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ENHANCED OIL BIODEGRADATION USING IMMOBILISED RHODOCOCCUS-DIETZIA CONSORTIUM ON AGRICULTURAL WASTE

Abstract: Oil contamination of soils remains an acute environmental problem, particularly in oil-producing regions such as western Kazakhstan. In this study, we explored whether a microbial consortium - specifically *Rhodococcus erythreus AT7* and *Dietzia maris 22K* - could work better when immobilised on agricultural waste like buckwheat and rice husks for cleaning up oil-contaminated soils. The adsorption immobilisation method was applied and compared with free cell systems over 45 days using model soils contaminated with crude oil from the Karazhanbas field. Our results showed that when we immobilised cells on buckwheat and rice husks, they achieved significantly higher TPH degradation (58.4 ±3.7) % and (52.1 ±4.2) %, respectively) compared to free cells. Kinetic modelling revealed first-order degradation kinetics ($R^2 > 0.95$) with rate constants of 0.0187 d^{-1} for buckwheat husks, 0.0162 d^{-1} for rice husks, and 0.0108 d^{-1} for free cells, representing 73 % enhancement for buckwheat husk. Why did this work so well? We found several reasons: excellent cell retention (92.3 ±2.1) %, better moisture retention (55 % compared to just 38 % in controls), and - perhaps most interestingly - favourable chemical properties, especially higher antioxidant content. Under optimal conditions (10 % carrier ratio, 60 % - 70 % moisture, pH 7.0 - 7.5), projected cleanup timelines of 3-4 months are achievable. These findings suggest that agricultural waste carriers represent a promising and cost-effective approach for bioremediation of oil-contaminated soils.

Keywords: bioremediation, buckwheat husk, immobilised microorganisms, oil degradation, oil-contaminated soils, rice husk, western Kazakhstan

Introduction

Oil pollution affects more than 5 million km^2 of land globally, posing a serious environmental threat [1, 2]. In Kazakhstan, particularly in the Caspian regions, more than 43,000 km^2 of disturbed lands have been identified, including 6,000 km^2 of territories contaminated with petroleum products [3]. Petroleum hydrocarbons (PHs) exhibit carcinogenic effects and alter the physical and chemical properties of soil, thereby reducing water permeability and fertility [4].

Traditional physical and chemical remediation methods are effective but energy-intensive and may cause secondary contamination [5-7]. Bioremediation using

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immobilised microbial consortia represents a more sustainable approach [8]. Immobilised cells demonstrate significant advantages over free cells in terms of survival and retention at contaminated sites [8, 9].

Recently, researchers have become increasingly interested in agricultural wastes as biodegradable carriers for immobilisation. Previous work has shown promising results with materials like corn cobs and wheat straw [10-13], and even carrot peel [14] has proven effective for hydrocarbon breakdown. Among oil-degrading microorganisms, *Rhodococcus* and *Dietzia* strains [2, 15] are of particular interest due to their capability to degrade various hydrocarbons [16, 17].

Agricultural wastes have been widely employed as immobilisation carriers in various biotechnological applications due to their biodegradability, low cost, and favourable surface properties. In particular, rice husk and buckwheat husk have been successfully utilised for the immobilisation of industrial enzymes such as lipases, cellulases, and other hydrolases, demonstrating their potential as effective bio-support materials. Lignocellulosic carriers have also been investigated in certain bioremediation studies for bacterial immobilisation and hydrocarbon degradation [10-13].

However, the targeted application of these specific agricultural byproducts for immobilising petroleum-degrading microbial consortia remains limited, especially under the extreme continental climate conditions characteristic of Central Asia. This study addresses this gap by evaluating the performance of buckwheat and rice husks as microbial carriers in the context of oil-contaminated soils typical of the region.

The purpose of this study was to assess the effectiveness of an immobilised consortium of *R. erythreus AT7* and *D. maris 22K* on plant-based substrates for bioremediation of oil-contaminated soils in western Kazakhstan. The specific objectives included: (1) comparison of the efficiency of immobilised and free cells, (2) evaluation of the effect of different substrates on consortium activity, and (3) analysis of degradation kinetics. The strains of *Rhodococcus erythreus AT7* and *Dietzia maris 22K* are patented [18] and adapted to Kazakhstan's climate. Kazakhstan produces 38-45 thousand tonnes of rice husks and 28-36 thousand tonnes of buckwheat husks [3], which have high porosity and are deemed safe for biotechnological applications. The hypothesis was that immobilisation on plant-based carriers would provide enhanced cell viability and accelerate oil degradation processes.

Materials and methods

Bacterial strains and cultivation

A consortium of hydrocarbon-oxidising bacteria consisting of *Rhodococcus erythreus AT7* and *Dietzia maris 22K* strains was used in this study. These strains were previously isolated from oil-contaminated soils of western Kazakhstan and stored under cryogenic conditions at –30 °C.

For cultivation, the consortium was grown in modified Voroshilov-Dianova medium of the following composition: NaCl - 10 $g \cdot L^{-1}$, K_2HPO_4 - 1 $g \cdot L^{-1}$, $MgSO_4 \cdot 7H_2O$ - 0.2 $g \cdot L^{-1}$, $CaCl_2$ - 0.01 $g \cdot L^{-1}$, NH_4NO_3 - 1 $g \cdot L^{-1}$, distilled water - up to 1 L. The medium was sterilised by autoclaving at 121 °C for 15 min. Autoclaved crude oil was added at a concentration of 5 $mL \cdot L^{-1}$ as the sole source of carbon and energy. The strains were inoculated in equal proportions (1:1 ratio) and incubated at 28°C for 48 h on an orbital shaker at 150 rpm. We monitored microbial growth spectrophotometrically at 600 nm using

a Shimadzu UV-1800 spectrophotometer [19]. This proved to be a reliable method, though we had to be careful about timing the measurements consistently.

Preparation plant-based substrates

Rice husk (RH) and buckwheat husk (BH) were the two kinds of agricultural wastes that were utilised as immobilisation carriers. Foreign particles were first removed from the starting ingredients. The plant carriers were prepared by treating them with 1 % NaOH and HCl solutions after being cleaned with distilled water.

The chemical composition of carriers was determined using standard analytical methods: moisture content was measured gravimetrically by drying at 105 °C for 24-48 hours [20], Ash content was determined by calcination at 550 °C in a muffle furnace [21], protein content was analysed by the Kjeldahl method [22, 23], fat content was determined by the Soxhlet method [24], and fibre and lignin content were analysed by the Van Soest method [25]. Starch content was determined by the enzymatic method [26]. Vitamins A, B_1, B_2, and E were analysed by high-performance liquid chromatography with ultraviolet and fluorescence detection [27]. Fourier transform infrared spectroscopy in the range of 4000 cm^{-1} - 400 cm^{-1} was used to identify functional groups on the carrier surface according to standard procedures [28].

Oil adsorption capacity

Sorption capacity of the prepared carriers was evaluated using an adapted method. A 1.0 g sample of dry carrier was placed in a container with 20 mL of crude oil and incubated at (25 ±2) °C for 30 min with periodic stirring. Subsequently, the free oil was removed by filtration through a funnel with pre-weighed filter paper. Sorption capacity, Q_s [$g \cdot g^{-1}$] was calculated using equation [29]:

$$Q_s = \frac{m_2 - m_1}{m_1} \tag{1}$$

where: m_1 - mass of dry carrier [g], m_2 - mass of carrier after saturation with oil and removal of excess liquid [g]. All measurements were repeated five times to ensure statistical reliability, and the results were averaged.

Microbial immobilisation

Immobilisation of bacterial cells was carried out by the adsorption method. The prepared substrates (5 g dry weight) were mixed with the active microbial suspension (25 mL , ~10^8 CFU·mL^{-1}) in sterile 250 mL Erlenmeyer flasks, maintaining a 1 : 5 (w/v) ratio. The mixture was incubated at 28 °C on an orbital shaker at 150 rpm for 24 h to maximise cell attachment to the substrate surface. Immobilisation efficiency was determined indirectly by measuring the decrease in the optical density of the cell suspension before and after contact with the carrier material. Cell suspension samples (1 mL) were taken after (0, 6, 12, and 24) h, and the optical density was measured at λ = 600 nm. The Immobilisation efficiency *IE* [%] was calculated according to:

$$IE = \frac{OD_0 - OD_f}{100} \tag{2}$$

where: OD_0 - the initial optical density, OD_f - the final optical density after 24 hours of contact.

Immobilisation efficiency was confirmed by scanning electron microscopy (SEM). Carrier samples were fixed with 2.5 % glutaraldehyde in phosphate buffer (pH = 7.2) for 2 h at 4 °C, dehydrated through graded ethanol series (30, 50, 70, 90, 100) % v/v, critical point dried using CO_2, and sputter-coated with gold-palladium (10 nm thickness). SEM examination was performed using a scanning electron microscope at accelerating voltages of (5-20) kV.

Experimental design

A sandy loam soil sampled in western Kazakhstan was used for modelling the bioremediation process. The soil was characterised by the following basic properties: pH = 7.8 ±0.2 (water extract 1 : 5), organic matter content (2.1 ±0.3) %, water holding capacity (28 ±2) %, and granulometric composition: sand 65 %, silt 25 %, clay 10 %.

Artificial soil contamination was carried out by careful mixing of crude oil from the Karazhanbas field with soil in the ratio of 0.5 g of oil per 50 g of soil (1 % w/w). The selected 1 % (w/w) contamination level represents realistic field conditions documented in western Kazakhstan, where TPH contamination typically ranges from (0.5 - 2.8) % in operational oil fields [3, 4]. This level aligns with previous bioremediation studies using agricultural carriers [11, 12] and ensures sufficient substrate availability for microbial degradation while remaining within the optimal range for aerobic biodegradation processes [1].

The experimental design included six treatments, each with five independent replications ($n = 5$):

- Control (C): contaminated soil without microbial treatment
- Abiotic control (AB): autoclaved contaminated soil (121 °C)
- Carrier control (CC): contaminated soil + 5 g sterilised agricultural carriers to evaluate adsorption and physical effects
- Free cells (FC): contaminated soil + 5 mL of free bacterial suspension ($\sim 10^8$ CFU·mL^{-1})
- Buckwheat husk (BH): contaminated soil + 5 g immobilised consortium on buckwheat husk
- Rice husk (RH): contaminated soil + 5 g immobilised consortium on rice husk

The carrier control was designed to isolate physical adsorption effects from biological degradation processes.

To ensure statistical independence and avoid pseudo-replication, a sacrificial sampling design was employed. A total of 120 experimental units were prepared (6 treatments × 4 time points × 5 replicates), with each unit consisting of 50 g of contaminated soil in sterile 250 mL glass containers. Separate containers were designated for sampling at T_0 (day 0, baseline), T_1 (day 15), T_2 (day 30), and T_3 (day 45). At each time point, 30 containers (5 replicates per treatment) were completely sacrificed for analysis, with 5 g of soil taken from each container for TPH determination and additional samples collected for microbial and enzymatic analyses.

Incubation was performed under controlled conditions at (25 - 28) °C with relative air humidity maintained at (60 - 70) %. Soil moisture was maintained at (60 - 70) % of full moisture capacity throughout the experiment.

Analytical methods

The concentration of total petroleum hydrocarbons was determined by infrared spectrometry using an FTIR spectrometer (IRAffinity-1S, Shimadzu). Calibration was performed using eight concentration points (0 - 1000) mg·L^{-1} with $R^2 \geq 0.998$.

Soil samples were dried at 60 °C, pulverised, and extracted with hexane (20 mL) in an ultrasonic bath (30 min, 40 kHz). Fractional analysis of hydrocarbons was carried out by IR spectrometry using characteristic absorption bands:

- BTEX: (3030 – 3100) cm^{-1} (aromatic C-H)
- Alkanes: (2850 – 2960) cm^{-1} (aliphatic C-H)
- PAHs: (1600, 1500) cm^{-1} (aromatic C=C)

In Eq. (3) the degradation of TPH was described by first-order kinetics:

$$C(t) = C_0 \cdot e^{-kt} \quad (3)$$

where: $C(t)$ - concentration of TPH [mg·kg^{-1}] at time t, C_0 - initial concentration [mg·kg^{-1}], k - degradation rate constant [d^{-1}], t - time [d].

The model parameters were determined by the nonlinear regression method. The half-life was calculated as $t_{1/2} = \ln(2) \cdot k^{-1}$.

Viable cells were counted by serial dilutions with plating on:

- Nutrient agar for total microbial counts
- Mineral medium with 0.5 % oil (hydrocarbon-oxidising bacteria)

Catalase activity was determined by the gasometric method, measuring the volume of released oxygen. To 1 g of soil sample, 2 mL of 3 % H_2O_2 solution was added, and the volume of released O_2 was measured for 3 min using a graduated gasometric tube (Bach-Oparin method). The activity was expressed in mL $O_2 \cdot g^{-1}$ soil·$(3\ min)^{-1}$.

Lipase activity was determined by the gasometric method through hydrolysis of triolein with titration of the formed fatty acids using 0.05 M NaOH [μmol fatty acids·$g^{-1} \cdot h^{-1}$]. Analyses were performed in triplicate at 15 d, 30 d, and 45 d.

Soil moisture content was measured gravimetrically at each sampling time point. Fresh soil samples (2 - 3) g were weighed immediately after collection, then dried at 105 °C for 24 h until constant weight was achieved. Moisture content [%] was calculated as:

$$MC = \frac{W_w - W_d}{W_d} \cdot 100 \quad (4)$$

where: MC - moisture content, W_w - wet weight, W_d - dry weight.

Measurements were performed in triplicate for each experimental unit.

Statistical analysis

All data are presented as mean ± standard deviation (SD), based on five replicates ($n = 5$). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to assess pairwise differences. A significance level of $p < 0.05$ was considered statistically significant.

Results and discussion

Characterisation of plant-based substrates

The chemical composition of the carriers (Table 1) revealed important differences that help explain their biodegradation performance. Rice husk showed higher content of

structural polymers - cellulose (34.3 - 43.1) % and lignin (19.2 - 47.0) % - which contribute to mechanical stability and durability during long-term use. These components are typical for lignocellulosic materials and support microbial attachment and immobilisation [10, 11]. Similar findings have been reported for wheat straw and corn cob-based carriers [12].

However, the most significant difference was the substantially higher rutin content in buckwheat husk - 28.8 mg·(100 g)$^{-1}$ compared to 15.0 in rice husk, representing a 92 % increase. This compositional difference may have functional implications, as flavonoids like rutin are established antioxidants that could provide cellular protection during hydrocarbon degradation. Petroleum oxidation, particularly through monooxygenase pathways, generates reactive oxygen species (ROS) that can compromise microbial viability. The elevated rutin content may mitigate oxidative stress and support sustained metabolic activity. Notably, the potential antioxidant effects of plant-based carriers have received limited attention in previous biodegradation studies [10, 13, 14], suggesting this mechanism warrants systematic investigation.

Table 1

Content of organic components and vitamins in buckwheat husks and rice husks

Component	**Rice husk [% by weight] or [mg·(100 g)$^{-1}$]**	**Buckwheat husk [% by weight] or [mg·(100 g)$^{-1}$]**
Humidity	(3.75 - 24.08) % by weight	(8.0 - 14.0) % by weight
Ash content	(11.86 - 31.78) % by weight	(2.7 - 4.0) % by weight
Pentosans	(4.52 - 37.00) % by weight	(12.5 % by weight
Cellulose	(34.32 - 43.12) % by weight	(20.0 - 27.0) % by weight
Lignin	(19.20 - 46.97) % by weight	(8.0 - 15.0) % by weight
Proteins	(1.21 - 8.75) % by weight	(3.3 - 7.0) % by weight
Fats	(0.30 - 6.62) % by weight	2.2 % by weight
Starch	9.76 % by weight	0.6 % by weight
Vitamin A	0.04 mg · (100 g)$^{-1}$	0,003 mg · (100 g)$^{-1}$
Vitamin B_1	0.45 mg · (100 g)$^{-1}$	0.16 mg · (100 g)$^{-1}$
Vitamin B_2	0.1 mg · (100 g)$^{-1}$	0.084 mg · (100 g)$^{-1}$
Vitamin P (rutin)	15.0 mg · (100 g)$^{-1}$	28.8 mg · (100 g)$^{-1}$
Vitamin E	1.6 mg · (100 g)$^{-1}$	2.3 mg · (100 g)$^{-1}$

The compositional differences directly explain the superior performance of buckwheat husk. The 92 % higher rutin content (28.8 vs 15.0 mg · (100 g)$^{-1}$) provides antioxidant protection against oxidative stress during petroleum biodegradation, while the lower lignin content (8.0 - 15.0) % vs (19.2 - 47.0) % enables better microbial accessibility. These biochemical advantages, combined with higher protein content for microbial nutrition, create a more favourable microenvironment that correlates with the observed 58.4% vs 52.1% TPH degradation efficiency.

Immobilisation efficiency

Both carriers demonstrated high immobilisation efficiency after 24 hours of contact - (92.3 ±2.1) % for buckwheat husk and (87.6 ±3.4) % for rice husk (Fig. 1). These values are comparable to more complex immobilisation systems and confirm that agricultural wastes can serve as efficient, cost-effective microbial carriers [9, 29]. The efficiency falls within the range expected for biofilm-based systems, where porous, hydrophilic surfaces promote cell attachment and retention [8].

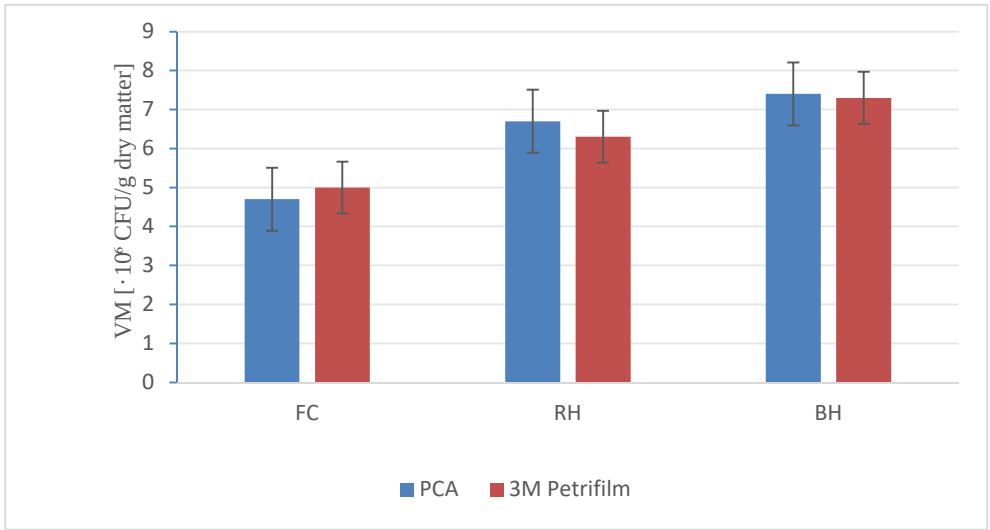


Fig. 1. Comparison of viable microorganisms (VM) on different carriers after immobilisation. Error bars represent standard deviations (n = 5). Different letters above bars indicate statistically significant differences (p < 0.05, Tukey HSD test). PCA = plate count agar, 3M Petrifilm = rapid enumeration method

Scanning electron microscopy (Fig. 2) confirmed this observation through visual and quantitative analysis. Buckwheat husk supported a high cell density of (3.2 ±0.4) · 10^6 cells·cm^{-2} and (42 ±5) % surface coverage, indicating successful colonisation. The dense, clustered attachment pattern is consistent with previous reports of biofilm formation on lignocellulosic substrates used in environmental applications [11, 13], and confirms that these natural materials can provide suitable microenvironments for microbial persistence.

TPH degradation kinetics

Over the 45-day experiment, TPH biodegradation showed a clear treatment hierarchy: buckwheat husk achieved (58.4 ±3.7) %, followed by rice husk at (52.1 ±4.2) %, and free cells at (38.3 ±5.8) % (Fig. 3). Minimal TPH loss in control setups (5.8-12.5) % confirmed that the observed removal was predominantly biological, not due to abiotic factors or sorption alone.

Kinetic modelling revealed first-order degradation across all treatments, with rate constants of k = 0.0187 d^{-1} for buckwheat husk, k = 0.0162 d^{-1} for rice husk, and k = 0.0108 d^{-1} for free cells (Table 2). These values are consistent with those reported for other immobilised systems [1, 11], and represent a ~73 % improvement in degradation rate due to carrier-based immobilisation. The performance of buckwheat husk, in particular, compares favourably to other plant-based carriers like wheat straw and corn cob [10, 12, 13], confirming its potential as a cost-effective, high-performance alternative.

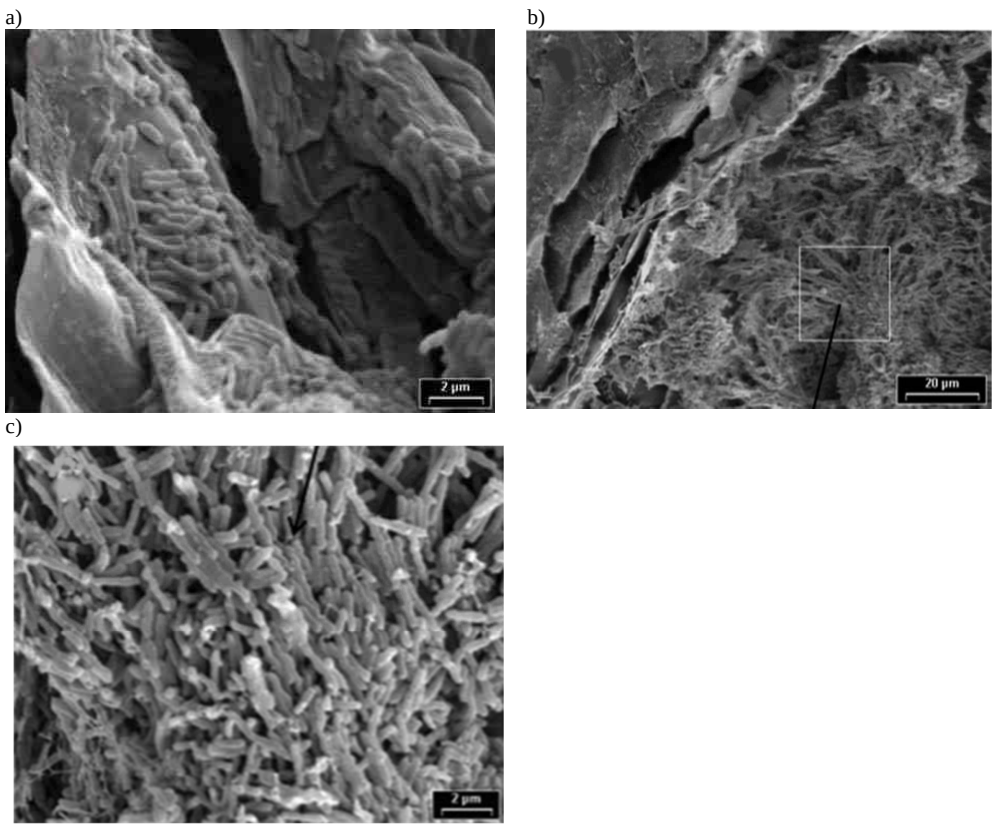


Fig. 2. SEM characterisation of buckwheat husk carrier: a) Native porous structure showing high surface area available for microbial attachment (scale bar: 20 μm); b) Higher magnification revealing interconnected microporosity favourable for cell retention (scale bar: 2 μm); c) Successful bacterial immobilisation demonstrating cell attachment and biofilm formation (arrows indicate bacterial cells, scale bar: 2 μm)

The enhanced degradation can be attributed to synergistic interactions between the two strains used. As demonstrated by Chen et al. [2], Dietzia efficiently degrades saturated hydrocarbons, while Rhodococcus is known for its broad catabolic range, particularly for aromatic compounds [15, 16]. This metabolic complementarity enabled the consortium to effectively degrade the complex Karazhanbas crude oil, which contains both aliphatic and aromatic fractions.

Fraction-specific analysis (Fig. 4) confirmed this pattern: BTEX compounds were degraded most efficiently, followed by short-chain and medium-chain alkanes, while PAHs were least affected. This selectivity is consistent with established patterns in hydrocarbon biodegradation - lighter and more bioavailable compounds are more readily biodegraded, whereas heavier or polycyclic compounds are more resistant due to lower solubility and higher toxicity.

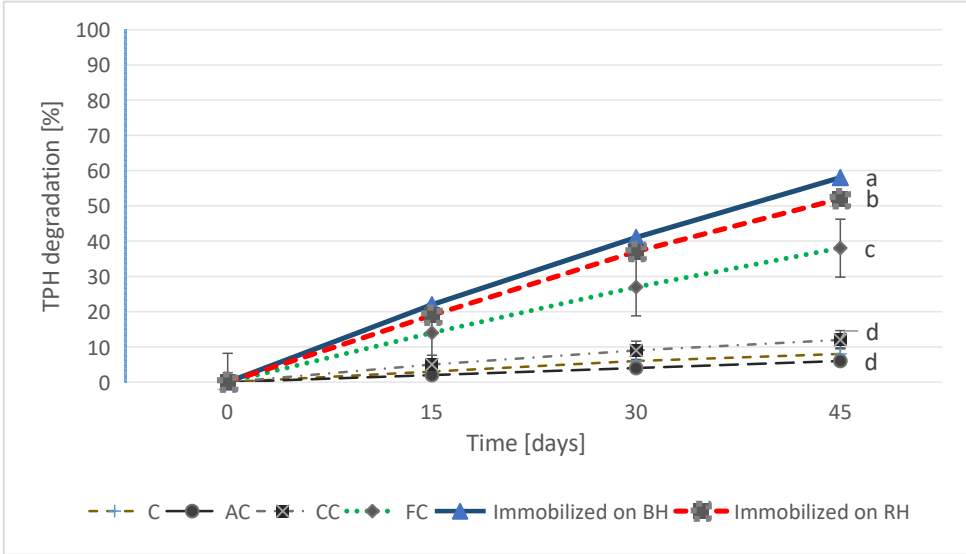


Fig. 3. TPH decomposition over time with different treatments (% of initial oil mass). Error bars represent standard deviations (n = 5). Letters at Day 45 indicate statistically significant differences between treatments (p < 0.05, Tukey HSD test). BH = buckwheat husk, RH = rice husk, FC = free cells, CC = carrier control, AC = abiotic control, C = control

Table 2

Kinetic parameters for first-order TPH degradation

Treatment	**Rate constant k [d^{-1}]**	**R^2 [-]**	**Half-life [days]**	**Statistical group***
Control	0.0018 ±0.0004	0.943	385 ±85	d
Abiotic control	0.0012 ±0.0003	0.952	578 ±95	d
Carrier control	0.0028 ±0.0005	0.964	247 ±45	c
Free cells	0.0108 ±0.0018	0.976	64.2 ±10.8	b
Immobilised on buckwheat husk	0.0187 ±0.0032	0.987	37.1 ±6.4	a
Immobilised on rice husk	0.0162 ±0.0027	0.981	42.8 ±7.1	a

*Different letters indicate statistically significant differences (p < 0.05, Tukey HSD test). Rate constants determined by non-linear regression analysis (n = 5). The differences between rate constants are statistically significant (p < 0.001). The 73 % enhancement in degradation rate for immobilised cells compared to free cells demonstrates improved microbial activity through cell protection and optimal substrate concentration maintenance on carrier surfaces

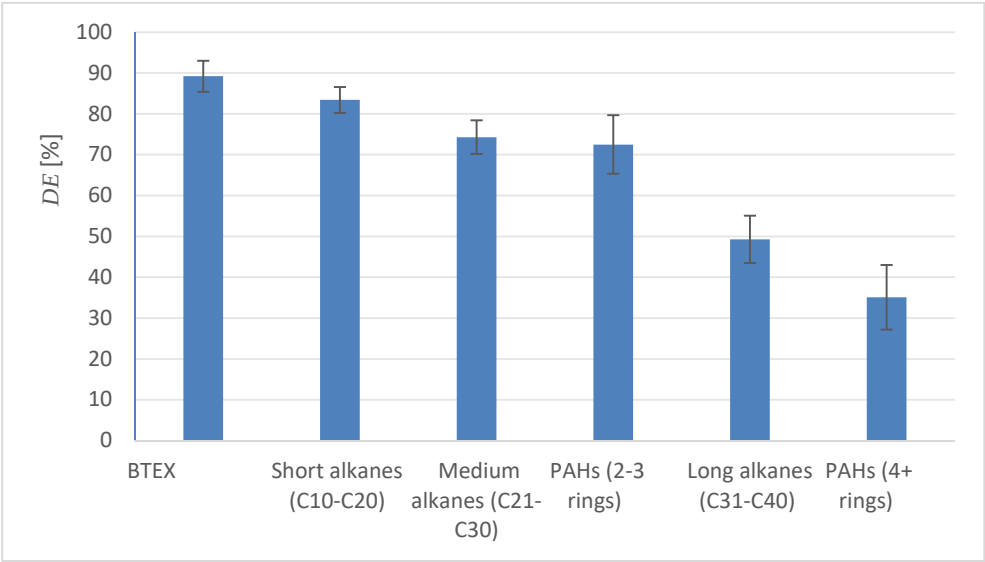


Fig. 4. Degradation efficiency, *DE* of different hydrocarbon fractions by immobilised bacteria on BH after 45 days. Error bars represent standard deviations (n = 5). Different letters above bars indicate statistically significant differences between hydrocarbon fractions ($p < 0.05$, Tukey HSD test). BTEX - benzene, toluene, ethylbenzene, xylenes; PAHs - polycyclic aromatic hydrocarbons

The abundance of hydrocarbon-oxidising bacteria was monitored in parallel with TPH degradation assessment. Initial bacterial abundance in inoculated treatments was ~10^8 CFU·g^{-1} soil (Table 3).

Table 3

Dynamics of hydrocarbon-oxidising bacteria abundance [10^7 CFU·g^{-1} soil]

Variant	Day 0	Day 15	Day 30	Day 45
Control	0.08 ±0.02^b	0.11 ±0.03^c	0.14 ±0.04^c	0.17 ±0.05^c
Abiotic control	0.05 ±0.01^b	0.03 ±0.01^c	0.02 ±0.01^c	0.01 ±0.01^c
Carrier control	0.12 ±0.03^b	0.28 ±0.05^c	0.35 ±0.08^c	0.22 ±0.04^c
Free cells	10.0 ±1.2^a	18.4 ±2,3^b	24.6 ±3.1^b	14.2 ±2.0^b
Immobilised on buckwheat husk	10.2 ±1.1^a	48.3 ±5.2^a	76.5 ±7.3^a	62.4 ±6.1^a
Immobilised on rice husk	10.5 ±0.9^a	35.7 ±4,1^a	58.2 ±5,8^a	47.3 ±5.2^a

Values with different superscript letters within each column are significantly different ($p < 0.05$, Tukey HSD test). Statistical analysis performed using one-way ANOVA followed by Tukey's post-hoc test (n = 5).

Hydrocarbon-oxidising bacteria were monitored throughout the experiment (Table 3), starting with similar inoculum levels across all treatments (~10^8 CFU·g^{-1} soil). Immobilised systems demonstrated enhanced microbial persistence. By day 30, buckwheat husk reached a peak of (76.5 ±7.3) · 10^7 CFU·g^{-1}, followed by rice husk at (58.2 ±5.8) · 10^7 CFU·g^{-1}, while the free-cell system showed lower abundance at (24.6 ±3.1) · 10^7 CFU·g^{-1}. Subsequently, populations stabilised but remained consistently higher in the immobilised systems.

These findings confirm that lignocellulosic carriers offer physical protection and a favourable microenvironment for cell survival. The sustained high biomass aligns with the known advantages of biofilm-based biodegradation systems - including stress buffering, retention in soil, and metabolic cooperation [8, 9, 29].

Enzymatic activity

Enzymatic activity trends correlated with the biodegradation results. Both catalase and lipase activities increased steadily across all biotic treatments (Table 4), confirming active microbial metabolism rather than passive adsorption. By day 45, catalase activity in immobilised systems reached (4.7 ±0.6) mL $O_2 \cdot g^{-1}$ soil·(3 min)$^{-1}$ for buckwheat husk and (3.9 ±0.5) mL $O_2 \cdot g^{-1}$ soil·(3 min)$^{-1}$ for rice husk - 68 % and 39 % higher than in the free-cell system (2.8 ±0.7) mL $O_2 \cdot g^{-1}$ soil·(3 min)$^{-1}$, respectively. Lipase followed a similar pattern, with up to 80 % higher activity in immobilised systems (13.5 ±2.0) μmol fatty acids·g^{-1} soil·h^{-1}) compared to free cells.

Table 4

Catalase and lipase activity in soil samples during bioremediation experiment

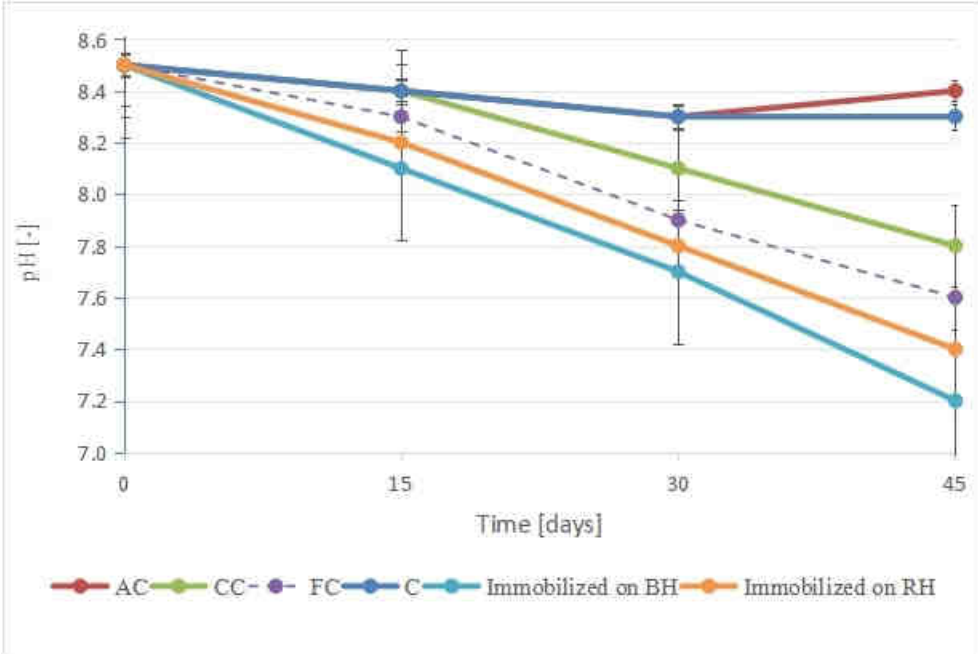
Catalase [mL $O_2 \cdot g^{-1}$ soil·(3 min)$^{-1}$]			
	Day 15	Day 30	Day 45
Control	0.1 ±0.0^{d}	0.1 ±0.0^{d}	0.1 ±0.0^{d}
Abiotic control	0.1 ±0.0^{d}	0.1 ±0.0^{d}	0.1 ±0.0^{d}
Carrier control	0.8 ±0.1^{c}	1.1 ±0.2^{c}	1.3 ±0.2^{c}
Free cells	1.8 ±0.3^{b}	2.3 ±0.4^{b}	2.8 ±0.7^{b}
Immobilised on buckwheat husk	3.2 ±0.4^{b}	4.1 ±0.5^{b}	4.7 ±0.6^{a}
Immobilised on rice husk	2.6 ±0.3^{a}	3.4 ±0.4^{a}	3.9 ±0.5^{b}
Lipase [μmol fatty acids·g^{-1} soil·h^{-1}]			
	Day 15	Day 30	Day 45
Control	0.8 ±0.3^{d}	0.7 ±0.2^{d}	0.6 ±0.3^{d}
Abiotic control	0.3 ±0.1^{d}	0.2 ±0.1^{d}	0.2 ±0.1^{d}
Carrier control	2.1 ±0.4^{c}	2.8 ±0.5^{c}	3.2 ±0.6^{c}
Free cells	6.3 ±1.1^{b}	9.8 ±1.4^{b}	13.5 ±2.0^{b}
Immobilised on buckwheat husk	12.4 ±1.8^{b}	18.6 ±2.1^{b}	24.3 ±2.7^{b}
Immobilised on rice husk	9.7 ±1.5^{a}	14.2 ±1.9^{a}	19.1 ±2.3^{a}

Values with different superscript letters within each enzyme assay and time point are significantly different ($p < 0.05$, Tukey HSD test). Statistical analysis performed using one-way ANOVA followed by Tukey's post-hoc test ($n = 5$). Data represent means ± standard deviations.

This consistent enzymatic boost mirrors the TPH degradation patterns and strongly supports the conclusion that biodegradation was biologically driven. The elevated activity also highlights the benefits of immobilisation - enhanced stress tolerance, better substrate accessibility, and improved metabolic performance under carrier protection.

Catalase and lipase were selected as key enzymatic markers due to their direct involvement in hydrocarbon degradation pathways. Catalase (EC 1.11.1.6) decomposes hydrogen peroxide, a reactive byproduct formed during alkane oxidation via monooxygenase enzymes, and serves as an indicator of microbial adaptation to oxidative stress. Elevated catalase activity reflects active detoxification processes associated with aerobic hydrocarbon metabolism.

a)



b)

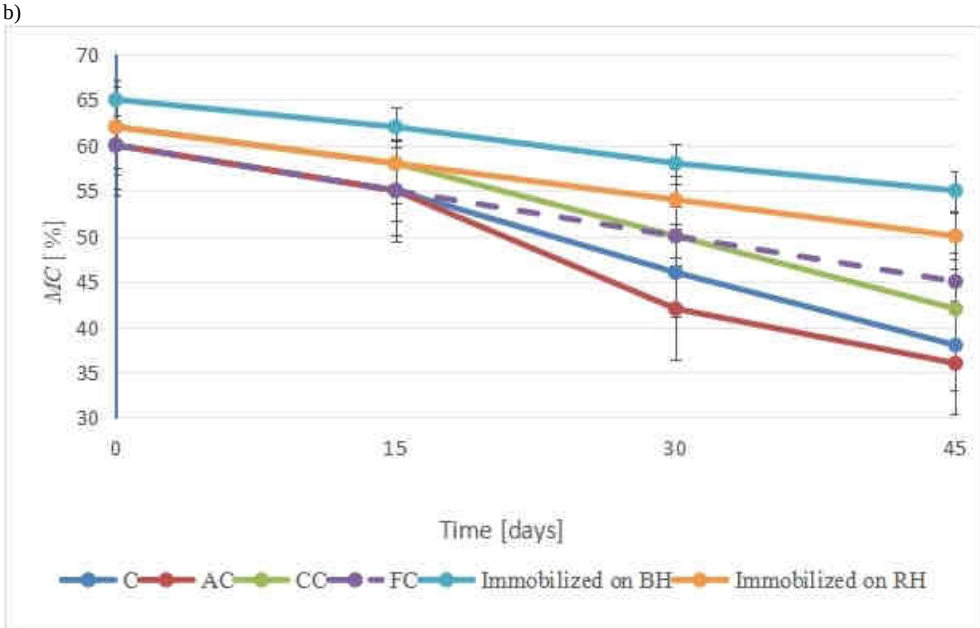


Fig. 5. Dynamics of a) soil pH and b) moisture content during 45-day bioremediation experiment. Error bars represent standard deviations (n = 5). Letters at day 45 indicate statistically significant differences between treatments ($p < 0.05$, Tukey HSD test). C = control, AC = abiotic control, CC = carrier control, FC = free cells, BH = buckwheat husk, RH = rice husk

Lipase (EC 3.1.1.3) catalyses the hydrolysis of ester bonds in petroleum-derived lipid fractions, facilitating the release and solubilisation of hydrophobic hydrocarbons. This enhances substrate bioavailability and supports subsequent microbial degradation.

The progressive increase in both enzymatic activities observed during the experiment confirms sustained microbial metabolism and distinguishes active biodegradation from passive removal processes. These enzymatic trends also highlight the favourable conditions provided by carrier-based immobilisation systems for microbial function and persistence.

Soil parameters

Soil pH steadily decreased in all microbial treatments, with buckwheat husk showing the most pronounced shift - from 8.5 to 7.4 by day 45, compared to a slight drop in the control (8.5 to 8.2) (Fig. 5a). This acidification likely reflects organic acid production during hydrocarbon metabolism, a well-established indicator of active biodegradation.

Moisture retention patterns showed consistent trends. By the end of the experiment, buckwheat husk treatments maintained (55.2 ±3.1) % soil moisture content - significantly higher than the (38.4 ±2.7) % observed in the control treatment ($p < 0.05$, Fig. 5b). Rice husk showed intermediate moisture retention at (47.8 ±2.9) %. This improved water-holding capacity likely helped sustain microbial activity, particularly under fluctuating conditions, and may partially explain the superior performance of immobilised systems [29].

Collectively, these results highlight how agricultural waste carriers offer multiple synergistic advantages: higher cell retention, more stable populations, elevated enzyme activity, and a more favourable microenvironment. In particular, the superior performance of buckwheat husk can be attributed not only to its structure, but also to its antioxidant profile - a mechanism that has received limited attention and may provide new strategies for optimising natural carriers in bioremediation.

Conclusion

This study demonstrates that immobilising *Rhodococcus erythreus AT7* and *Dietzia maris 22K* on agricultural waste carriers significantly enhances oil biodegradation efficiency. Buckwheat husk achieved the highest TPH removal (58.4 ±3.7) % with a degradation rate constant of $k = 0.0187$ d^{-1}, representing a 73 % improvement over free-cell systems. These kinetic parameters compare favourably with previously reported values for biochar [11] and other lignocellulosic materials [10, 12, 13], while demonstrating superior degradation rates under comparable conditions.

The enhanced efficiency is attributed to the metabolic complementarity of the bacterial consortium: *Dietzia* species demonstrate superior capability for saturated hydrocarbon degradation [2], while *Rhodococcus* strains exhibit broad catabolic versatility, particularly for aromatic compound metabolism [15, 16]. This synergistic relationship enabled comprehensive degradation of the complex Karazhanbas crude oil matrix, containing both aliphatic and aromatic hydrocarbon fractions.

The superior performance of buckwheat husk is fundamentally linked to its distinct biochemical profile. The 92 % higher rutin content (28.8 vs 15.0 mg·[100 g]$^{-1}$ compared to rice husk) provides a mechanistic explanation for enhanced biodegradation efficiency. Rutin-mediated cellular protection operates through multiple antioxidant pathways: direct reactive oxygen species neutralisation, transition metal chelation, and membrane lipid

stabilisation [31, 32]. This protection is particularly critical during petroleum biodegradation, where alkane monooxygenases and aromatic dioxygenases generate oxidative stress that can compromise microbial viability and enzymatic function. The flavonoid's antioxidant capacity, well-documented across diverse molecular structures [32], creates a protective microenvironment that sustains metabolic activity under hydrocarbon stress conditions. This biochemical enhancement mechanism represents a novel approach to carrier optimisation, extending beyond traditional physical and structural considerations. Additionally, buckwheat husk demonstrated superior cell retention ($3.2 \pm 0.4) \cdot 10^6$ cells$\cdot$cm^{-2}) and moisture preservation (55 % vs 38 % in controls), both critical factors for sustained microbial performance.

Biochemical validation confirmed active biodegradation rather than passive sorption. Progressive increases in catalase activity (reaching (4.7 ± 0.6) mL $O_2 \cdot g^{-1}$ soil$\cdot$(3 min)$^{-1}$) and lipase activity (24.3 ± 2.7) μmol fatty acids$\cdot g^{-1}$ soil$\cdot h^{-1}$) demonstrated enhanced enzymatic function in immobilised systems. The concurrent pH shift from 8.5 to 7.4 indicates organic acid production during active hydrocarbon metabolism [8-10]. The minimal TPH removal in carrier control (k = 0.0028 d^{-1}) compared to immobilised systems (k = 0.0187 d^{-1}) confirms that enhancement is predominantly biological. These enzymatic biomarkers provide quantitative evidence that rutin-rich carriers enhance microbial detoxification capacity while supporting sustained metabolic activity.

This investigation advances the field through several key innovations: (1) systematic optimisation of regionally abundant agricultural substrates for petroleum-degrading consortia under Central Asian climatic conditions, (2) first quantitative demonstration of antioxidant-mediated enhancement as a carrier selection criterion, introducing rutin content as a predictive parameter for biodegradation efficiency, (3) integration of locally adapted strains with indigenous waste materials suitable for Kazakhstan's extreme temperature variations (–30 °C to +45 °C), and (4) comprehensive performance characterisation incorporating enzymatic biomarkers and fractional hydrocarbon analysis for mechanistic validation.

The correlation between rutin content and degradation performance establishes antioxidant capacity as a fundamental design criterion for agricultural waste carriers, representing a paradigm shift from purely physical optimisation to biochemically-informed carrier engineering [31, 32]. This approach recognises that flavonoid compounds serve multiple functional roles in biological systems, including cellular protection and metabolic enhancement.

Under optimised operational parameters (10 % carrier-to-soil ratio, (60 - 70) % moisture content, pH 7.0-7.5), the system achieved a hydrocarbon half-life of 37 days, enabling complete remediation within 3-4 months. The antioxidant-mediated enhancement mechanism identified here establishes a framework for systematic evaluation of flavonoid-containing agricultural residues, potentially enabling structure-activity relationship development for next-generation carrier design [32].

Future research priorities include: field-scale validation under extreme climatic conditions, optimisation for diverse regional crude oil compositions (Tengiz, Kashagan fields), and comprehensive techno-economic analysis for industrial implementation. The successful demonstration of biochemical carrier enhancement opens new avenues for sustainable bioremediation technology development using regionally abundant agricultural waste streams.

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