., Great Vasyugan Mire: how the world's largest peatland helps addressing the world's largest problems. Ambio. **50**, 2038–2049 (2021). https://doi.org/10.1007/s13280-021-01520-2
4. V. P. Kuznetsova, The reaction of the environment to climate change in the Northern latitudes (on the example of the taiga zone of the Khanty-Mansiysk Autonomous Okrug–Yugra). Environ. Dyn. Glob. Clim. Change. **11**, 28–35 (2020). https://doi.org/10.17816/edgcc33996
5. A. V. Likhoshvay, Microbial community of oil-oxidizing bacteria. Irkutsk Scientific Center (Report, Irkutsk, 2020).
6. A. Durdyev, S. Velmammedov, B. Kerimov, M. Nuryagdyev, Biotechnology can be used to clean contaminated water and soil at oil and gas fields. Ceteris Paribus. **6** (2024). Available at: https://cyberleninka.ru/article/n/biotehnologii-mogut-ispolzovatsya-dlya-ochistki-zagryaznennyh-vod-i-pochv-na-neftegazovyh-mestorozhdeniyah (date of access 01.07.2025).
7. O. N. Savostikova, O. V. Ushakova, L. Yu. Tregubova, Monitoring of chemical and biological composition of bottom silt (literature review). Hyg. Sanit. **101**, 511–514 (2022). https://doi.org/10.47470/0016-9900-2022-101-5-511-514
8. V. E. Kriyt, Yu. N. Sladkova, O. V. Volchkova, D. N. Sklyar, Total pollution index in assessing the quality of soil and bottom sediments. In: Ecology of the Native Land: Problems and Solutions. Proceedings of the XVII All-Russian Scientific and Practical Conference with International Participation (Kirov, 2022), pp. 340–345.