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Analysis of the Viability of Oil-Oxidizing Microorganisms After Long-Term Storage and the Effect of Humate Concentration on Their Activity

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ABSTRACT

This study evaluated the viability of oil-oxidizing microorganisms after long-term refrigerated storage. It determined how different concentrations of potassium humate affect their growth, activity, and bioremediation potential in oil-contaminated soil. Four bacterial strains, S3, S4, S6, and A1, previously isolated from oil-contaminated soils, were examined after one year of storage. Their viability was assessed by cultivation in meat-peptone broth and on meat-peptone agar, followed by visual observation, microscopy, Gram staining, and spectrophotometric measurement of optical density at 600 nm. Potassium humate obtained from oxidized brown coal was tested at concentrations of 0.5%, 1%, 5%, and 10%. The efficiency of soil purification was evaluated by measuring the mass fraction of petroleum products during a 90-day experiment. After storage, strains S3, S4, and A1 retained viability and showed active growth, whereas S6 did not recover under standard cultivation conditions. The response to humate depended on both strain and concentration. S3 recorded the highest optical density at 10% humate, while A1 showed growth at 1% and 10%. In the soil experiment, S4 with potassium humate achieved the greatest reduction of petroleum products, reaching 77.11% purification after 90 days. S3 with humate and Bakoyl with humate also showed high efficiencies of 75.49% and 76.07%, respectively. The results confirm that potassium humate can enhance the activity of selected oil-oxidizing microorganisms and support the development of combined biopreparations for reclaiming oil-contaminated soils.

Keywords: *Oil-oxidizing microorganisms, Humate, Concentration, Viability, Oil-contaminated soil*

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INTRODUCTION

Oil and petroleum product pollution is one of the most complex forms of anthropogenic environmental impact. Currents can transport oil entering water bodies for hundreds to thousands of kilometers from the discharge site, penetrate the water column, and accumulate in bottom sediments, affecting all groups of organisms. Modern bioremediation technologies use oil-oxidizing microorganisms (OOMs) to treat hydrocarbon-contaminated soils and water bodies. These microorganisms can degrade a wide range of hydrocarbon compounds, including alkanes, aromatic hydrocarbons, and petroleum derivatives, converting them into less toxic substances (Daffonchio *et al*., 2013). However, biodegradation efficiency largely depends on microbial viability after storage and on the environmental conditions into which they are introduced (Bashkin *et al*., 2010). One important approach to increasing the stability and activity of OOMs after long-term storage is using growth stimulants, such as humic substances, particularly humates. Humic acids, a key organic component of soils, natural waters, and peat, show increased reactivity toward oil and its components because of their hydrophobic aromatic structure. This reactivity allows humic acids to play an important role in self-purification processes and in mitigating the environmental impact of oil pollution. The efficiency of bioremediation of oil-contaminated

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water bodies can be significantly improved by using biological preparations that combine humic acids with microorganisms capable of degrading crude oil. As dispersing agents, humic acids help break down oil emulsions into smaller components, which microorganisms can then degrade more easily. Under abiotic stress, when microorganisms' uptake of essential nutrients slows, humic acids serve as a substrate that provides bacteria with components necessary for their vital activity.

Microorganisms can partially utilize molecules of humic substances and peripheral fragments represented by oligosaccharides and peptides, thereby supplying nutrients. Humates are natural organic compounds obtained from peat, brown coal, and sapropel and are known for their biostimulating and adaptogenic properties (Yakimov *et al*., 2007; Dmitrieva *et al*., 2017; Kassenova *et al*., 2024). Their application can not only maintain microbial metabolic activity but also enhance the biodegradation of petroleum pollutants (Chugunov *et al*., 2000).

Nevertheless, the effect of humate concentration on the viability and oil-oxidizing activity of microorganisms after long-term storage remains insufficiently studied. Systematic studies are needed to determine optimal storage conditions and stimulation approaches for microbial consortia intended for subsequent effective application in bioremediation technologies (Manucharova *et al*., 2021).

The use of oil-oxidizing microorganisms (OOMs) in environmental purification biotechnologies has become one of the most promising and environmentally safe methods for eliminating oil pollution. Such microorganisms include bacteria of the genera *Pseudomonas*, *Rhodococcus*, *Acinetobacter*, and *Alcanivorax*, which are capable of degrading hydrocarbons through specialized enzymatic systems (Chugunov *et al*., 2000). The process of bioremediation of ecosystems contaminated with oil and petroleum products is based on the ability of microorganisms to decompose complex organic compounds of petroleum. However, a major limitation of using OOMs under field conditions is their decreased activity and abundance after long-term storage, especially at low temperatures or under lyophilization conditions (Viruel *et al*., 2011; Vaccari *et al*., 2019).

To improve the efficiency of bioremediation preparations, it is necessary to use approaches that help preserve the viability of microbial cells and restore their metabolic activity. One such approach is using humic substances, which can stimulate microbial growth and enzymatic activity and protect cells from stress factors (Cong *et al*., 2020). Humates may also serve as a nutrient source and pH regulator, promoting better microbial adaptation in hydrocarbon-contaminated ecosystems (Al-Zahrani & Idris 2010).

Despite individual studies on the use of humates in agricultural technologies and microbiology, the effect of different concentrations of these compounds on the oil-oxidizing activity of microorganisms after long-term storage remains poorly understood. This is especially relevant for developing effective long-shelf-life biopreparations suitable for use under extreme climatic conditions (Ichor *et al*., 2014).

This study aims to address this gap by comprehensively assessing the viability of oil-oxidizing microorganisms after storage under different conditions and evaluating their activity in the presence of humates at different concentrations. The obtained results may be used to develop new generations of biopreparations intended for the ecological rehabilitation of oil-contaminated areas (Kassenova *et al*., 2024).

MATERIALS AND METHODS

The study focused on oil-oxidizing microorganisms, namely strains S3, S4, S6, and A1, previously isolated from soils contaminated with petroleum products.

The following components were used in the study: meat-peptone agar (MPA) for cultivation and isolation of pure cultures; meat-peptone broth (MPB) for obtaining microbial suspension and assessing growth in liquid medium; and potassium humate at concentrations of 0.5%, 1%, 5%, and 10% as an additive to evaluate its effect on the growth activity of the studied strains.

The nutrient media were prepared according to the manufacturer's instructions. The media were poured into Petri dishes under sterile conditions in Class II biological safety laminar flow cabinets. For 90-mm Petri dishes, the poured medium volume was 18–20 cm^3, corresponding to a layer thickness of approximately 3 mm and meeting the requirements of the relevant standard (Interstate Council for Standardization, Metrology and Certification (ISC) 2017).

The nutrient media were sterilized by autoclaving at 110 °C and a pressure of 0.5–1.1 atm for 30 minutes. Glassware was sterilized in a drying oven at 170 ± 10 °C for 2 hours or by autoclaving at 125 °C and 1.1–2 atm for 15–30 minutes (State Committee of the USSR for Standards 1976).

The study included the following microbiological methods:

- Gram staining to assess the morphological and tinctorial properties of cells;
- inoculation of microorganisms onto nutrient media for quantitative and qualitative characterization of growth;
- identification based on a combination of morphological features, colony morphology, and Gram-negative or Gram-positive reaction.

After inoculation, the Petri dishes were incubated in a thermostat at 28 ± 1 °C for 48–72 hours. Changes in colonies and biomass were analyzed visually and using a microscope (State Committee of the USSR for Standards 1976).

To study the effect of humate, growth activity tests were performed in MPB supplemented with humate at different concentrations. Activity was assessed by measuring the optical density of the culture liquid using spectrophotometry at 600 nm.

The following laboratory equipment was used in the experiment:

- TS-1/80 SPU thermostat for incubation of microbial cultures;
- Olympus BX43 binocular microscope (Japan, 2017) for microscopy and identification;
- Class II biological safety cabinet to ensure sterility when working with cultures;
- laboratory autoclave for sterilization of nutrient media and instruments;
- shaker for preparing homogeneous suspensions;
- spectrophotometer for measuring optical density and assessing biomass.

In this study, potassium humate was obtained from oxidized brown coal mined from the Sarykol deposit in Kazakhstan. The coal was preliminarily ground to less than 0.5 mm. The following characteristics of the obtained coal were determined, expressed as mass percentages: Ad — 66.09; Wr — 5.73; Vd — 17.78; Std — 0.71; Ctd — 21.01; Htd — 1.68; Ntd — 2.09; Na — 0.61; Al — 0.89; K — 0.58; Ca — 0.31; Ti — 0.22; Fe — 1.11; Zr — 0.08. The coal particle size distribution was as follows: 2.95 μm — 10%, 63.8 μm — 50%, and 452 μm — 90%.

Humate was obtained by processing the raw material in a rotary-pulsation apparatus and an ultrasonic reactor. These processes reduced coal particles to sizes ranging from 19.2 nm to 3.57 μm. In both cases, air was supplied to the reactor, promoting coal oxidation and increasing humic-substance content. During grinding and ultrasonic treatment, the mixture temperature did not exceed 50–55 °C, which is acceptable for coal oxidation by atmospheric oxygen and for extracting the resulting salts of humic, fulvic, and other acids.

As a result of air oxidation, a micellar dispersion formed, namely a solution of humic substances with particle sizes below one micrometer. This technology is based on cavitation dispersion of humate-containing substances, particularly coal, using electrophysical treatment (Yermagambet *et al*., 2021).

RESULTS AND DISCUSSION

After one year of storage under refrigerated conditions, an assessment of the vital activity of four strains of oil-oxidizing microorganisms was carried out: S3, S4, S6, and A1. All cultures were transferred under sterile conditions into MPB liquid culture medium (100 ml per 500 ml flask), then placed on a shaker and incubated at 32 °C for two days **(Figure 1)**.

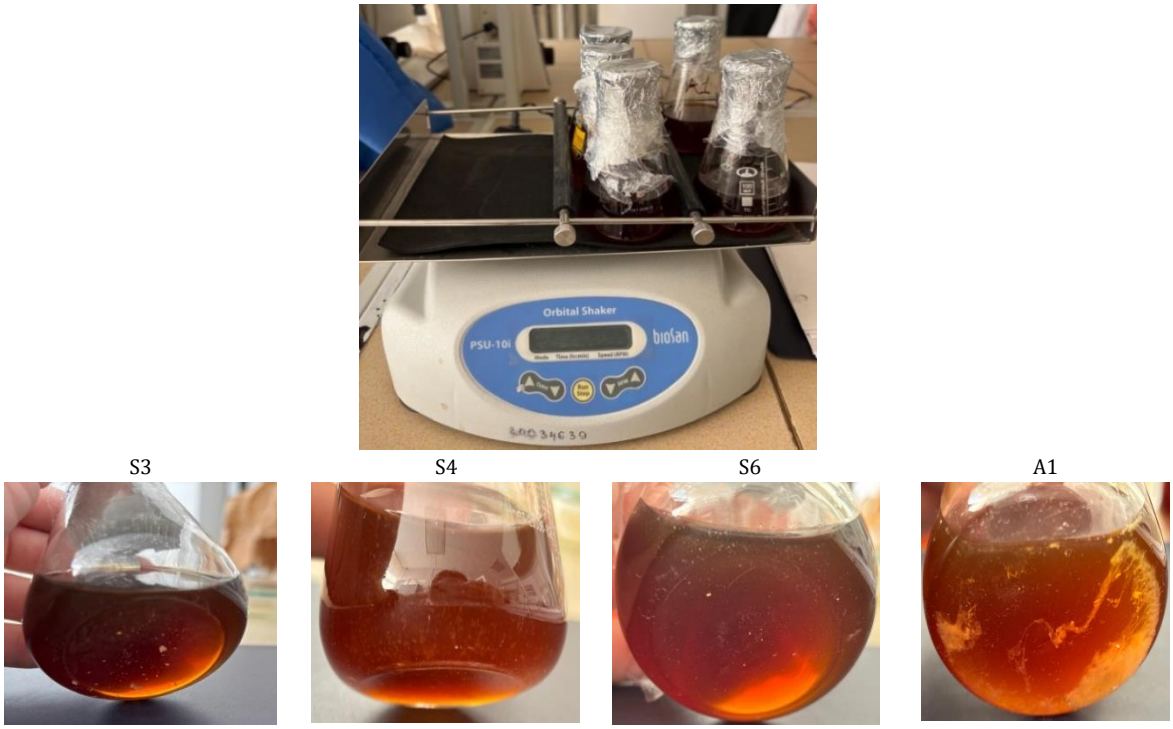


Figure 1. Analysis of the viability of microorganisms preserved in containers (S3, S4, S6, A1)

As can be seen from **Figure 1**, on the second day the following was recorded:

- intensive growth of strains S3, S4, and A1, accompanied by sediment formation and a change in the color of the medium to a lighter shade;
- Absence of growth in strain S6, indicating loss of viability.

Therefore, the preservation of microorganism viability after storage is determined by both their physiological characteristics and external conditions:

1. Stability of cellular structures: Strain S6 may have undergone cell membrane disruption or DNA degradation during long-term storage, even at low temperatures, leading to a complete loss of vital activity.
2. Metabolic activity: Strains S3, S4, and A1 retained the ability to metabolize and use MPB components, which is confirmed by active reproduction. This indicates their resistance and potential for adaptation after a dormant period.
3. Role of MPB: Meat-peptone broth contains the necessary amino acids, peptides, and carbon sources that contribute to the rapid recovery of metabolic activity in viable strains. It acts as both an energy source and a growth regulator.
4. Possibility of damage during preservation: If storage conditions were unstable, for example, temperature fluctuations or insufficient density of the preservation medium, this could have led to the inactivation of sensitive strains such as S6.

After cultivation in MPB liquid nutrient medium, all strains were transferred to solid MPA nutrient medium to confirm viability. The inoculation results showed that strains S3, S4, and A1 actively developed on agar, forming characteristic colonies. At

the same time, strain S6 again showed no signs of growth, which confirms its loss of viability even after transfer to a more favorable nutrient medium. This indicates severe damage to strain S6 cells during storage, preventing it from restoring metabolic activity in either liquid or solid medium **(Figure 2)**.

Conclusion on the viability of oil-oxidizing microorganisms: among the four studied oil-oxidizing strains, S3, S4, and A1 retained viability after long-term refrigerated storage. Strain S6 was non-viable, which may reflect individual sensitivity to storage conditions or a loss of adaptive potential. The MPB medium proved effective as a restorative nutrient base for reviving bacteria, promoting metabolic activation in viable cultures. These data should be considered when selecting strains for bioremediation technologies and when developing storage strategies for collection cultures.

In addition, strain S6 requires additional conditions to activate or restore viability.

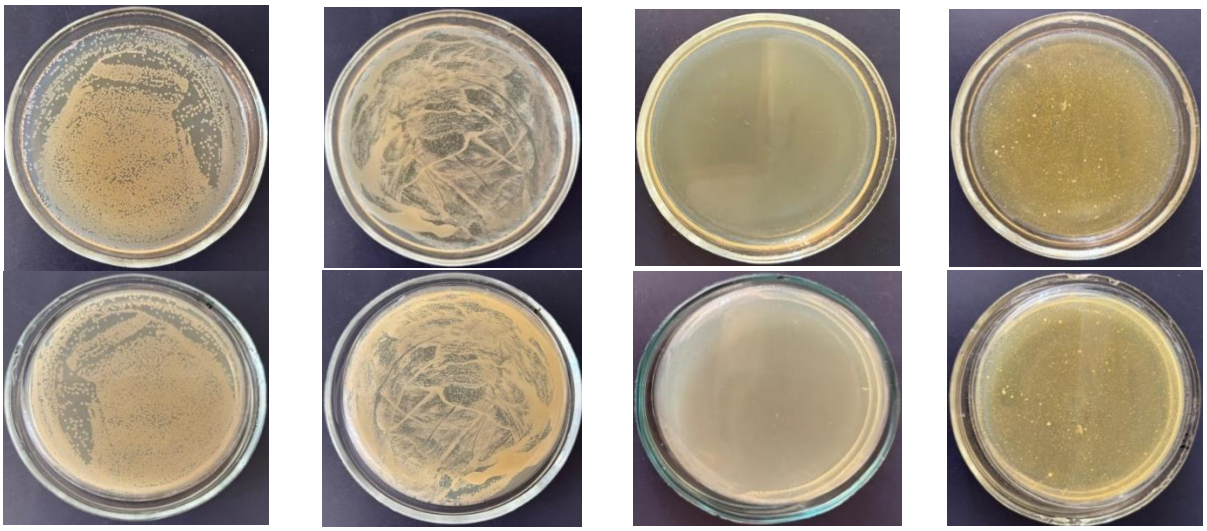


Figure 2. Growth of cultures on solid nutrient medium

Influence of oil-oxidizing bacteria on the properties of humate at different concentrations.

Soil contamination with petroleum products is one of the most acute environmental problems in oil-producing regions. Using oil-oxidizing microorganisms that degrade hydrocarbons and restore microbiological balance in soil is considered an effective bioremediation method. One factor contributing to improved microbial activity is humate, an organic compound that improves soil structure and stimulates microflora growth. The study focused on bacterial strains S3, S4, S6, and A1. Sodium humate was used in four concentrations. Bacterial growth was assessed visually and also by spectrophotometry based on optical density. Microorganisms were morphologically identified by microscopy after Gram staining **(Figure 3)**.

As can be seen from **Table 1**, the growth of cultures on humate at different concentrations showed dependence on the strain:

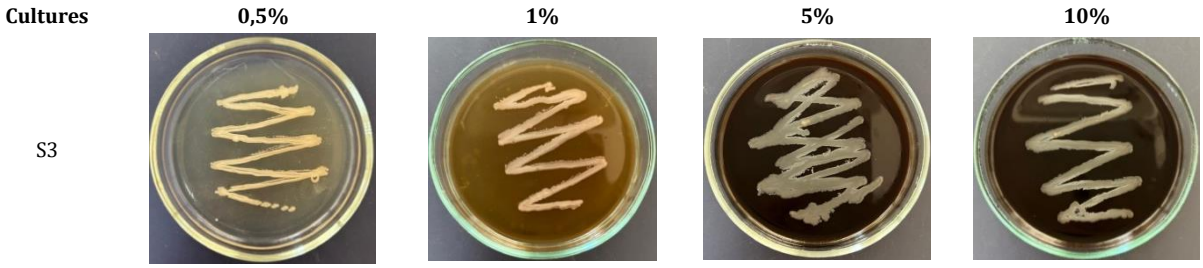
Strain S3 showed the most active growth at 10% humate (OD = 246).

Strain A1 showed growth at 1% and 10% (OD = 130 and 114, respectively).

Strain S4 demonstrated negative OD values, which may indicate an inhibitory effect of humate.

Strain S6 did not show growth at 1%, but showed OD = 127 and 107 at 0.5% and 10%, respectively.

A slight decrease in the pH of humate solutions was observed as concentration increased up to 10% (7.75 → 7.58), followed by an increase at 20% (pH = 7.85). This may indicate the buffering properties of humate at high concentration.



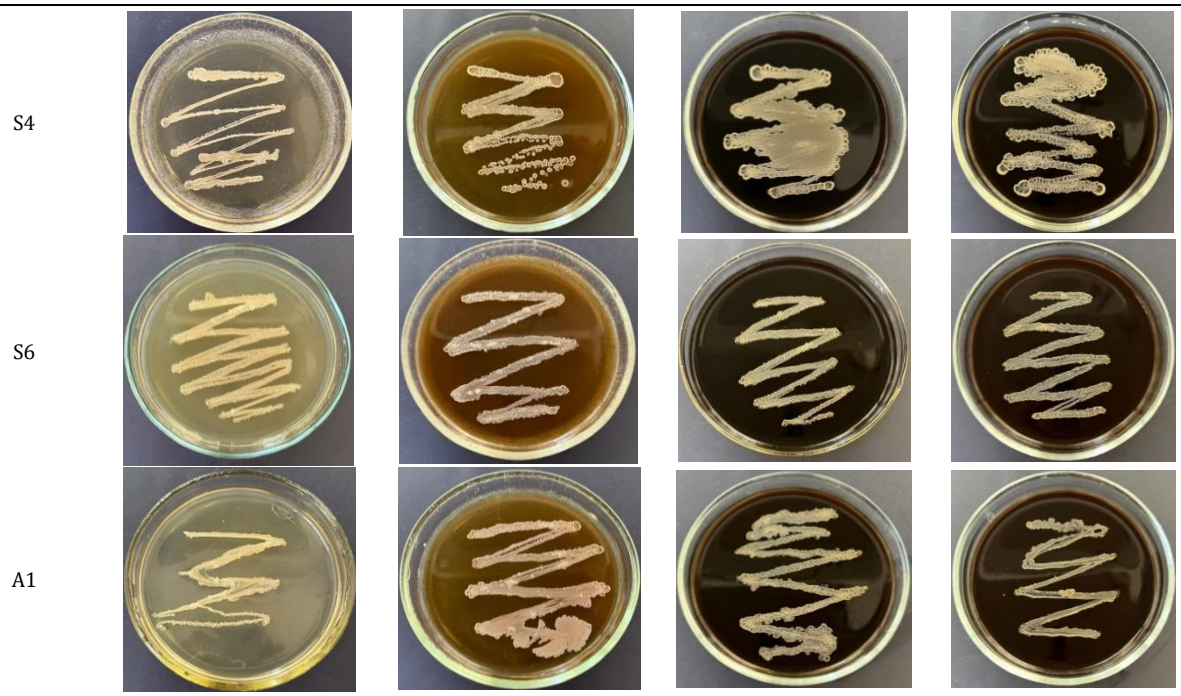


Figure 3. Growth of oil-oxidizing microorganism strains on nutrient media with different humate concentrations (0.5%, 1%, 5%, 10%)

Table 1. Results of microorganism OD measured by spectrophotometer

Cultures	0,5%	1%	5%	10%
S3	174	75	73	246
S4	-0,105	-0,161	-0,90	-0,154
S6	127	-0,117	0,144	107
A1	-0,081	130	-0,142	114

This behavior can be explained by the following factors: Presence of weakly acidic functional groups: Humates contain various functional groups, such as carboxyl (-COOH) and phenolic (-OH) groups, which can dissociate in diluted solutions, releasing hydrogen ions (H^+) and slightly acidifying the medium. Therefore, as concentration increases up to 10%, available H^+ increases, causing a decrease in pH. Buffering properties of humates: With a further increase in concentration up to 20%, the buffering properties of humates come into effect. At high concentrations, humate solutions can absorb or neutralize both hydrogen ions (H^+) and hydroxide ions (OH^-) because their polyfunctional groups retain ions in coordination bonds. This stabilizes or even increases pH.

Simply put, at high concentrations, humate acts as a buffer system, counteracting changes in the medium's acidity. Ionic interactions and structural organization: At high concentrations, humate molecules can form aggregates or associates, in which the availability of functional groups for dissociation may decrease. This reduces the amount of free H^+ ions in solution and, consequently, increases pH. Influence of alkaline cations: Humates often contain alkali metals, such as Na^+ or K^+, especially in sodium or potassium humates. As humate concentration increases, so does the content of these cations, which can shift pH toward the alkaline side through ion exchange.

Thus, the decrease in pH up to 10% concentration is associated with an increase in the number of dissociating weakly acidic groups. In contrast, the increase in pH at 20% is caused by activated buffering properties, reduced availability of acidic groups, and increased influence of alkaline components in the solution.

The mass fraction of petroleum products in soil with the introduction of humic preparations was determined by the gravimetric method according to GOST RD 52.18.647-2003. The results obtained are presented in **Table 2**.

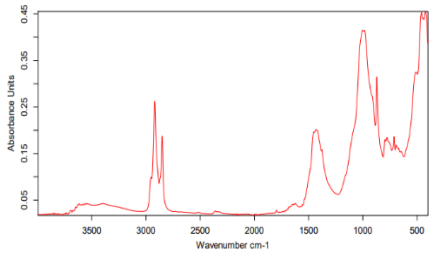
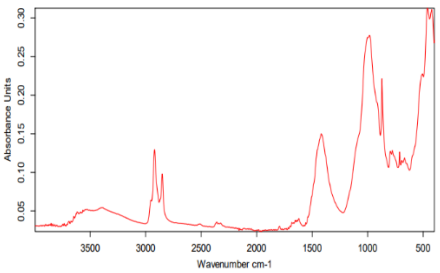
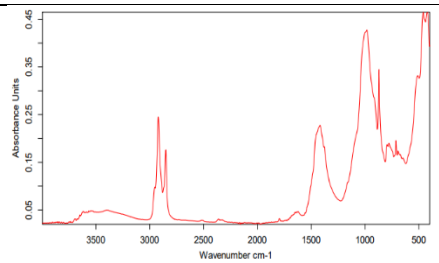
Table 2. Efficiency of oil-contaminated soil purification under the action of humate-containing biopreparations based on different microorganism strains

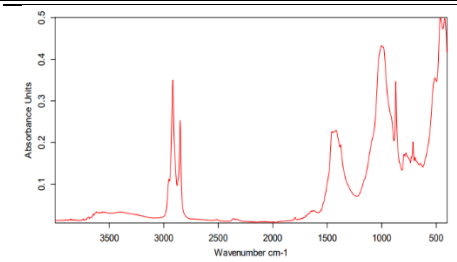
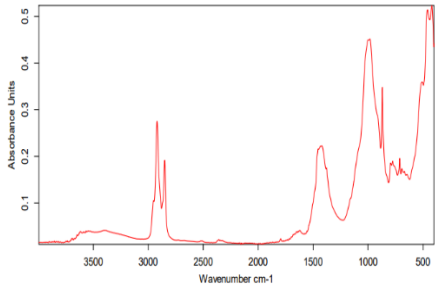
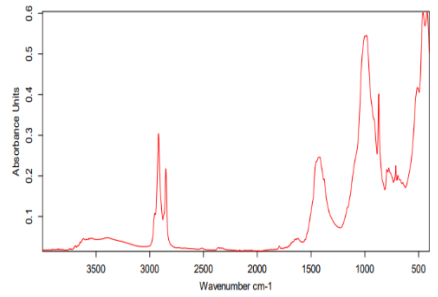
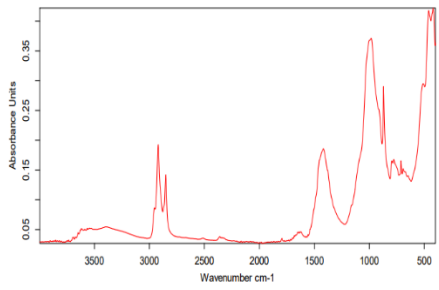
Sample name	Mass fraction of petroleum products, mg/kg			Degree of purification, %
Initial oil-contaminated soil	86528			0
Days	30th day	60th day	90th day	%
A1 + Potassium humate (1:1)	49000	38400	24400	71.80
S3 + Potassium humate (1:1)	48300	36900	21200	75.49
S4 + Potassium humate (1:1)	46000	35100	19800	77.11

S6 + Potassium humate (1:1)	50400	38900	25100	70.99
Bakoil (30 ml) + Potassium humate	48000	36000	20700	76.07
Bakoil (30 ml)	53000	40400	25900	70.06

Analysis of the data in **Table 2** shows the effectiveness of biopreparations that combine potassium humate with oil-oxidizing microorganism strains in treating soil contaminated with petroleum products. On days 30 and 60, all variants with humate and bacteria showed a significant decrease in petroleum product content compared with the initial concentration (86528 mg/kg). The variant with Pseudomonas zhaodongensis (S4) + potassium humate recorded the highest degree of purification (59.44%), indicating the high biodegradation potential of this strain in a humate-containing medium. The variant with the industrial biopreparation "Bakoil" + potassium humate also showed comparable efficiency (58.39%). Strains *Kocuria rosea* (A1) and *Cytobacillus firmus* (S3) also demonstrated a high level of purification, more than 55%. Even strain *Bacillus atrophaeus* (S6), previously recognized as non-viable in recovery tests, showed a purification degree of 55.04% in the presence of potassium humate, suggesting possible residual metabolic activity or humate involvement in the physicochemical sorption of oil. These results confirm the high efficiency of humate-containing bacterial preparations for bioreclaiming oil-contaminated soils. This diagram confirms that adding potassium humate significantly enhances the biodestructive activity of both individual strains and ready-made preparations and should be considered an important element of the bioremediation strategy. The S4 + potassium humate variant showed the highest degree of purification (46.83%), while Bakoil without humate showed the lowest (38.74%). All variants with potassium humate addition showed improved decomposition of petroleum products compared with the control. **Table 3** presents the IR spectroscopy results for the samples.

Table 3. Results of IR spectroscopy of the samples

IR spectrum of the sample	Main bands and interpretations
Initial soil from the "Zhetybay" deposit	
	~3420–3440 cm^{-1} – broad band: corresponds to O–H stretching vibrations. Typical for hydroxyl groups of alcohols, phenols, or carboxylic acids. ~2920–2850 cm^{-1} – weak to medium intensity bands: correspond to C–H (sp^3) stretching vibrations of alkanes. ~1720–1710 cm^{-1} – strong absorption: characteristic of the C=O (carbonyl group). May belong to ketones, aldehydes, or carboxylic acids. ~1620–1600 cm^{-1} – vibrations of C=C bonds in aromatic rings or conjugated systems. ~1380–1450 cm^{-1} – deformation vibrations of $–CH_2$ and $–CH_3$ groups. ~1100–1050 cm^{-1} – strong band: corresponds to C–O stretching vibrations (alcohols, ethers, phenols)
A1 + Potassium humate (1:1)	
	~3392 cm^{-1} – Broad O–H stretching vibration (alcohols or phenols) or N–H stretching (amines). ~2920 - 2851 cm^{-1} – C–H stretching vibrations of aliphatic groups ($–CH_2$, $–CH_3$). ~2360 cm^{-1} – Possible CO_2 interference or stretching of –C≡N / –C≡C groups. ~1794 cm^{-1} – Strong absorption of C=O stretching (carbonyl group, e.g., ester, anhydride, or acid chloride). ~1621 cm^{-1} – C=C stretching (aromatic ring or alkene) or amide C=O. ~1417 cm^{-1} – C–H bending ($–CH_2$/$–CH_3$ scissoring). ~983, 870, 777, 712 cm^{-1} – Out-of-plane bending of aromatic C–H or alkene =C–H (substitution pattern of aromatic rings). ~668 cm^{-1} – Possible aromatic C–H bending (monosubstituted or para-substituted benzene). ~459 - 423 cm^{-1} – Fingerprint region, may correspond to skeletal vibrations of aromatic or inorganic components.
S3 + Potassium humate (1:1)	
	~3401 cm^{-1} – Broad O–H (alcohol/phenol) or N–H (amine/amide) stretch. ~2919 - 2851 cm^{-1} – Aliphatic C–H stretches ($–CH_2$/$–CH_3$). ~2360 cm^{-1} – Likely CO_2 (atmospheric) artifact. ~1623 cm^{-1} – C=C stretch of an aromatic ring (or amide C=O if N–H is present). ~1418 cm^{-1} – CH_2/CH_3 bending (scissoring). ~983, 871, 777, 712, 693 cm^{-1} – Aromatic C–H out-of-plane bends; pattern suggests a substituted benzene ring (often consistent with meta-substitution). ~459 - 423 cm^{-1} – Fingerprint/ skeletal vibrations.
S4 + Potassium humate (1:1)	

	~3566 cm^{-1} – Sharp "free" O–H stretch (non-H-bonded alcohol/phenol). ~2918 - 2849 cm^{-1} – Aliphatic C–H stretches (–CH_2/–CH_3). ~1419 cm^{-1} – CH_2/CH_3 bending (scissoring). ~1003 cm^{-1} – C–O stretch of an alcohol/phenol or ether. ~871, 777, 712, 693 cm^{-1} – Aromatic C–H out-of-plane bends; pattern consistent with a meta-substituted benzene ring. ~512, 461, 420 cm^{-1} – Skeletal/fingerprint vibrations.
S6 + Potassium humate (1:1)	
	~3401 cm^{-1} — Broad O–H stretch (alcohol/phenol) or N–H stretch (primary/secondary amine or amide). ~2919 - 2851 cm^{-1} — Aliphatic C–H stretching (–CH_2, –CH_3). ~1622 cm^{-1} — C=C stretch (aromatic) or possibly amide I (C=O of amide) if N–H present; not a typical ester/ketone C=O (which appears ~1700 cm^{-1}). ~1419 cm^{-1} — C–H bending / CH_2/CH_3 scissoring or aromatic ring breathing contribution. ~983, 871, 777, 712, 693 cm^{-1} — Aromatic C–H out-of-plane bending modes — pattern consistent with a substituted benzene (these bands can help identify substitution pattern; presence of multiple bands around 700–900 cm^{-1} often indicates disubstitution, e.g., meta- or para- types). ~458 - 420 cm^{-1} — Fingerprint/skeletal vibrations (low-energy ring or heavy-atom bends).
Bakoil (30 mL) + Potassium humate	
	~3399 cm^{-1} — Broad O–H stretch (alcohol/phenol) or N–H stretch (amine/amide). ~2918 - 2850 cm^{-1} — Aliphatic C–H stretching (–CH_2, –CH_3). ~1623 cm^{-1} — C=C stretch (aromatic ring) or possibly amide I (if N–H present); not a typical saturated carbonyl (~1700 cm^{-1}). ~1419 cm^{-1} — C–H bending / CH_2/CH_3 scissoring; can also include aromatic ring breathing contributions. ~984 cm^{-1} — C–H out-of-plane bending / =C–H wag (often associated with substituted alkenes or aromatic substitution patterns). ~871, 777, 712, 693 cm^{-1} — Aromatic C–H out-of-plane bending modes — pattern suggests a disubstituted benzene (commonly meta- or para- substitution; exact pattern leans toward meta-type when multiple bands appear in this region). ~510, 458, 420 cm^{-1} — Fingerprint / skeletal vibrations (heavy-atom bends, ring deformations).
Bakoil (30 ml)	
	~3402 cm^{-1} — Broad O–H stretch (alcohol/phenol) or N–H stretch (primary/secondary amine or amide). ~2920 - 2851 cm^{-1} — Aliphatic C–H stretching (–CH_2, –CH_3), indicating alkyl chains. ~2359 cm^{-1} — Likely atmospheric CO_2 artifact (common) — not a structural feature unless the sample contains CO_2-derived species. ~1636 cm^{-1} — C=C stretching (conjugated/ aromatic) or amide I (C=O) if an amide is present; less typical for unconjugated ester/ketone (which usually appear closer to ~1700 cm^{-1}). ~1418 cm^{-1} — CH_2/CH_3 bending (scissoring) and/or aromatic ring breathing contribution. ~983, 871, 777, 712, 693 cm^{-1} — Aromatic C–H out-of-plane bending modes — pattern consistent with a disubstituted (likely meta- or mixed-substitution) benzene ring. ~460 - 421 cm^{-1} — Low-frequency fingerprint/skeletal vibrations (ring deformations or heavy-atom bends).

All spectra contain a broad intense signal in the region of 3400–3560 cm^{-1}, indicating the presence of O–H groups, alcohols or phenols, or N–H groups, amines or amides. In the region of 2920–2850 cm^{-1}, bands of stretching vibrations of aliphatic C–H bonds (–CH_2, –CH_3) are recorded, indicating the presence of alkyl fragments. Characteristic bands at ~1620–1635 cm^{-1} correspond to vibrations of C=C bonds of the aromatic ring or amide groups (C=O + N–H). Bands at 1415–1420 cm^{-1} are associated with deformation vibrations of –CH_2/–CH_3 and breathing of the aromatic ring.

In the region of 900–690 cm^{-1}, intense peaks are observed that are characteristic of out-of-plane deformations of aromatic ring C–H bonds, indicating the presence of substituted benzene rings. The combination of several bands in this region mainly corresponds to meta- or para-substituted aromatic structures. Low-frequency bands (~510–420 cm^{-1}) belong to the "fingerprint" region and correspond to skeletal vibrations of the aromatic nucleus or heavy atoms.

Thus, based on the totality of the data, it can be concluded that the studied samples are aromatic compounds with substituted

benzene rings, containing aliphatic chains and, probably, functional groups of the –OH type, phenolic or alcoholic, and/or –NH type, amine or amide. Clearly expressed bands characteristic of ketones, aldehydes, or ester C=O groups (1700–1750 cm^{-1}) were not detected in the spectra.

CONCLUSION

The study established that the strain *Bacillus atrophaeus* (S6), stored in meat-peptone broth (MPB) at +4 °C for one year, lost viability. The absence of growth after subculturing indicates degradation of the cell population. This is because MPB is not intended for long-term strain storage, as it lacks cryoprotectants and buffer components needed to stabilize cells. In addition, the accumulation of toxic metabolites and changes in medium pH during long-term storage contribute to bacterial death. This case emphasizes the need for specialized preservation methods, such as storage in glycerol at –20 °C or lyophilization, to preserve microbial viability.

It was also established that oil-oxidizing bacteria interact with humates, changing their properties depending on concentration. This indicates mutual influence between microorganisms and humic substances, which should be considered when developing biopreparations and bioremediation technologies. At low humate concentrations, the medium pH decreased, likely due to the activation of weakly acidic functional groups in humic substances. At 20% humate, pH increased, which may indicate humate buffering and changes in its colloidal structure influenced by bacterial metabolites.

The results in **Table 2** clearly show that the maximum degree of purification of oil-contaminated soil is achieved by combining potassium humate with active oil-oxidizing bacteria, especially strain S4. This confirms the feasibility of developing combined biopreparations for reclaiming oil-contaminated areas, with an emphasis on selecting an effective strain and humic base. Thus, the interaction of oil-oxidizing bacteria with humates is accompanied by changes in the acid-base balance, indicating their ability to modify the physicochemical characteristics of humic substances. These data may be useful for developing biopreparations for reclaiming contaminated soils and biostimulation systems based on humic compounds.

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