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COMPREHENSIVE EVALUATION OF THE EFFECTIVENESS OF SORBENTS BASED ON IMMOBILIZED HYDROCARBON-OXIDIZING BACTERIAL STRAINS (FIELD AND LABORATORY STUDIES)

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- Environmental contamination by petroleum products, which in modern Ukraine has assumed a catastrophic military nature due to damage to critical infrastructure, poses a threat of nationwide ecocide. The high toxicity and carcinogenicity of petroleum hydrocarbons migrating through trophic chains necessitate the implementation of effective "green" accelerated biodegradation technologies using sorbents with microorganism-degraders.
- **OBJECTIVE.** To evaluate the effectiveness of a biopreparation based on an oil-absorbing sorbent with immobilized active strains of hydrocarbon-oxidizing actinobacteria (Dietzia maris, Gordonia rubripertincta, Rhodococcus erythropolis) under various application algorithms, and to compare the degree of petroleum hydrocarbon biodegradation under laboratory and field conditions.
- **MATERIALS AND METHODS.** Studies were conducted under laboratory (modeling) and field (in situ) conditions at industrial power substation sites in Dnipro. Sanitary-chemical analyses of oil content (transformer and waste motor oils) were performed using gravimetric and IR spectroscopic methods. Microbiological parameters were assessed using plating and culture techniques on selective media. Statistical processing of data was carried out using SPSS 16.0 and STATISTICA 8.0 software packages.
- **RESULTS.** In the field experiment under conditions of severe initial contamination (up to 94,700.0 mg/kg) and application of an integrated algorithm (biosorbent application of 2–4 kg/m^2, loosening to a depth of 40 cm, and moistening up to 60%), the degree of transformer oil biodegradation reached 83.4–97.9% after 9 months. Under laboratory conditions (ex situ), autonomous treatment of waste motor oil without moistening or nutrient maintenance ensured reliable spatial localization of the spill (at sorbent doses of 60–80% of the pollutant mass), but the total hydrocarbon elimination after 180 days reached only 10.1–21.8% due to moisture and nutrient deficiencies.
- **CONCLUSIONS.** The evaluated biopreparation demonstrates high bioremediation potential; however, the rate and extent of biodegradation critically depend on environmental conditioning. To achieve the full metabolic potential of immobilized strains, adherence to protocols involving aeration, moistening, and nutrient supplementation is mandatory.
- **KEYWORDS:** *sorbents; bioremediation, petroleum, bacteria, cells, immobilized, soil pollutants, environmental restoration technologies.*

КОМПЛЕКСНА ОЦІНКА ЕФЕКТИВНОСТІ СОРБЕНТІВ НА ОСНОВІ ІММОБІЛІЗОВАНИХ ВУГЛЕВОДНЕВО-ОКИСНЮВАЛЬНИХ ШТАМІВ БАКТЕРІЙ (НАТУРНІ ТА ЛАБОРАТОРНІ ДОСЛІДЖЕННЯ)

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- Забруднення довкілля нафтопродуктами, яке в сучасній Україні набуло катастрофічного воєнного характеру через ураження об’єктів критичної інфраструктури, створює загрозу загальнонаціонального екоциду. Висока токсичність та канцерогенність нафтових вуглеводнів, що мігрують трофічними ланцюгами, потребують впровадження ефективних «зелених» технологій прискореної біодеструкції за допомогою сорбентів із мікроорганізмами-деструкторами.
- **МЕТА.** Визначити ефективність біопрепарату на основі нафтопоглинального сорбенту з іммобілізованими активними штамами вуглеводнево-окиснювальних актинобактерій (Dietzia maris, Gordonia rubripertincta, Rhodococcus erythropolis) за різних алгоритмів застосування та порівняти ступінь біодеструкції нафтопродуктів у лабораторних і натурних умовах.
- **МАТЕРІАЛИ ТА МЕТОДИ.** Дослідження проводили у лабораторних (науково-модельних) та натурних (in situ) умовах на промислових майданчиках електропідстанцій м. Дніпро. Санітарно-хімічні аналізи вмісту нафтопродуктів (трансформаторної та відпрацьованої моторної олив) виконували гравіметричним та ІЧ-спектрометричним методами. Оцінку мікробіоценозу здійснювали за допомогою мікробіологічних методів посіву та вирощування на вибіркових середовищах. Статистичну обробку результатів проводили з використанням програм SPSS 16.0 та STATISTICA 8.0.
- **РЕЗУЛЬТАТИ.** У натурному експерименті за умов високого вихідного забруднення (до 94 700,0 мг/кг) та застосування комплексного алгоритму (внесення біосорбенту 2–4 кг/м2, розпушування на 40 см, зволоження до 60%) ступінь біодеструкції трансформаторної оливи через 9 місяців досяг 83,4–97,9%. У лабораторних умовах (ex situ) автономна обробка відпрацьованої моторної оливи без зволоження та агротехнічного обслуговування забезпечила стабільну просторову локалізацію розливу (при дозах сорбенту 60–80% від маси полютанту), але кінцева деградація вуглеводнів за 180 діб становила лише 10,1–21,8% через дефіцит вологи та біогенів.

ВИСНОВКИ. Досліджений біопрепарат має високий потенціал біоремедіації, проте швидкість і повнота біодеструкції критично залежать від кондиціонування середовища. Для реалізації повного метаболічного потенціалу іммобілізованих штамів є обов'язковим дотримання регламенту із забезпечення аерації, зволоження та внесення додаткових біогенних елементів.

КЛЮЧОВІ СЛОВА: сорбенти, біоремедіація, нафтові забруднення, бактерії, іммобілізовані клітини, ґрунтові забруднювачі, технології відновлення довкілля.

INTRODUCTION

Environmental pollution caused by oil and oil products (OP) remains one of the most pressing environmental and health issues of our time. The high toxicity, bioaccumulative potential and long-term persistence of these compounds in the environment (soil, groundwater and surface water) pose a direct threat to ecosystems and human health. Crude oil is a complex mixture of hydrocarbons, among which polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, ethylbenzene and xylenes (the BTEX complex) pose a particular danger.

When oil products enter the soil, they disrupt its structure, inhibit the vital functions of the indigenous microflora and block self-purification processes. Recent studies confirm that the hydrophobic nature of petroleum hydrocarbons leads to their persistent sorption by the soil matrix, making them long-term sources of secondary pollution of atmospheric air (through evaporation) and adjacent aquifers [1, 2].

Whilst, on a global scale, chronic oil pollution of ecosystems has traditionally been linked to operational spills during extraction, accidents on ageing main pipelines or logistical failings within the industry, in modern-day Ukraine this environmental problem has taken on a catastrophic, war-related character. Systematic and targeted strikes on critical infrastructure — oil depots, oil refineries and major industrial hubs (in particular, there are documented cases of large-scale disasters at fuel storage facilities in the Kyiv, Zhytomyr, Rivne and Dnipropetrovsk regions, as well as in Kharkiv, and an attack on the Dnipro Hydroelectric Power Station) — have transformed local incidents into a threat of nationwide ecocide.

According to monitoring data and consolidated analysis from the «Slovo i Dilo» portal (based on verified information from the Ministry of Environmental Protection and Natural Resources and the State Environmental Inspection of Ukraine), the total environmental damage caused by the hostilities has now exceeded the critical threshold of 126 billion euros. Within the breakdown of these losses, the consequences of damage to fuel infrastructure rank among the most significant. For instance, as a result of large-scale fires, over 4.1 million tonnes of burnt petroleum products were released into the atmosphere, accompanied by the deposition of toxic particulates and the subsequent accumulation of soot, oxides and benzo(a)pyrene in the upper soil layers. Specifically, the area of chemically verified contaminated soil in the country reached 1.4 million square metres, whilst the total damage to the land fund caused by littering and pollution is estimated at 26.4 billion euros [3–5].

From the perspective of preventive medicine, the greatest danger lies in the ability of petroleum components to migrate through food chains. Many of these compounds have proven carcinogenic, mutagenic and endocrine-disrupting properties. Benz(a)pyrene and other PAHs are officially classified by the WHO and the International Agency for Research on Cancer (IARC) as Group 1 carcinogens. Even at low concentrations, when ingested over a prolonged period (via drinking water or food), petroleum products can cause dysfunction of the hepatobiliary, immune and nervous systems [6].

The scale and duration of this negative impact call for a fundamental review of traditional approaches to the comprehensive assessment of the environmental condition of affected areas. The search for and implementation of high-tech, environmentally safe and economically viable methods for the remediation of oil pollution are becoming particularly important. Traditional physico-chemical remediation methods (soil excavation, incineration, chemical oxidation) are costly, energy-intensive and often lead to secondary disruption of the ecological balance. In this regard, the focus of modern medical ecology is on the development of green technologies, in particular remediation (biodegradation) using effective strains of degrading microorganisms and environmentally safe sorbents.

In Ukraine, biosorbents such as «Ekolan-M», «Ekonadin», «Rodex-T», «Deivoroil», «Putidoil», «Destroyl», «Desna» and «Rodoil» have come into widespread use. These sorbents belong to the class of biodestructive sorbents, which are capable of localising oil pollution and breaking down adsorbed petroleum products using biological methods.

The degradation of petroleum products occurs through the breakdown, by microorganisms, of the complex hydrocarbon components of crude oil into less toxic substances, which they utilise as a source of nutrition. Such microorganisms adsorb onto the surface of petroleum products and then absorb them through their cell membranes. Within the cells of the microorganisms, complex hydrocarbons are broken down into simpler compounds, such as fatty acids, alcohols and others. The breakdown products are released from the cells into the environment, where they can subsequently be broken down by other microorganisms that form part of the microbial community in the water body or soil, down to the simplest inorganic compounds. An important condition for the effectiveness of bioremediation processes is the maintenance of optimal environmental parameters for the growth of microorganisms, in particular: the presence of oxygen (for aerobic microorganisms); the presence of essential accompanying nutrients (nitrogen, phosphorus, potassium); the presence of trace elements; stable temperature and humidity corresponding to the optimal conditions for specific microorganisms [7–10].

In view of this, the search for and justification of highly effective, environmentally safe, integrated methods for the accelerated biodegradation of petroleum hydrocarbons is a pressing issue in modern hygiene and ecology.

The objective of the study to determine the effectiveness of a biological preparation based on an oil-absorbing sorbent containing immobilised active strains of hydrocarbon-oxidising actinobacteria of the species Dietzia maris, Gordonia rubripertincta, Rhodococcus erythropolis under various application protocols, and to compare the degree of biodegraction of petroleum products under laboratory and field conditions.

MATERIALS AND METHODS

To achieve the set objective and address specific tasks identified in the study, a comprehensive methodological approach was employed, which involved the following groups of research methods: sanitary-chemical and analytical methods — to determine the initial and residual content of petroleum products (transformer and motor oils, diesel fuel) in soil and biosorbent (gravimetric method, infrared spectroscopy); microbiological methods — for qualitative and quantitative analysis of the state of the microbiocenosis during bioremediation (ultrasonic disintegration method, limit dilution method on solid media, most probable number method on liquid mineral media); statistical analysis methods — to ensure the reliability of the results obtained, assess data variability and determine the significance of differences between experimental and control groups. The data obtained were processed using the SPSS 16.0 software package, MS Excel v.8.0.3 and the STATISTICA 8.0 software package with an open licence for non-commercial use.

The study of the destructive activity of the biological agent under investigation in natural conditions (in situ experiment and assessment of initial contamination) was carried out at industrial sites of power substations in the city of Dnipro. These sites were characterised by systematic anthropogenic (technogenic) load in the form of contamination with transformer oil (TO) resulting from draining and refilling operations, as well as from chronic fluid leaks through leaks and gaps at the joints of structural elements and units.

To carry out an initial assessment of the extent of environmental and sanitary-hygienic problems in the area, prior to the application of the bioagent, the representative spot sampling method was used. In accordance with current regulatory requirements, nine soil samples were collected using the «envelope» method to determine the initial concentration of petroleum products (PP) in the contaminated layers of the industrial sites. Quantitative analysis of the petroleum products content in the collected samples was carried out using the gravimetric method in accordance with *Measurement Procedure No. 081/12-0116-03 «Soils. Procedure for measuring the mass fraction of petroleum products by the gravimetric method»*.

In order to intensify mass transfer processes, optimise the air-water regime and ensure aerobic conditions for the activity of degrading microorganisms, a mechanical aeration method was applied: the contaminated areas were loosened to a depth of 40 cm and subsequently moistened until optimal soil moisture parameters were achieved (60 per cent of full moisture capacity). Simultaneously with the loosening process, a biological preparation was applied uniformly at a rate of 2–4 kg/m^2 [11].

To determine the optimal parameters for the spatial retention (localisation) of petroleum products and to assess the potential for their subsequent biodegradation, a series of laboratory experiments was carried out (*laboratory modelling of the processes of localisation and autonomous degradation of petroleum products*). The experimental design involved three variants of the ’sorbent/petroleum products’ mass ratio: the addition of biosorbent in amounts of 40 per cent, 60 per cent and 80 per cent of the total mass of the pollutant. Used engine oil (UEO) with a fixed mass of 100 g was used as a model ecotoxicant. According to preliminary calculations, the surface area of a free spill of this quantity of fluid on a dense substrate is approximately 0.10 m^2.

A key methodological decision was to recreate laboratory modelling conditions that were as close as possible to real-life scenarios for the clean-up of accidental spills (in situ) at industrial sites. The modelling was carried out on a solid surface, which had been pre-covered with a waterproofing material (polyethylene wrap) to prevent the vertical migration (seepage) of hydrocarbons.

The biological agent was applied in accordance with the manufacturer's technical recommendations. The composite sorbent was initially applied along the outer edge of the spill (extending 0.5–1.0 cm beyond its boundaries to prevent lateral spread), with a gradual concentric shift towards the centre until the entire contaminated area was completely covered.

Following completion of the primary sorption phase (24 hours after contact), experimental models of conical mounds (cone-shaped piles) were mechanically formed from the resulting organomineral mixture (used engine oil + sorbent). In accordance with the research programme, no additional components (structure-forming agents, soil conditioners or mineral fertilisers) were deliberately added to the mixture, and the substrate was not artificially moistened, in order to assess the preparation's autonomous capacity and potential for self-regulation. To maintain a minimum aerobic condition inside the mounds, the mixture was subjected to thorough mechanical mixing (aeration) at intervals of once every 14 days.

In this experiment, the residual content of petroleum products in the mass of the biosorbent was quantified over time at 2, 30 and 180 days from the start of the experiment using the gravimetric method, in accordance with Methodology No. 081/12-0724-10 «Waste. Methodology for measuring the mass fraction of petroleum products (non-polar hydrocarbons) using the gravimetric method».

RESULTS AND DISCUSSION

Field experiment (*in situ*) and assessment of the dynamics of the reduction in initial contamination. The exposure of the biological agent under investigation to natural conditions lasted for nine months. Following this period, nine soil samples were collected for monitoring purposes with a view to conducting a comprehensive assessment of the degradation dynamics of petroleum products (PPs).

Laboratory and analytical studies have shown that, within the industrial sites of power substations, the transformer oil (TO) content in all samples analysed–taken both from the top 30 cm layer as well as from the deeper 30–60 cm layers of the soil profile, had undergone a significant reduction compared with the baseline data.

In particular, the residual content of PP in the samples studied ranged from 895.52 mg/kg (sample No. 2) to 3,988.03 mg/kg (sample No. 5). The average data showed a clear trend towards the elimination of hydrocarbons: in samples No. 1 and No. 7, the residual content was approximately 1,984.00 mg/kg and 1,988.07 mg/kg respectively; in samples No. 4 and No. 6 — around 2,994.01 mg/kg and 2,985.07 mg/kg; samples No. 3 and No. 8 showed values of 3,984.06 mg/kg and 996.01 mg/kg respectively (*Table 1*).

These figures indicate that the preparation has a high destructive capacity, as the residual amount of PP relative to the initial contamination level did not exceed 16.6 per cent in any of the cases.

Numerous environmental and hygiene studies have scientifically demonstrated that when petroleum products migrate into deeper layers of the soil profile — 30–60 cm or more — an oxygen deficiency arises and the conditions for self-purification deteriorate. It has also been established that, given the nature of this ecological effect, the most technologically sound approach is the use of consortia of degrading microorganisms immobilised on an oil-absorbing sorbent. This formulation design ensures a prolonged effect, enables «targeted» delivery of the bioagent to the required depth within the ecotope, and allows its destructive impact to be reduced with sufficient precision.

Thus, the results of the field experiment demonstrated that the biological preparation under investigation exhibits pronounced destructive activity even under conditions of a critical level of anthropogenic oil pollution (up to 94,700.0 mg/kg), ensuring a reduction in the concentration of transformer oil 9 months after introduction to a level of 4.2–16.6 per cent relative to the initial values [11].

Laboratory modelling of the processes of localisation and autonomous degradation of used engine oil. Visual and laboratory monitoring of the geometric parameters of the formed mixtures showed that a dosage of 40 per cent by mass of the pollutant is insufficient for reliable spatial localisation of petroleum products. In this scenario, secondary seepage and desorption-induced spreading of engine oil onto the adjacent surface beyond

Table 1. **Changes in the concentration of transformer oil in the soil of industrial sites under field experiment (*in situ*) conditions**

Soil sample number	Sampling depth (cm)	Initial PP concentration (mg/kg)	Residual content of PP after 9 months (mg/kg)	Proportion of PP in stock (%)	Degree of biodegradation (%)
Sample № 1	0–30	94,700.0	1,984.00	2.10	97.90
Sample № 2	0–30	23,800.0	895.52	3.76	96.24
Sample № 3	30–60	24,000.0	3,984.06	16.60	83.40
Sample № 4	0–30	45,000.0	2,994.01	6.65	93.35
Sample № 5	30–60	24,000.0	3,988.03	16.62	83.38
Sample № 6	0–30	35,000.0	2,985.07	8.53	91.47
Sample № 7	0–30	32,000.0	1,988.07	6.21	93.79
Sample № 8	0–30	23,800.0	996.01	4.18	95.82
Sample № 9	30–60	28,000.0	1,850.20	6.61	93.39
M ± m	—	36,700.0 ± 7,420.0	2,407.22 ± 388.40	7.92 ± 1.75	92.08 ± 1.75

Note: The baseline contamination levels are based on background monitoring of power substation sites. The remediation process involved the application of a biosorbent (2–4 kg/m^2), mechanical aeration (loosening to a depth of 40 cm) and moistening (to 60 per cent of water-holding capacity).

the primary boundary were observed. For this reason, the investigation of samples containing 40 per cent sorbent was discontinued on day 180, as this system configuration proved to be technically unfeasible.

In contrast, the use of the biological agent in quantities of 60 per cent and 80 per cent of the mass of used engine oil ensured the effective retention of petroleum products within the structure of the formed conical embankment, establishing stable spatial boundaries for the waste and demonstrating the system's full technical capability regarding the spatial containment of the spill.

An analysis of the destructive potential of the introduced preparation revealed a moderate decline in hydrocarbon concentration at the intermediate stages of the experiment. On the 30th day of exposure, a reduction in the content of PP was recorded compared with the initial data obtained on the 2nd day: in the variant using 60 per cent sorbent by mass of the pollutant, the concentration decreased from 404.6 g/kg to 364.6 g/kg, and in the variant using 80 per cent sorbent, it fell from 439.6 g/kg to 342.9 g/kg.

However, between days 30 and 180, a marked stabilisation of the process was observed, with the concentration reaching a steady state: the NP concentration remained virtually unchanged, standing at 363.8 g/kg for 60 per cent sorbent and 343.9 g/kg for 80 per cent sorbent. An overall assessment of the preparation's effectiveness over the entire 180-day observation period showed that the net degradation (elimination) of PP in samples containing 60 per cent and 80 per cent sorbent amounted to only 10.1 per cent and 21.8 per cent of the initial level, respectively (*Table 2*).

Such a slow rate of biodegradation over a prolonged period is attributable to limiting environmental factors. As the experiment was conducted in an autonomous mode — without artificial humidification or optimisation of the salt (nitrogen-phosphorus) balance — the metabolic activity of the immobilised degrading microorganisms was limited by a deficiency of moisture and biogenic elements. This led to the inhibition of the prolonged phase of biodegradation of the heavy, hard-to-access fractions of engine oil.

For a more accurate interpretation of the results we have obtained and a better understanding of the potential limits of the effectiveness of the technology for the biodegradation of petroleum products using a system of hydrocarbon-oxidising microorganisms immobilised on a sorbent, it is appropriate to present data from a similar experiment conducted under conditions of minor soil contamination, including the necessary details of the experimental conditions in this case [12]. The experiments were carried out under laboratory conditions using air-dried sod-podzolic soil, to which moderate amounts (1 per cent and 5 per cent) of crude oil, or 5 per cent of diesel fuel (DF), were added separately. The soil samples were additionally fertilised with NPK) at a rate of 0.65 g/kg of soil for 1 per cent contaminant and 2.65 g/kg for 5 per cent. The initial cell count of hydrocarbon-oxidising bacteria in the preparation was at least 1×10^6 cells/g of soil. Control samples consisted of clean and contaminated soil without the addition of the biologi-

Table 2. **Trends in the degradation of used motor oil (UMO) under conditions of an autonomous laboratory test (*ex situ*)**

Experimental variant (sorbent dose)	PP content on the 2nd day (g/kg)	PP content on the 30th day (g/kg)	PP content on day 180 (g/kg)	Overall elimination of PP over 180 days (%)	Technical assessment of the spill's location
40 per cent of the mass of the UMO	412.5 ± 12.1	398.0 ± 11.4	—	—	**Unsatisfactory** (desorption spreading)
60 per cent of the mass of the UMO	404.6 ± 8.5	364.6 ± 7.2	363.8 ± 6.9	10.1%	**Effective** (secure retaining wall)
80 per cent of the mass of the UMO	439.6 ± 9.2	342.9 ± 6.8	343.9 ± 6.4	21.8%	**High** (complete localisation of the spill)

Note: The study of the 40 per cent variant on day 180 was terminated due to secondary fluid seepage. The experiment was conducted in autonomous mode (without artificial humidification or the introduction of additional sources of NPK).

cal preparation. Soil samples for further analysis were collected at intervals of 1, 10, 30, 60 and 90 days from the start of the experiment.The hydrocarbon content was determined using infrared (IR) spectroscopy with carbon tetrachloride extraction. As in our experiments, a significant increase in the purification of the soil matrix was observed following the addition of the biological preparation, compared with natural self-purification (biological control). By day 30, the introduced microflora had facilitated rapid primary degradation of the hydrocarbon fractions. Under conditions of 1 per cent oil contamination, the degradation rate was 56.4 per cent (control — 32.1 per cent), whilst under conditions of a massive 5 per cent oil load, it was 43.7 per cent (control — 25.5 per cent). The highest initial kinetics were recorded in the variant with 5 per cent diesel fuel contamination, where the degree of purification reached 63.1 per cent compared with 40.3 per cent in the control, which may be due to the greater availability of linear alkanes in diesel fuel for microbial metabolism. By the end of the experiment (day 90), the difference between the test variants and the control had reached its maximum. Thus, under other similar conditions, this experiment yielded comparable results; in particular, a significant improvement in the natural degradation rates of hydrocarbons (on average 1.6 times) was observed compared with the control variants. At the same time, it should be noted that such efficiency of biodegradation was observed only under conditions where a very moderate amount (up to 5 per cent) of petroleum products had been introduced into the soil.

An analysis of the comparative effectiveness of the biodegradation of petroleum products (*Fig. 1*) demonstrates the crucial role played by the remediation process parameters.

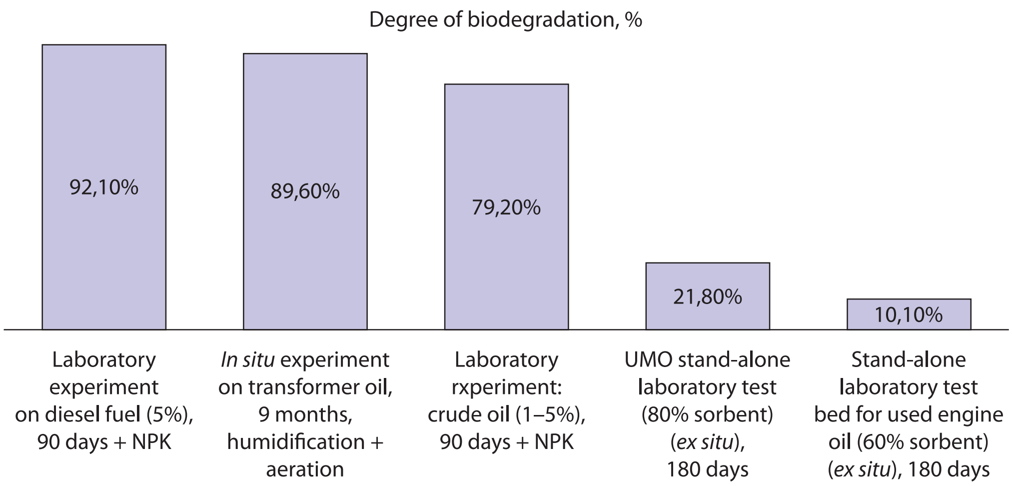

Fig. 1. Comparison of the overall efficiency of the biodegradation of PP under different process conditions

The optimised process, involving the addition of NPK and aeration, ensures a high level of removal of both light fractions (diesel fuel — 92.1 per cent) and medium-weight oils (transformer oil — 89.6 per cent; crude oil — 79.2 per cent).

An autonomous mode requiring no operational maintenance during the treatment of used motor oil (UMO) achieves only 10.1–21.8 per cent total destruction.Thus, in the event of a UMO spill, the biosorbent primarily serves to immobilise and localise the contamination; for in-depth purification, however, a comprehensive phyto- and bioremediation algorithm must be employed.

CONCLUSIONS

1. A comparative analysis of the results of field, laboratory and vegetation experiments leads to the comprehensive conclusion that the biological agent under investigation has high remediation potential and is capable of ensuring the thorough remediation of habitats from various types of hydrocarbons (up to 72.8–85.5 per cent for crude oil, 92.1 per cent for diesel fuel and up to 83.4–95.8 per cent for transformer oil) even under conditions of critical anthropogenic loading (94,700.0 mg/kg).

2. The nature and rate of biodegradation depend critically on the operating conditions and the system's architecture. Thus, in an open soil matrix (field and growing seasons), mass transfer processes, mechanical aeration and the maintenance of moisture at 60 per cent ensure the prolonged and dynamic activity of degrading microorganisms. In contrast, under conditions of an isolated laboratory disposal pile, the autonomous activity of the preparation is limited by a deficiency of moisture and biogenic substances, leading to the stabilisation of the process with a biodegradation plateau at 10.1–21.8 per cent.

3. It has been scientifically established that, in order to fully realise the biotic potential of the introduced strains under any spill remediation scenario (both in situ and ex situ), it is essential to adhere to the technical regulations stipulating environmental conditioning (moistening, aeration, biostimulation), which, together with the high succession-based self-regulation of the microbiocenosis, guarantees an environmentally safe and effective restoration of the environment without the risk of secondary biological contamination.

REFERENCES

1. **Singha LP, Pandey P.** Rhizosphere assisted bioengineering approaches for the mitigation of petroleum hydrocarbons contamination in soil. *Crit Rev Biotechnol*. 2021;**41**(5):749–66.
doi: https://doi.org/10.1080/07388551.2021.1888066
2. **Chettri B, Singha NA, Singh AK.** Efficiency and kinetics of Assam crude oil degradation by *Pseudomonas aeruginosa* and *Bacillus* sp. *Arch Microbiol*. 2021;**203**(9):5793–803.
doi: https://doi.org/10.1007/s00203-021-02567-1
3. **Splodytel A, Holubtsov O, Chumachenko S, Sorokina L.** *The impact of Russia's war against Ukraine on the state of Ukrainian soils*. Kyiv: Centre for Environmental Initiatives Ecoaction; 2023. 32 p. Available from: https://en.ecoaction.org.ua/wp-content/uploads/2023/05/Impact-of-war-on-soil-Ecoaction.pdf
4. **Biliavska L, Iutynska G, Loboda M, Ropotilov B, Skrotskyi S.** Diagnostics and bioremediation of soils affected by military operations in Ukraine. *Biological Systems: Theory and Innovation*. 2024;**15**(3):67–78.
doi: https://doi.org/10.31548/biologiya/3.2024.67
5. Slovo i Dilo. Environmental damage to Ukraine as a result of hostilities: analytical report [Internet]. 2024 [cited 2026 Jun 9].
6. **Lammel G.** Polycyclic aromatic compounds — atmospheric fate and environmental exposure [keynote lecture]. In: Book of abstracts: *24th European Meeting on Environmental Chemistry* (EMEC24); 2024 Nov 26–29; Alicante, Spain. 2024. p. 22. Available from: https://rua.ua.es/dspace/bitstream/10045/149821/3/EMEC-24-Book_abstracts.pdf
7. **Shi L, Liu Z, Yang L, Fan W.** Effect of plant waste addition as exogenous nutrients on microbial remediation of petroleum-contaminated soil. *Ann Microbiol*. 2022;**72**:22.
doi: https://doi.org/10.1186/s13213-022-01679-3
8. **Kebede G, Tafese T, Abda EM, Kamaraj M, Assefa F.** Factors influencing the bacterial bioremediation of hydrocarbon contaminants in the soil: mechanisms and impacts. *J Chem*. 2021;**2021**:9823362.
doi: https://doi.org/10.1155/2021/9823362
9. **Ablieieva IYu, Pliatsuk LD, Berezhna IO, Habbasova SM,** inventors; Sumy State University, assignee. Sposib bioremediatsii naftozabrudnenykh obiektiv [Method for bioremediation of oil-contaminated objects]. Ukrainian patent UA 146472 U. 2021 Feb 24. Ukrainian. Available from: https://essuir.sumdu.edu.ua/handle/123456789/85806
10. **Mekonnen BA, Aragaw TA, Genet MB.** Bioremediation of petroleum hydrocarbon contaminated soil: a review on principles, degradation mechanisms, and advancements. *Front Environ Sci*. 2024;**12**:1354422.
doi: https://doi.org/10.3389/fenvs.2024.1354422
11. **Kulahin OO.** Pokrashchennia sanitarnoho stanu gruntiv naselenykh mists iz zastosuvanniam metodu bioremediatsii [Improvement of sanitary state of soils in the populated areas using bioremediation method]. *The Medical and Ecological Problems*. 2016;**20**(1–2):20–2. Ukrainian. Available from: https://ecomed-journal.org/index.php/journal/article/view/7
12. **Dumanska TU, Nohina TM, Pidhorskyi VS.** Bioremediatsiia zabrudnenoho naftoiu ta dyzelnym palyvom gruntu [The bioremediation of oil and fuel oil contamination soil]. *Scientific Bulletin of Uzhhorod University. Series Biology*. 2008;(22):122–5. Ukrainian. Available from: https://dspace.uzhnu.edu.ua/jspui/handle/lib/15549

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