

Article

Pilot-Scale Evaluation of an Immobilised *Rhodococcus*–*Dietzia* Consortium on Agricultural Carriers for Petroleum-Contaminated Soil Remediation Under Arid Field Conditions in Kazakhstan

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Abstract

Oil contamination of soils in arid regions of Kazakhstan is a critical environmental problem, as extreme temperatures, low humidity and salinity limit traditional bioremediation. A pilot-scale 45-day field experiment at the Karazhanbas oil field (Mangistau Region, Kazakhstan) provided a first field validation of an adsorption-immobilisation bioremediation technology. A consortium of *Rhodococcus erythropolis* AT7 and *Dietzia maris* 22K was immobilised on buckwheat and rice husk carriers. Four treatments were tested on 1 × 1 m plots (initial petroleum products 3725 mg/kg): the consortium immobilised on buckwheat husks, on rice husks, free cells, and an untreated control. Petroleum products were measured by FTIR spectroscopy on days 0, 15, 30 and 45. The buckwheat husk variant showed the highest observed degradation efficiency among the tested treatments—94.0 ± 0.5% (3725 to 223 ± 18 mg/kg)—1.6 times higher than rice husk (57.9%) and 1.7 times higher than free cells at the standard dose (54.6%). Degradation followed first-order kinetics ($k = 0.0355 \text{ day}^{-1}$; $t_{1/2} = 19.5 \text{ days}$), and hydrocarbon-oxidising microorganisms increased to $3.4 \times 10^8 \text{ CFU/g}$ in the immobilised treatments. The higher performance of buckwheat husks may be associated with lower lignin content and improved carrier properties, with phenolic compounds such as rutin as a plausible additional factor. These pilot results provide a basis for larger-scale validation in Western Kazakhstan.

Keywords: bioremediation; oil-contaminated soil; immobilisation of microorganisms; *Rhodococcus erythropolis*; *Dietzia maris*; buckwheat husks; field experiment; arid climate; Kazakhstan; hydrocarbon-oxidising microorganisms



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1. Introduction

Oil contamination of soils is a critical environmental problem in oil-producing regions worldwide, causing damage to terrestrial ecosystems, agricultural productivity and public health [1–3]. Kazakhstan, one of the world's largest oil producers (84.2 million tonnes in 2022) [4], has over 180,000 hectares of land contaminated with petroleum products, of which more than 60% is concentrated in the Mangistau and Atyrau regions [5]. The situation is exacerbated by the arid climate of Western Kazakhstan: extreme temperatures

(up to +45 °C), low humidity (30–40%) and soil salinisation hinder the natural recovery of ecosystems, prolonging the degradation of hydrocarbons by 20–30 years or more [6,7].

Existing remediation methods are classified as physico-chemical and biological [8,9]. Physico-chemical methods—soil removal and disposal (270–460 USD/t) and thermal treatment (23–330 USD/t)—provide rapid remediation with an efficiency of >95%, but irreversibly destroy soil structure and microbial communities [10,11]. Biological methods—landfarming (30–50% over 6–12 months) and phytoremediation (20–40% over 2–5 years)—are environmentally safe and cost-effective (21–119 USD/t), but demonstrate limited effectiveness under extreme climatic conditions [12–14]. Recent reviews emphasise that traditional bioremediation strategies often require a comprehensive approach to eliminate residual toxicity and assess the impact on soil biota [15,16].

The immobilisation of oil-degrading microorganisms on solid substrates represents a promising strategy for overcoming these limitations [17–19]. This approach ensures: (i) protection of cells from environmental stresses; (ii) a 100–1000-fold increase in active biomass density; (iii) prolonged cell viability (up to 90 days); (iv) targeted delivery of degraders to contamination sites [20–22]. Organic carriers based on agro-industrial waste attract particular attention due to their low cost, biodegradability and ability to serve as an additional carbon source [23–25]. Wang et al. (2025) reported 72.8% removal of total petroleum hydrocarbons from soil over 120 days using an immobilised microbial consortium in combination with Sudan grass [26].

Bacteria of the genera *Rhodococcus* and *Dietzia* (Actinomycetota) are recognised as benchmark degraders of aliphatic (C10–C35) and aromatic hydrocarbons, possessing thermotolerance and halotolerance, the ability to produce biosurfactants, and the presence of multiple alkane hydroxylase and dioxygenase genes [27–30]. It is of fundamental importance that strains isolated from local contaminated ecosystems demonstrate excellent adaptation to regional conditions: studies of oil-degrading strains isolated from Kazakhstani oil fields support their effective degradation of hydrocarbons and adaptation to the extreme climatic conditions of Central Asia [31].

Despite an extensive laboratory infrastructure, field validation of immobilised bioremediation technologies—particularly in arid regions with extreme temperatures and saline soils—remains insufficient [32–38]. A key debate in the literature concerns whether immobilisation increases or decreases the effectiveness of bioremediation under real field conditions. A number of studies report that the advantage of immobilised cells is strain-dependent and can diminish over time: Rivelli et al. (2013) found that immobilisation on corn cob powder accelerated hydrocarbon degradation by *Pseudomonas* sp. in the short term but conferred no benefit on *Rhodococcus* sp., whose cells aggregate into clusters and are poorly adsorbed onto the porous matrix [39]. This debate remains unresolved due to a lack of rigorous comparative field studies under extreme climatic conditions. Table 1 summarises representative studies across the range of scales at which this technology has been reported.

The present study addresses this gap through a pilot-scale field validation of a bioremediation technology using a consortium of *Rhodococcus erythropolis* AT7 and *Dietzia maris* 22K, immobilised on carriers made from agro-industrial waste (buckwheat and rice husks), at the Karazhanbas oil field (Mangistau Region, Kazakhstan). Specific research objectives were: (1) to assess the degradation efficiency of the immobilised consortium compared to free cells under field conditions; (2) to compare two organic carriers with different biochemical properties; (3) to quantitatively assess microbiological and biochemical parameters of soil remediation; (4) to identify factors explaining the observed differences in efficiency; and (5) to assess the economic feasibility for practical implementation. Given the pilot, proof-of-concept nature of this study, the experiment was designed to establish

the fundamental field efficacy of the technology and to identify the most promising carrier, rather than to provide a fully powered statistical comparison; the latter is the objective of subsequent larger-scale trials.

Table 1. Representative studies of bioremediation of petroleum-contaminated soil across scales, with reported outcomes and limitations. Rows are ordered by decreasing scale, with the present study last. The selection is illustrative rather than systematic.

Study	System	Soil	Scale	Key Result	Main Limitations
Pelaez et al., 2013 [33]	Autochthonous PAH degraders; biostimulation with slow/fast-release fertiliser + commercial surfactants	PAH-polluted soil, naphthalene-dominated	Field, 900 m ³ , 161 d (three-step: lab → pilot → field)	94.4% PAH reduction; concomitant selection of autochthonous PAH degraders (<i>Bacillus</i> , <i>Pseudomonas</i> dominant)	Biostimulation only—no immobilisation or bioaugmentation; PAH, not TPH
Li et al., 2002 [7]	Indigenous fungi immobilised on rice husk-bran carrier; windrow composting	Crude-oil soil, Liaohe field, 25,800–77,200 mg/kg	Field, 8 m ³ windrows, 53 d	38–57% TPH; inoculation added only +0.8–5.2 pp over the paired uninoculated control	n = 1 per soil type; biopiles under cover; TPH gravimetric; no carrier-only arm
Hosseini et al., 2025 [29]	Free-cell consortium, in situ biosurfactant producers; arid-region strains	Sterilised soil spiked at 100,000 mg/kg	Greenhouse pots, 2 kg, 120 d	64.7%; control ≈ 12%; GC-MS: 100% for C5–C9 but 28–46% for C17–C21	Autoclaved, freshly spiked soil; pot scale; no immobilisation; no field trial
Guarino et al., 2017 [34]	Indigenous free-cell consortium (10 ⁸ CFU g ^{−1}) vs. natural attenuation vs. landfarming	Aged diesel soil, refinery (Italy), 863–12,818 mg/kg	Tray mesocosms, 1 kg, n = 3, 90 d	89.2% from 3821 mg/kg initial; natural attenuation alone 57%; removal falls as initial concentration rises	Not a field trial; no immobilisation; no dispersion or statistics reported
Rivelli et al., 2013 [39]	<i>Pseudomonas</i> / <i>Rhodococcus</i> , free vs. immobilised on corn cob (carrier at 1% w/w); carrier-only control included	Garden soil spiked with six alkanes, 1000 mg/kg each	Microcosms, 60 g, n = 3, 30 d	Immobilisation helped <i>Pseudomonas</i> at day 15 only; no benefit for <i>Rhodococcus</i> , inferior by day 30; carrier-only control reached 28–65%	60 g microcosms; spiked model hydrocarbons; cell loads not stated as matched
This study	<i>R. erythropolis</i> AT7 + <i>D. maris</i> 22K immobilised on buckwheat/rice husk	Aged arid saline soil, Karazhanbas, 3725 mg/kg	Pilot field, 3 × 1 m ² plots, 45 d	94.0% (buckwheat husk); free cells 54.6%; control 12.0%	n = 3; no CFU-matched or carrier-only arm; 32–48× higher cell load than the free-cell arm; TPH by FTIR only

2. Materials and Methods

2.1. Characteristics of the Study Site

2.1.1. Field Trial Site

Field trials were conducted at the bioremediation site of Kazekoservice LLP within the Karazhanbas oil field (Mangistau Region, Republic of Kazakhstan; 43°45′ N, 52°18′ E). Climatic conditions during the experiment (September–October 2025) were: air temperature +22...+28 °C; soil temperature +25...+32 °C; relative air humidity 35–45%; no precipitation. These conditions correspond to the typical arid climate of Western Kazakhstan in the autumn.

2.1.2. Characteristics of the Contaminated Soil

Soil from the Karazhanbas field, which has historically been contaminated with oil, was used for the experiment. Soil type: light chestnut, sandy loam; particle size distribution: sand 62%, silt 26%, clay 12%; organic matter content 2.1%; pH 7.8 (slightly alkaline). The initial concentration of petroleum products was determined by IR spectrometry (see Section 2.4.1) and amounted to 3725 mg/kg of dry soil. The contamination is long-standing (weathered oil, >5 years).

2.2. Microorganisms and Biological Preparation

2.2.1. Microorganism Strains

A consortium of hydrocarbon-oxidising bacteria was used: *Rhodococcus erythropolis* AT7 (registration number B-RKM-0769) and *Dietzia maris* 22K (registration number B-RKM-0768). The strains were isolated from oil-contaminated soils in Western Kazakhstan, patented and adapted to the climate of Kazakhstan [40]. They are characterised by their ability to degrade aliphatic (C10–C35) and aromatic hydrocarbons, thermotolerance (activity up to +42 °C), halotolerance (growth at NaCl concentrations up to 10%) and belonging to the 4th pathogenicity group (non-pathogenic) [27].

2.2.2. Preparation of Organic Carriers

Agricultural by-products were used as carriers: buckwheat hulls (*Fagopyrum esculentum*)—a by-product of buckwheat groats production; and rice husks (*Oryza sativa*)—a by-product of rice production. Pre-treatment was carried out sequentially: washing with distilled water (removal of dust and water-soluble impurities); treatment with a 1% NaOH solution (30 min, removal of waxes); treatment with a 1% HCl solution (30 min, increase in porosity); three-fold washing with distilled water until pH neutral; drying at 60 °C to constant weight; and grinding to a particle size of 2–5 mm.

Characterisation of the Carriers

The chemical composition of the substrates was previously characterised [41] using standard analytical methods: moisture content was determined gravimetrically by drying at 105 °C; ash content by ashing in a muffle furnace at 550 °C; protein content by the Kjeldahl method; fat content by the Soxhlet method; fibre content (cellulose, pentosans) and lignin content by the Van Soest method; starch content by an enzymatic method; and rutin and vitamins A, B1, B2, E content by high-performance liquid chromatography (HPLC) with UV and fluorescence detection. Functional groups on the surface of the carriers were identified by Fourier transform infrared spectroscopy (FTIR) in the range 4000–400 cm^{−1}. The key components explaining the differences in bioremediation efficiency are shown in Table 2.

Table 2. Content of major organic components and vitamins in buckwheat and rice husks (according to [41]).

Component	Buckwheat Husks	Rice Husks
Moisture content, % by mass	8.0–14.0	3.75–24.08
Ash content, % by mass	2.7–4.0	11.86–31.78
Pentosans, % by mass	12.5	4.52–37.00
Cellulose, % by mass	20.0–27.0	34.32–43.12
Lignin, % by mass	8.0–15.0	19.20–46.97
Protein, % by mass	3.3–7.0	1.21–8.75
Fat, % by mass	2.2	0.30–6.62
Starch, % by mass	0.6	9.76
Vitamin A, mg/100 g	0.003	0.04
Vitamin B1, mg/100 g	0.16	0.45
Vitamin B2, mg/100 g	0.084	0.1
Vitamin P (rutin), mg/100 g *	28.8	15.0
Vitamin E, mg/100 g	2.3	1.6

* The rutin content is a key parameter explaining the differences in the antioxidant protection of the carriers. Values taken from [41] and presented as ranges or individual values as in the source.

2.2.3. Immobilisation of Microorganisms

Immobilisation was carried out by adsorption. A bacterial suspension was prepared by culturing the strains in liquid nutrient medium (glucose 10 g/L, peptone 5 g/L, yeast extract 3 g/L, mineral salts) at +28 °C and 150 rpm for 72 h, followed by centrifugation ($5000 \times g$, 15 min) and resuspension of the cells in saline to a concentration of 10^9 CFU/mL. Immobilisation was carried out at a carrier:suspension ratio of 1:5 (mass/volume), incubating at +28 °C with periodic stirring for 24 h; the liquid phase was then separated and the immobilised cells were dried at room temperature to a moisture content of ~30%.

The immobilisation efficiency (η) was calculated using Equation (1):

$$\eta = [(N_0 - N_s)/N_0] \times 100\% \quad (1)$$

where N_0 is the initial number of cells in the suspension (CFU) and N_s is the number of cells in the supernatant after immobilisation (CFU). The immobilisation efficiency was $92.3 \pm 2.1\%$ for buckwheat husks and $87.6 \pm 2.8\%$ for rice husks.

2.3. Field Experiment Design

2.3.1. Experimental Treatments

This study was designed as a pilot-scale, proof-of-concept field trial intended to establish the fundamental efficacy of the technology under real arid-zone conditions and to guide the design of future larger-scale experiments. The field experiment comprised four treatments (Table 3). Each treatment was carried out on three independent plots measuring 1×1 m ($n = 3$ biological replicates), which were spatially separated to rule out any mutual influence. The number of replicates was selected as appropriate for a pilot trial under field logistic constraints; accordingly, the statistical outcomes are interpreted as indicative trends to be confirmed in subsequent fully powered studies.

Table 3. Experimental treatments in the field trial.

Treatment	Description	Sample Reg. No.
1	Biopreparation on buckwheat husks	976-1, 976-2, 976-3
2	Biopreparation on rice husks	977-1, 977-2, 977-3
3	Control (untreated)	978-1, 978-2, 978-3
4	Free cells (without immobilisation)	979-1, 979-2, 979-3

2.3.2. Preparation of Experimental Plots

Plot size was 1×1 m, treatment depth approximately 0.1 m, and mass of treated soil 100–150 kg per plot (depending on soil density); the distance between plots was at least 1 m (to prevent cross-contamination). Contaminated soil from the Karazhanbas field, with an initial concentration of petroleum products of 3725 mg/kg, was evenly distributed over each plot.

2.3.3. Application of the Biological Agent

Application rate (pilot scale): immobilised biological agent—10% of soil mass (10–15 kg of agent per plot); free cells (Treatment 4)—bacterial suspension with a concentration of 10^9 CFU/mL, 1 litre per plot. The biological preparation was spread evenly over the surface of the plot, mixed with the soil to a depth of approximately 10 cm (by hand) and moistened to 60–70% of full moisture capacity. It should be noted that the immobilised (Treatments 1, 2) and free-cell (Treatment 4) variants were each administered at the standard application rate for the respective form of the product, and consequently differed in the absolute number of cells delivered: the immobilised treatments received approximately

$3.2\text{--}4.8 \times 10^{13}$ CFU per plot, whereas the free-cell treatment received 1.0×10^{12} CFU per plot—a difference of roughly 32- to 48-fold. The free-cell variant therefore reflects the traditional method of applying the suspension at its standard dose, rather than a control strictly matched to the immobilised variants in terms of cell count.

2.3.4. Experimental Conditions

The duration of the experiment was 45 days (September–October 2025). Moisture was maintained at 60–70% by periodic watering (every 3–5 days); aeration was provided by loosening the soil 2–3 times a week to a depth of 15–20 cm; and air and soil temperatures (at a depth of 15 cm) were recorded daily.

2.4. Analytical Methods

2.4.1. Determination of Petroleum Product Content

The concentration of total petroleum hydrocarbons (TPH) was determined by infrared spectrometry using an FTIR spectrometer (IRAffinity-1S, Shimadzu, Kyoto, Japan). Calibration was performed using eight concentration points in the range 0–1000 mg/L ($R^2 \geq 0.998$). Soil samples were collected using the envelope method (5 spot samples from the plot, combined sample ~500 g), dried at 60 °C, ground and extracted with n-hexane (20 mL) in an ultrasonic bath (30 min, 40 kHz). The relative contributions of the main structural regions were monitored qualitatively from their characteristic absorption bands (aromatic C–H, 3030–3100 cm^{-1} ; aliphatic C–H, 2850–2960 cm^{-1} ; aromatic C=C, 1500 and 1600 cm^{-1}); these bands provide an indicative structural fingerprint and were not used for absolute quantification of individual BTEX or PAH compounds, which requires chromatographic analysis (GC-FID/GC-MS).

Degradation efficiency was calculated using the formula $E = [(C_0 - C_t)/C_0] \times 100\%$, where C_0 is the initial concentration of petroleum products (mg/kg) and C_t is the concentration at time t (mg/kg). Samples were taken on days 0 (initial state), 15, 30 and 45.

2.4.2. Microbiological Analysis

The population of hydrocarbon-oxidising microorganisms (HOM) was determined by the serial dilution method followed by plating onto Raymond's agarised mineral medium containing crude oil (0.5% v/v) as the sole source of carbon and energy. Composition of the medium (g/L): Na_2HPO_4 —1.25; KH_2PO_4 —1.25; NH_4NO_3 —1.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ —0.2; FeCl_3 —0.05; agar—15.0. Soil samples (1 g) were suspended in 9 mL of sterile saline solution, tenfold serial dilutions (10^{-1} – 10^{-8}) were prepared, and 0.1 mL was inoculated onto Petri dishes in three analytical replicates. The cultures were incubated at +28 °C for 7–14 days, after which the colonies that had grown were counted and the count expressed as CFU/g of dry soil. Samples were taken on days 0, 15, 30 and 45.

2.4.3. Soil Biochemical Parameters

Catalase activity was determined by the gasometric method based on the volume of oxygen released during the decomposition of hydrogen peroxide [42]. To 1 g of soil, 2 mL of a 3% H_2O_2 solution was added, and the volume of O_2 released over 3 min at room temperature was measured using a graduated gas-measuring tube. Activity was expressed in mL of O_2 per 1 g of soil over 3 min.

Dehydrogenase activity was determined using the method of Casida et al. [43] based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenylformazan (TFF). A TTC solution was added to 5 g of soil, incubated at +30 °C for 24 h in the dark, the resulting TFF was extracted with ethanol, and the optical density was measured at $\lambda = 485$ nm. Activity was expressed in mg TFF per 1 g of soil per day (mg TFF/(g·day)). Enzymatic activity was determined on days 15, 30 and 45 in triplicate.

2.4.4. Kinetic Analysis

The degradation of petroleum hydrocarbons was described by a first-order kinetic model: $C_t = C_0 \cdot e^{-kt}$, where C_t is the concentration of petroleum products at time t (mg/kg), C_0 is the initial concentration (mg/kg), k is the degradation rate constant (day^{-1}) and t is time (days). The kinetic parameters (k , $t_{1/2}$, R^2 , 95% confidence interval) were determined using non-linear regression (GraphPad Prism 9, first-order exponential decay model) with fixed parameters $Y_0 = 3725$ mg/kg and plateau = 0. The half-life was calculated using the formula $t_{1/2} = \ln 2/k$.

2.5. Statistical Analysis

Statistical analysis was performed on the mean values of the plots ($n = 3$ plots per treatment; analytical subsamples were first averaged across the plot). Data are presented as the mean $\pm$ standard deviation ($M \pm SD$). Normality of distribution was tested using the Shapiro–Wilk test, and homogeneity of variances using Levene’s test. One-way analysis of variance (ANOVA) with Tukey’s post-hoc test was used for multiple comparisons; the significance level was $p < 0.05$. Kinetic parameters were calculated using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA).

Given the pilot design and the limited number of field replicates ($n = 3$ per treatment), the tests for normality (Shapiro–Wilk) and homogeneity of variances (Levene) have limited statistical power; therefore, the results of the inferential tests are reported as indicative rather than definitive, and the between-treatment differences should be interpreted as preliminary trends requiring confirmation in larger-scale trials.

3. Results

3.1. Dynamics of Petroleum Hydrocarbon Degradation Under Field Conditions Bioremediation Efficiency by Treatment

The results of the 45-day field experiment are presented in Table 4. In all variants where the biopreparation was applied, a marked reduction in the concentration of petroleum products was observed relative to the control (differences detected under pilot-scale conditions; nominal $p < 0.001$, interpreted as indicative).

Table 4. Residual petroleum product content and degradation efficiency under field conditions (45 days, $n = 3$).

Treatment	Number of Plots	Initial Concentration, mg/kg	Residual Concentration (45 Days), mg/kg	Efficiency, %
Buckwheat husks	3	3725 ± 12	223 ± 18^a	94.0 ± 0.5^a
Rice husks	3	3725 ± 12	1570 ± 124^b	57.9 ± 3.3^b
Free cells	3	3725 ± 12	1692 ± 156^b	54.6 ± 4.2^b
Control	3	3725 ± 12	3278 ± 89^c	12.0 ± 3.2^c

Data are presented as $M \pm SD$ ($n = 3$ plots). Different superscripts (a, b, c) indicate statistically significant differences between treatments on day 45 ($p < 0.05$, Tukey’s post-hoc test).

The dynamics of TPH concentration over the 45-day trial are shown in Figure 1. Treatment 1 (immobilised on buckwheat husks) showed the highest degradation efficiency— $94.0 \pm 0.5\%$ —reducing the concentration of petroleum products from 3725 to 223 ± 18 mg/kg, and showed the highest observed degradation efficiency among all treatments (indicative difference, nominal $p < 0.001$). Treatment 2 (rice husks) achieved a degradation rate of $57.9 \pm 3.3\%$, which is 36.1 percentage points lower than the buckwheat husk variant ($p < 0.001$). Treatment 4 (free cells) showed an efficacy comparable to that of rice husks— $54.6 \pm 4.2\%$ ($p > 0.05$ relative to rice husks)—which is 1.7 times lower than that of the immobilised consortium on buckwheat husks. In the control variant, the reduction in

concentration was only 12.0% and was statistically significantly lower than in all variants with the biopreparation ($p < 0.001$); this residual reduction is due to abiotic processes and the activity of the background soil microflora.

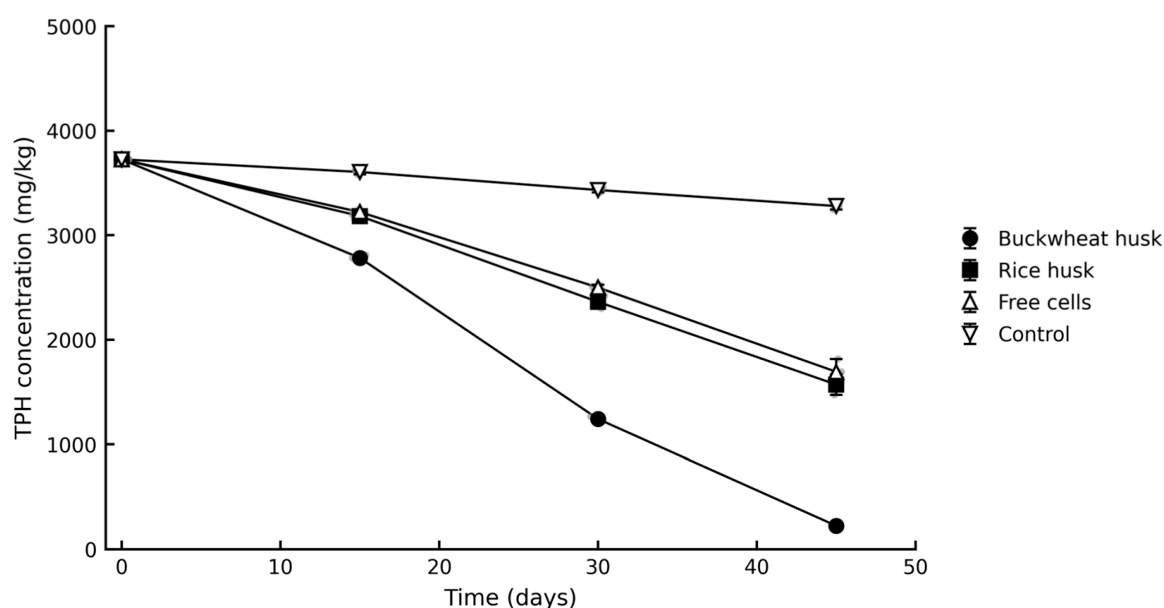


Figure 1. Dynamics of TPH concentration in soil over the 45-day field trial. Symbols show individual plot values ($n = 3$) and the treatment mean at each sampling day; error bars represent SD.

3.2. Biodegradation Kinetics

The degradation of petroleum hydrocarbons in all variants is satisfactorily described by first-order kinetics. The kinetic parameters, calculated using non-linear regression, are presented in Table 5.

Table 5. Kinetic parameters of TPH degradation (first-order model).

Treatment	k (Day ⁻¹)	t _{1/2} (Days)	R ²
Buckwheat husk	0.0355 (0.0268–0.0443)	19.5	0.913
Rice husk	0.0164 (0.0142–0.0185)	42.4	0.950
Free cells	0.0148 (0.0129–0.0168)	46.7	0.946
Control	0.0028 (0.0026–0.0029)	251.6	0.979

Parameters were determined using non-linear regression with fixed $Y_0 = 3725$ mg/kg and plateau = 0 (first-order exponential decay model, GraphPad Prism 9; $n = 3$ plots). Values in brackets are 95% confidence intervals. $t_{1/2} = \ln 2/k$.

The degradation rate constant for buckwheat husk ($k = 0.0355$ day⁻¹; 95% CI: 0.0268–0.0443) was 2.2 times higher than that for rice husk (0.0164 day⁻¹; 95% CI: 0.0142–0.0185) and 2.4 times higher than that for free cells (0.0148 day⁻¹; 95% CI: 0.0129–0.0168). Accordingly, the half-life ($t_{1/2}$) for buckwheat husk was 19.5 days—2.2 times shorter than for rice husk (42.4 days) and 2.4 times shorter than for free cells (46.7 days). In the control, the rate constant was very low ($k = 0.0028$ day⁻¹; $t_{1/2} \approx 252$ days), consistent with the limited extent of natural attenuation under arid conditions. The coefficient of determination for the buckwheat-husk treatment ($R^2 = 0.913$) was lower than for the other treatments ($R^2 = 0.946$ – 0.979), reflecting the initial lag followed by accelerating removal noted above; the first-order constants are therefore reported as empirical descriptors of overall removal rate rather than as evidence of a strictly first-order mechanism.

3.3. Microbiological Parameters

The dynamics of the hydrocarbon-oxidising microorganism (HOM) population over the 45-day trial are shown in Figure 2. The background population of hydrocarbon-oxidising microorganisms (HOM) in the soil prior to the application of the biological preparation was 2.3×10^5 CFU/g. In the treatments with the immobilised consortium, the HOM population was significantly higher throughout the experiment. In the buckwheat hulls treatment, the population reached a maximum by day 15 (3.4×10^8 CFU/g) and remained at a high level with a slight decrease by day 45 (2.5×10^8 CFU/g). A similar trend was observed for rice husks (peak of 2.8×10^8 CFU/g on day 15). In the variant with free cells, the HOM population was two orders of magnitude lower ($1.1\text{--}2.3 \times 10^6$ CFU/g), reflecting the absence of the carrier's protective effect. Since the free-cell treatment nonetheless achieved 54.6% removal (Table 4), degradation in this variant is attributed largely to the stimulated indigenous soil microflora rather than to the introduced free cells alone. In the control variant, the HOM population remained at background levels (2.3×10^5 CFU/g) throughout the experiment.

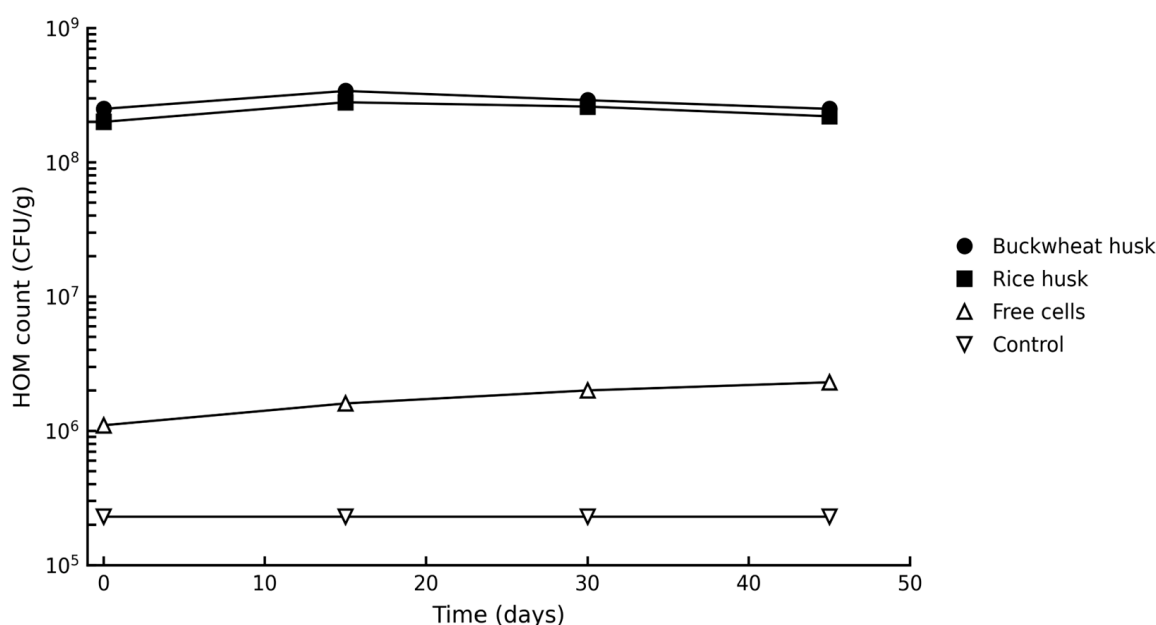


Figure 2. Changes in the number of hydrocarbon-oxidising microorganisms (HOM) in soil during the 45-day field experiment. The Y-axis is on a logarithmic scale. Data are presented as means $\pm$ SD ($n = 3$ plots).

3.4. Biochemical Parameters of Soil

3.4.1. Enzymatic Activity

Soil enzymatic activity is an integral indicator of its biological condition. The dynamics of catalase and dehydrogenase activity over the 45-day trial are shown in Figure 3. Catalase and dehydrogenase activity in all treatments with the biological preparation increased progressively throughout the experiment, indicating active microbial metabolism. By day 45, catalase activity in the buckwheat hull variant reached $3.9 \text{ mL O}_2/(\text{g} \cdot 3 \text{ min})$, exceeding the control ($0.5 \text{ mL O}_2/(\text{g} \cdot 3 \text{ min})$) by a factor of 7.8 ($p < 0.001$); dehydrogenase activity was 3.3 units compared with 0.6 in the control (5.5 times higher, $p < 0.001$). The hierarchy of enzymatic activity (buckwheat husks > rice husks > free cells > control) is consistent with the efficiency of petroleum product degradation and the dynamics of HOM abundance.

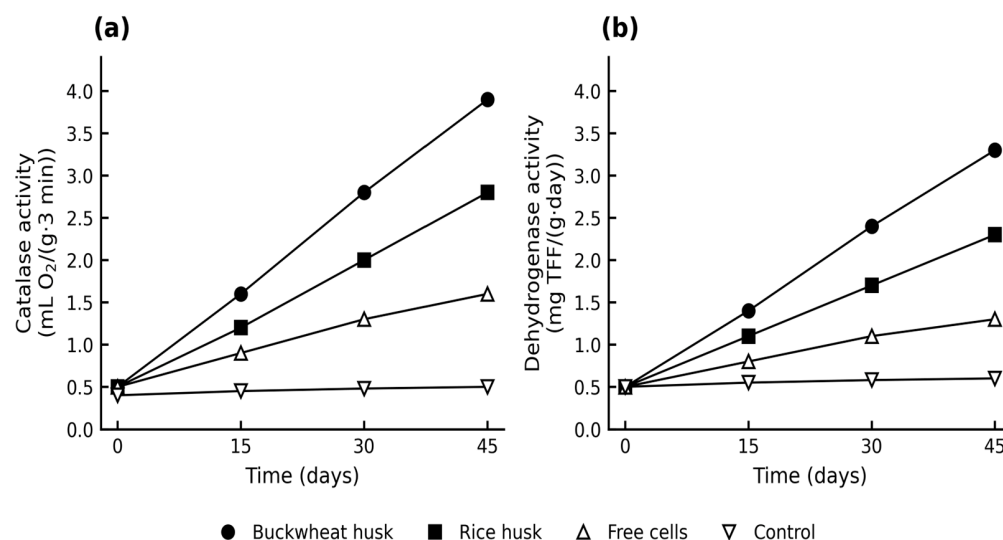


Figure 3. Dynamics of soil enzymatic activity during the 45-day field trial: (a) catalase activity; (b) dehydrogenase activity. Values are treatment means ($n = 3$ plots). Buckwheat husk and rice husk—immobilised consortium; free cells—non-immobilised consortium at standard dose; untreated control.

3.4.2. Water-Holding Capacity

The application of organic carriers increased the soil's water-holding capacity. By the end of the experiment, soil moisture was maintained at 55% in the buckwheat husk treatment, whereas in the control it was only 38%. The improvement in water-holding properties is of particular significance for the arid conditions of the Mangistau region, where moisture availability is a limiting factor in bioremediation, and may partly explain the higher efficiency of immobilised systems.

4. Discussion

4.1. Mechanisms Underlying the Higher Performance of Buckwheat Husks over Rice Husks

The results of the field trials demonstrate that buckwheat husks provide significantly higher bioremediation efficiency (94.0%) compared to rice husks (57.9%)—a difference of 36.1 percentage points. This effect is explained by a combination of structural and biochemical factors.

4.1.1. Structural and Nutrient Properties of the Carriers

The higher performance of buckwheat husks is largely associated with the composition of their lignocellulosic matrix. Buckwheat husks are characterised by a significantly lower lignin content (8.0–15.0%) compared to rice husks (19.2–47.0%) (Table 2) [41]. Lignin is the most recalcitrant and dense component of the cell wall, limiting substrate availability and surface colonisation; its lower content in buckwheat hulls ensures better microbial accessibility and a looser, more porous structure of the carrier [23,44]. Furthermore, buckwheat husks contain more protein (3.3–7.0%) with a significantly lower ash content; rice husks, by contrast, are characterised by a high ash content (up to 31.78%) due to a significant silica (SiO_2) content, which is biochemically inert and reduces the mass fraction of bioavailable organic components [25].

4.1.2. Possible Contribution of Phenolic Compounds

Buckwheat husks also contain markedly higher levels of the flavonoid rutin (28.8 mg/100 g) than rice husks (15.0 mg/100 g; Table 2) [41]. Since the catabolism of petroleum hydrocarbons via monooxygenase pathways is known to generate reactive oxy-

gen species that can impair degradative activity [45,46], polyphenolic antioxidants released during partial biodegradation of the carrier matrix may plausibly mitigate this oxidative stress and contribute to the longer persistence of active biomass observed in the buckwheat husk variant. Antioxidant compounds have been shown to mitigate oxidative stress and improve the activity of hydrocarbon-degrading bacteria [47,48]. However, oxidative stress markers were not assessed in the present study, and the contribution of rutin cannot be quantified or distinguished from the structural and nutritional factors discussed above. This mechanism is therefore proposed as a hypothesis warranting targeted investigation in future work, rather than as an established explanation.

4.2. Advantages of Immobilisation over Free Cells

The immobilised consortium on buckwheat husks outperformed free cells by a factor of 1.7 (94.0% versus 54.6%). This improved outcome is attributable to several factors. Firstly, the organic carrier creates a micro-niche protective effect [20,21]: the porous structure shields the cells from external stresses (temperature fluctuations of +25...+32 °C, drying of the upper soil layers, competition with indigenous microflora, toxic metabolites), whilst capillary forces retain moisture within the carrier. Secondly, immobilisation ensures a high local cell density, promoting cooperative hydrocarbon degradation by the consortium [18,23,49]. Thirdly, immobilised cells remain active throughout the entire bioremediation period [25,26]. By day 45, the HOM count in the free cell variant was 2.3×10^6 CFU/g, whereas in the buckwheat husk variant it was 2.5×10^8 CFU/g—approximately two orders of magnitude higher.

It should be noted that the variants with immobilised and free cells differed in the absolute number of cells introduced; therefore, the observed advantage of immobilisation reflects the cumulative effect of the technology (protection by the carrier, moisture retention, high local biomass density), rather than the isolated contribution of immobilisation at equal cell loading. Quantifying the contributions of immobilisation and inoculum dose requires further studies with an equalised number of cells introduced.

Within the field system itself, several factors plausibly contribute to the high observed efficacy: synergism of the introduced consortium with the indigenous microflora; the activating effect of diurnal temperature fluctuations; and the favourable substrate properties of weathered oil, which lacks the toxic light fractions that can inhibit microbial activity in fresh spills. Each of these is discussed in turn below.

4.3. Factors Contributing to High Field Efficiency and Comparison with Laboratory Data

A previously published laboratory study with the same immobilised consortium on buckwheat husks reported 58.4% degradation over 45 days on artificially contaminated model soil, whereas the present field trial yielded 94.0% on naturally weathered contamination [41]. It must be emphasised that these two studies are not directly comparable and the difference in the reported efficiency values should not be interpreted as a field-versus-laboratory improvement of the technology as such. The two systems differ in several first-order factors that independently affect biodegradation kinetics: (i) the nature of the contamination—fresh oil added to a sterile model matrix in the laboratory versus weathered oil residing in the natural soil for several years, which has already lost its volatile and most toxic light fractions; (ii) the initial concentration and contamination history—a single short-term spike in the laboratory versus a long-term, partly stabilised contamination in the field; (iii) the soil matrix itself—a sterile model substrate versus a natural soil hosting an established indigenous microbial community; and (iv) the physical environment—controlled constant laboratory conditions versus diurnal temperature fluctuations, natural aeration and a heterogeneous moisture regime. Accordingly, the 94.0% value should be interpreted

as the absolute field efficacy of the technology on aged Karazhanbas contamination, rather than as evidence that the technology is more efficient in the field than in the laboratory. A direct field-versus-laboratory comparison would require matched contamination type, concentration and matrix, and is beyond the scope of the present pilot trial. Hydrocarbon removal was quantified as total petroleum hydrocarbons (TPH) by FTIR; while appropriate and calibrated for TPH, this method does not resolve the molecular composition of the residual fraction, so the relative removal of aliphatic versus aromatic and polycyclic components cannot be established here. Chromatographic profiling (GC-FID/GC-MS) of the C10–C35 fraction is therefore an important next validation step to confirm that the measured reduction reflects degradation across hydrocarbon classes rather than preferential loss of the most bioavailable aliphatics.

4.3.1. Synergy with the Indigenous Microflora

The number of viable microorganisms in the variants with the biopreparation reached 10^8 CFU/g, reflecting both the introduced biomass of the consortium and the stimulation of microbial activity in the contaminated soil. It is assumed that synergy with indigenous degraders is achieved through the complementarity of degradation pathways, the exchange of intermediate metabolites (cross-feeding) and the cooperative formation of mixed biofilms [14,35]. The identification of specific taxa within the indigenous microbial community requires molecular analysis and is the subject of further research.

4.3.2. Temperature Cycles

Daily fluctuations in soil temperature under field conditions (+25...+32 °C) may have contributed to the activation of adaptive mechanisms in the microorganisms—the induction of heat shock proteins, increased expression of alkane hydroxylase and dioxygenase genes, accelerated metabolism with rising temperature (temperature coefficient $Q_{10} \approx 2$) and improved oxygen solubility during night-time cooling [2]. It should be stressed that the antioxidant (rutin), indigenous-synergy and temperature-activation mechanisms discussed here are plausible interpretations consistent with the observed data; none was directly tested in the present study, and each requires targeted experimental verification (oxidative-stress markers, molecular community analysis, and controlled temperature-response assays, respectively).

4.3.3. Pollution Characteristics

High concentrations of fresh oil containing light fractions cause substrate inhibition and toxic stress [16]. Weathered oil from the Karazhanbas field (3725 mg/kg), devoid of volatile toxic components and consisting predominantly of medium- and high-molecular-weight C17–C35 hydrocarbons, does not exert a pronounced inhibitory effect and serves as a favourable substrate for *Rhodococcus* and *Dietzia* [27].

Accordingly, the 94.0% efficiency achieved here should be understood as specific to aged, low-volatility contamination of this type, in which the most toxic and recalcitrant light fractions have already dissipated and the residual C17–C35 hydrocarbons remain comparatively bioavailable. Lower efficiencies should be expected for fresh spills containing inhibitory volatile fractions, for very high initial loadings, or for asphaltene-rich residues; site-specific bioavailability and contamination history therefore govern the transferability of these results to other contaminated sites.

4.4. Economic Aspects and Prospects for Application

Bioremediation using immobilised microorganisms on agro-industrial waste is generally regarded as one of the more cost-effective approaches to cleaning up oil-contaminated soils. For context, physico-chemical methods cost approximately 270–460 USD/t (soil

removal and disposal) and 23–330 USD/t (thermal treatment), whereas biological methods (landfarming, biopiling) fall within approximately 20–120 USD/t [10,11]. The carriers used here are favourable in this respect: buckwheat and rice husks are agricultural residues available at no material cost, and Kazakhstan produces 28–36 thousand tonnes of buckwheat husks and 38–45 thousand tonnes of rice husks annually [3], so carrier supply is not a constraint on scale-up. The cost of the technology is therefore governed not by the carrier but by the biomass production required, which scales with the application rate. Because the present trial was conducted at a single application rate (10% *w/w*, adopted from our earlier laboratory work [41]) and did not vary it, the dataset does not establish a dose–response relationship, and the lowest rate that retains acceptable removal efficiency under field conditions—the quantity that governs economic viability—cannot be inferred here. A quantitative economic assessment is therefore deferred until this rate has been established, which is a scheduled task in the approved work plan of the funding project (AP25796069).

4.5. Adaptation to an Arid Climate

The technology is adapted to the extreme conditions of Western Kazakhstan. The strains *R. erythropolis* AT7 and *D. maris* 22K remain active at temperatures up to +42 °C, which corresponds to summer soil temperatures in the Mangistau region. The immobilised consortium remains active at NaCl concentrations of up to 10%, which is important for saline oil-producing soils, where most biological preparations lose their effectiveness at 3–5% [6]. Furthermore, organic carriers (particularly buckwheat husks) increase the soil's water-holding capacity (from 38% to 55%), which is critical under conditions of low air humidity (30–40%).

4.6. Limitations and Directions for Further Research

As this work was conceived as a pilot-scale, proof-of-concept field study, it has a number of limitations that define the scope of its conclusions. The primary limitation is the small number of replicates ($n = 3$ plots per treatment), inherent to pilot field trials, which limits the statistical power of the inferential analyses; the reported differences should therefore be regarded as preliminary trends. Pilot trials were conducted on plots of 1 m²; validation on a larger scale is required to assess the logistics of application. Long-term monitoring (1–2 years) of residual concentrations and vegetation recovery is required. The previously reported laboratory value of 58.4% degradation [41] and the present field value of 94.0% refer to systems that differ in contamination type (fresh versus weathered oil), initial concentration, soil matrix (sterile model substrate versus natural soil) and environmental conditions; these values therefore characterise two distinct experimental contexts and cannot be used to quantify a field-versus-laboratory improvement of the technology.

Furthermore, the immobilised and free-cell variants were not matched in the absolute number of cells applied: the immobilised treatments received approximately 32- to 48-fold more CFU per plot than the free-cell treatment (Section 2.3.3). Nor did the design include a carrier-only treatment (buckwheat husk without inoculum). Consequently, the specific effect of immobilisation cannot be separated from inoculum density, and the abiotic contribution of the husk itself (oil sorption, moisture retention, additional carbon) cannot be quantified in the present dataset; for reference, a carrier-only control at 1% *w/w* loading has been reported to reach 28–65% removal [39], indicating that this contribution is unlikely to be negligible at the 10% loading used here. A direct assessment of oxidative stress markers confirming the presumed antioxidant role of rutin was not carried out, and confirmation of the results by GC-MS together with characterisation of the microbial community by 16S rRNA sequencing remain priority tasks for further research. A

full control matrix—untreated soil, carrier only, CFU-matched free cells, and immobilised cells—together with independent extraction-recovery validation and a closed carbon balance (CO₂ evolution) to confirm mineralisation, constitutes the core design of the planned larger-scale trial.

The application rate of 10% *w/w* was adopted from our earlier laboratory work [41] and was not varied in this trial; the present dataset therefore does not permit extrapolation to lower application rates under field conditions. Establishing a dose–response relationship, and hence an application rate that is both effective and economically viable, is a scheduled task in the approved work plan of the funding project (AP25796069) and a primary objective of the follow-up trial.

It should also be emphasised that a reduction in total petroleum hydrocarbons is a chemical endpoint and does not, by itself, demonstrate a corresponding reduction in ecological risk. Partial oxidation of hydrocarbons can generate polar and oxygenated metabolites that may remain bioavailable and phytotoxic even as measured TPH declines, so residual toxicity may persist despite the high removal efficiencies reported here. The present pilot focused on establishing chemical (TPH) and microbiological field efficacy as a prerequisite and did not include ecotoxicological endpoints; assessment of residual toxicity through phytotoxicity testing with native, arid- and salt-adapted plant species characteristic of the Mangystau region—white wormwood (*Artemisia terrae-albae*) and black saxaul (*Haloxylon aphyllum*) [50]—together with standard soil-ecotoxicity bioassays, is an explicit objective of the planned larger-scale trial.

5. Conclusions

When applied at the standard rate of the biopreparation, the immobilised-carrier formulation on buckwheat husks showed higher performance than the conventional free-cell suspension by a factor of 1.7 (94.0% versus 54.6%) and maintained a high HOM count in the buckwheat husk variant (2.5×10^8 CFU/g by day 45). Since the formulations were not matched for the absolute number of cells applied, this advantage should be attributed to the immobilised-carrier preparation as a complete applied technology—combining cell protection, moisture retention and a high local biomass density—rather than to immobilisation as an isolated factor; disentangling these contributions at an equalised cell load is a task for subsequent studies. Degradation in all variants followed first-order kinetics; the rate constant for buckwheat husks ($k = 0.0355 \text{ day}^{-1}$; $t_{1/2} = 19.5$ days) was 2.2–2.4 times higher than for rice husks and free cells. Catalase activity increased 7.8-fold, and dehydrogenase activity 5.5-fold relative to the control ($p < 0.001$); water-holding capacity increased from 38% to 55%. As a pilot-scale, proof-of-concept field validation, this study establishes the fundamental efficacy of the technology under arid conditions and provides a solid basis for subsequent larger-scale trials with increased replication, GC-MS confirmation and molecular characterisation of the microbial community.

6. Patents

Khozhanepessova, F.M.; Serikbayeva, A.K. Method for Immobilising Oil-Oxidising Microorganisms on a Buckwheat Husk Carrier. Patent of the Republic of Kazakhstan for Utility Model No. 11908, 13 March 2026.

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Conflicts of Interest: The authors hold intellectual property rights related to the immobilisation technology (Utility Model Patent of the Republic of Kazakhstan No. 11908), and the bacterial preparation “KazBioRem” is associated with commercial development. However, this study was designed, conducted, analysed and reported independently, and this interest did not influence the study design, data collection, analysis, interpretation, or the decision to publish. The funders had no role in the design of the study; in the collection, analysis or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. Author Nazira Moldagulova was employed by the company Scientific and Production Center of Ecological and Industrial Biotechnology LLP. Beyond this disclosure, the authors declare no further competing interests.

Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of Variance
BTEX	Benzene, Toluene, Ethylbenzene, Xylenes
CFU	Colony Forming Unit
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography–Mass Spectrometry
HOM	Hydrocarbon-Oxidising Microorganisms
HPLC	High-Performance Liquid Chromatography
PAH	Polycyclic Aromatic Hydrocarbon
ROS	Reactive Oxygen Species
TFF	Triphenylformazan
TPH	Total Petroleum Hydrocarbons
TTC	2,3,5-Triphenyltetrazolium Chloride

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