

Comparative Assessment of The Bioremediation Potential of Indigenous Microorganisms Isolated from The Industrial Zone of The Shargun Coal Mine

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Abstract: This study investigates the quantitative and qualitative characteristics of indigenous microorganisms present in soil and water sources within the industrial zone of the Shargun coal mine in Surkhandarya region. Physiological and enzymatic activities, metabolic adaptability, and carbon-assimilation capacities of the isolated strains were evaluated in nutrient media containing coal as the sole carbon source. *Bacillus simplex*, *Bacillus subtilis*, and *Pseudomonas fluorescens* demonstrated high growth rates, substantial protein synthesis, and notable hydrolytic activity towards complex substrates (starch, cellulose, tannin). These findings indicate that the isolated strains can be effectively employed for bioindication and bioremediation of soils contaminated with coal and coal-derived waste in industrial areas.

Keywords: Shargun coal mine; microorganisms; bacteria; *Bacillus simplex*; bioindication; enzymatic activity; coal substrate; environmental monitoring; indigenous strains.

Introduction:

The rapid expansion of various industrial sectors worldwide, particularly coal mining operations, has exerted significant adverse impacts on the environment. In particular, the industrial zones surrounding the Shargun coal mine are increasingly contaminated due to the accumulation of dust particles, toxic elements, and heavy metals in local soils and water bodies, leading to a drastic decline in biological diversity. This situation necessitates continuous ecological monitoring and the implementation of bioremediation strategies in such areas.

Globally, the environmental challenges associated with coal extraction have become a pressing issue. Notably, both active and abandoned coal mining sites

frequently experience severe degradation in soil, water, and air quality. Such contamination not only alters physical and chemical parameters but also significantly affects microbial diversity and the functional activity of indigenous microbiota. Consequently, the development and application of microbial monitoring and bioremediation measures remain among the key priorities of the global scientific community [1].

For instance, a study conducted in a mining area in Brazil (CNPq, 2015) demonstrated that soil microbial biomass and enzymatic activities significantly declined following coal extraction activities, but showed a tendency for natural recovery over time. Microbial groups such as Proteobacteria, Acidobacteria, and Firmicutes were identified as

dominant components, and their shifts reflected a disturbance in the microbial community equilibrium and an increase in biotic stress within the mining zone [2].

Similarly, Zhou et al. (2024) reported the highly efficient bioremediation of heavy metals such as Cd and Pb using *Bacillus pasteurii* in combination with hydrothermal carbon. Their 30-day experiments demonstrated reductions in Cd and Pb concentrations by 89.4% and 87.8%, respectively. The bacteria facilitated the formation of carbonate precipitates that immobilized the heavy metal ions, a process explained by the microbially induced calcium carbonate precipitation (MICP) mechanism [3].

Microbial bioconversion of coal waste is considered an environmentally safe and efficient method. In the study by Unitsky et al. (2022), *Bacillus* and *Enterobacter* strains exhibited high growth rates and solubilization activity in a 5% coal-containing medium. The application of *Trametes versicolor* significantly increased the yield of humic acids. The bioconversion process is based on a two-stage mechanism involving enzymatic reactions, representing a promising solution for the production of humic substances and for soil amelioration purposes [4].

Fotina et al. (2021) investigated the bioremediation and phytoremediation potential to mitigate environmental problems associated with coal waste, particularly polycyclic aromatic hydrocarbons (PAHs) and heavy metals, in the Kemerovo region. The *Pseudomonas stutzeri* strain demonstrated effective biosorption of Cu(II) ions, while the combined use of plants and mycorrhizal fungi accelerated coal degradation. This study proposed a technical-biological approach for reclamation and provides a basis for the development of biologically safe remediation strategies [5].

According to Cherdantseva and Gavrilova (2016), spore-forming bacteria, particularly *Bacillus cereus* and *Bacillus subtilis* strains, when applied in combination with organo-mineral substrates, enhanced plant survival rates by up to 70–90%. The high enzymatic activity of substrates enriched with wastewater sludge (WWS) contributed to the increased biological effectiveness of reclamation processes [6].

One of the major environmental issues in coal mining areas is dust pollution. In a study conducted in China, the *Bacillus X4* strain demonstrated effective dust suppression by cementing dust particles through the urease-mediated microbially induced calcium carbonate precipitation (MICP) technique. Physico-

chemical analyses (SEM, EDS, FTIR, XRD) confirmed the strong binding of dust particles. Furthermore, as soil dust concentrations increased, bacterial activity and the cementation effect were also enhanced [7].

The activity of indigenous strains isolated from the Shargun coal mine, such as *Bacillus simplex*, in lowering pH and modifying the substrate is consistent with the MICP (microbially induced calcium carbonate precipitation) mechanism. This finding supports the evaluation of microbiological dust suppression technologies as an environmentally safe and economically feasible approach. The present study focuses on elucidating the distribution, dominant groups, and biotechnological potential of indigenous microorganisms isolated from soil and water resources in the Shargun mine area. The primary aim is to identify these strains and assess their potential for mitigating environmental hazards in contaminated industrial zones. Strains belonging to the genera *Bacillus*, *Glutamicibacter*, and *Pseudomonas* exhibited high performance in terms of growth, enzymatic activity, and detoxification capacity. This investigation represents an independent experimental contribution aligned with existing literature, tailored to local conditions and providing a context-specific scientific approach. The following section details the microbiological, physicochemical, and enzymatic analysis methods employed in this research.

METHODS

1. Study Area and Sampling Procedure

This research was conducted within the industrial zone of the Shargun coal mine, located in the Surkhandarya region of Uzbekistan. The site is characterized by intensive coal mining activities, which significantly impact the surrounding environment. A total of five different types of samples were collected from locations with the highest degree of anthropogenic influence, including water, soil, coal of varying grades, and coal waste.

The sampling points were selected as follows:

- entrance area of the coal extraction shaft;
- coal transportation route;
- designated waste accumulation site;
- contaminated pond area.

Soil samples were collected under sterile conditions using the standard stratified method commonly applied in microbiological studies. For each sampling site, soil was collected at three depths: 0–5 cm (surface layer), 5–15 cm (intermediate layer), and 15–30 cm (deep layer). Approximately 500 grams of soil per layer were placed in sterile polyethylene bags,

properly labelled with relevant metadata, and transported to the laboratory under refrigerated conditions.

Coal and Water Sampling

Coal samples were collected separately from various types of coal differing in quality, commonly found within different zones of the site:

- high-grade coal fragments;
- coal mixed with rock debris;
- coal waste material.

An adequate quantity of each coal type was collected, clearly labelled, and handled to prevent cross-contamination. Water samples were taken from stagnant ponds and from accumulation sites adjacent to waste disposal areas. Each water sample was collected in sterile 500 mL containers, ensuring minimal contact with the external environment. During sampling, measurement units, sample type, precise location coordinates, and time were meticulously recorded. Throughout transportation, a cold chain was maintained to preserve the integrity of the native microbial communities. All sampling procedures adhered to international standards for microbiological sampling and handling [8].

3. Microbiological Isolation and Identification

Upon delivery to the laboratory, the collected soil and water samples were processed for microbiological examination. To isolate microorganisms, appropriate dilutions were prepared from the soil samples. Specifically, 10 grams of soil were mixed thoroughly in sterile physiological saline solution, and serial dilutions (10^5 , 10^6) were prepared. Water samples were either directly plated or diluted when necessary prior to further processing.

Each dilution was inoculated onto Petri dishes containing selective nutrient media. The following culture media were primarily used:

- Nutrient Agar (HiMedia): for cultivating general heterotrophic bacteria;
- Czapek Agar: for isolating actinomycetes and filamentous bacteria and for restricting the growth of certain unwanted microorganisms.

All Petri dishes were prepared under sterile conditions and incubated in a thermostat at 28–30 °C immediately after inoculation. Colonies were monitored daily, and their growth and morphology were analysed over a period of 48–72 hours. Colonies were selected based on distinct morphological characteristics, such as shape, colour, margin appearance, and surface structure, and subsequently purified to obtain pure cultures. The taxonomic

identification of the selected pure strains was performed using the modern MALDI-TOF mass spectrometry technique. For this purpose, biomass from pure cultures was applied onto a dedicated MALDI plate, treated with an appropriate matrix, and identified based on the acquired spectral data.

4. Assessment of Microbiological Activity

The bioconversion potential and enzymatic activity of the isolated indigenous bacterial strains in transforming coal waste were investigated under controlled laboratory conditions. For this purpose, a mineral nutrient medium supplemented with pulverized coal as the sole carbon source was used for each strain, while a mineral medium with sucrose served as a control.

The experimental setup was as follows:

- the strains were inoculated into sterile 250 mL Erlenmeyer flasks containing 100 mL of the prepared mineral nutrient medium;
- the flasks were incubated at 28–30 °C with continuous shaking at 150 rpm for 14 days.

Microbial growth was assessed based on biomass formation, colony counts, and cell density measurements. Biomass growth was determined photometrically, while colony numbers were quantified using standard plate count techniques. Certain strains also demonstrated the ability to degrade additional polysaccharides (starch and cellulose) and tannins in Raymond medium, indicating their enhanced substrate decomposition capability.

5. Assessment of Enzymatic Activity

The enzymatic activity of the isolated strains was evaluated using the following indicator reactions:

- Starch hydrolysis zone: colonies grown on starch-containing medium were treated with Lugol's iodine solution; the appearance of a clear halo around the colony indicated starch hydrolysis.
- Cellulose hydrolysis zone: the hydrolysed area on cellulose-enriched medium was detected using a specific indicator reagent.
- Tannin degradation: the ability of strains to degrade tannins in tannin-containing medium was visually assessed based on a colour reaction with ferric chloride.

The results of these tests provided an objective basis for concluding the strains' potential to grow on coal-based substrates, their enzymatic capabilities, and their efficiency in assimilating carbon sources.

Microbiological Diagnostics and Strain Selection

To facilitate biological monitoring of environmentally degraded industrial areas and to evaluate their biotechnological resources, the collection of isolated indigenous strains was analysed based on several key indicators. These criteria included the total number of strains, their origin from different pollution sources, their tolerance to coal waste as a carbon substrate, and their enzymatic activity.

Among the isolated microorganisms, strains exhibiting high growth rates, strong enzymatic activity, and efficient substrate utilization were grouped and prioritised. Through this selection process, bacteria such as *Bacillus simplex* were identified as having broad biotechnological potential and were therefore considered suitable for subsequent bioconversion experiments.

These findings highlight the dual application of the selected isolates: firstly, as bioindicator strains within ecological monitoring systems, and secondly, as promising agents for industrial biotechnology, particularly for the biological treatment and conversion of coal waste.

Data Analysis

The results obtained within the scope of this study were comprehensively analysed, and detailed characterisations were developed for each isolate. Average values for biomass growth, cell density, and colony counts were calculated and summarised in relevant tables. Additionally, indicators of enzymatic activity—specifically the dimensions of starch and cellulose hydrolysis zones and the tannin degradation zone—were measured visually and presented in diagrammatic form.

Based on the experimental data, the bioconversion potential of each strain in transforming coal waste was evaluated, and a list of promising strains for further selection was compiled. Notably, *Bacillus simplex*, *Pseudomonas chlororaphis*, and *Glutamicibacter arilaitensis* demonstrated superior growth performance, pronounced enzymatic activity, and high substrate tolerance.

The methods and evaluation criteria employed in this study were based on the scientific approaches and findings reported by renowned researchers, including Yokimiko David, Unitsky A.E., Zhao Y.Y., Fotina N.V., and others, as cited above. Additionally, all methodological procedures were adapted and applied in practice with careful consideration of the specific characteristics of the local ecosystem.

Isolation of Microorganisms

Within the scope of the microbiological investigations, a total of five soil samples collected from different sources were examined. Samples were collected in stratified layers from depths of 0.0–5.0 cm and 0–30 cm. The number of microorganisms isolated from each sample was evaluated based on their growth on nutrient media. For this purpose, suitable selective media were chosen for each bacterial group, and the samples were inoculated accordingly. The inoculated plates were incubated at 28–30 °C, and growth indicators were recorded after 48–72 hours.

The total number of isolated microorganisms and their distribution by source are presented in Table 1 below.

Table 1. Microbiological analysis of various sources within the Shargun coal mine area

Description (Location)	Soil Horizons, cm	Number of Isolates Obtained ($H \times 10^5$ KOE/r)
Foot of the hill at the entrance of the mine shaft	0-5,0	575×10^5
Foot of the hill at the entrance of the mine shaft	30	387×10^5
Coal Transportation Route (50 m)	Surface Layer	553×10^5
Roadside (50 m)	0-5,0	317×10^5
Waste Dump Site (100 m)	0-30	504×10^5

Analysis of Microbial Activity Indicators.

According to the results of the conducted analyses, the highest microbial activity indicators were observed in soil samples collected at a depth of 0–5.0 cm from the entrance area of the mine, where the total cell count reached 575×10^5 CFU/g. Similarly,

samples collected from the coal transportation route also exhibited high activity, with a total cell count of 553×10^5 CFU/g. The relatively elevated biotic activity in these locations can be attributed to several factors. Specifically, the mine entrance and the coal transportation area are characterised by a balanced moisture level and an abundance of organic

substrates and diverse carbon sources. These conditions create a favourable environment for microbial growth and proliferation, significantly influencing the total population density [9]. In contrast, samples obtained from deeper soil layers and the waste dump site demonstrated comparatively lower cell counts, indicating limited nutrient availability and reduced moisture levels in these zones.

Isolation and Identification of Indigenous Microorganisms.

In the subsequent stage of the study, cultures isolated from various ecosystems of the Shargun coal mine, including soil and aquatic environments, were identified based on their morphological characteristics and using MALDI-TOF mass spectrometry.

The results revealed that the isolated strains belong to families such as *Bacillus*, *Pseudomonas*, *Glutamicibacter*, *Kocuria*, *Acinetobacter*, *Mycobacterium*, and *Pseudarthrobacter*. Among these, *Bacillus simplex*, *Pseudomonas chlororaphis*, and *Glutamicibacter arilaitensis* were distinguished by their high adaptability to the local biotope, pronounced enzymatic activities, and robust growth rates. These strains demonstrated active proliferation in coal-containing and mineral media and showed notable activities of enzymes such as protease, cellulase, and tannase.

Analysis of the water samples from the study area further indicated variations in the distribution of microorganisms depending on geographic location and contamination levels. *Pseudomonas veronii* was isolated from a water source near the railway, *Glutamicibacter arilaitensis* from mountain spring waters at the mine entrance and the conveyor water system, and *Pseudomonas chlororaphis* from the vicinity of the coal transportation route. Notably, *Bacillus cereus* was identified in the highly polluted wastewater accumulation site, highlighting the resilience of this species to heterogeneous

environments.

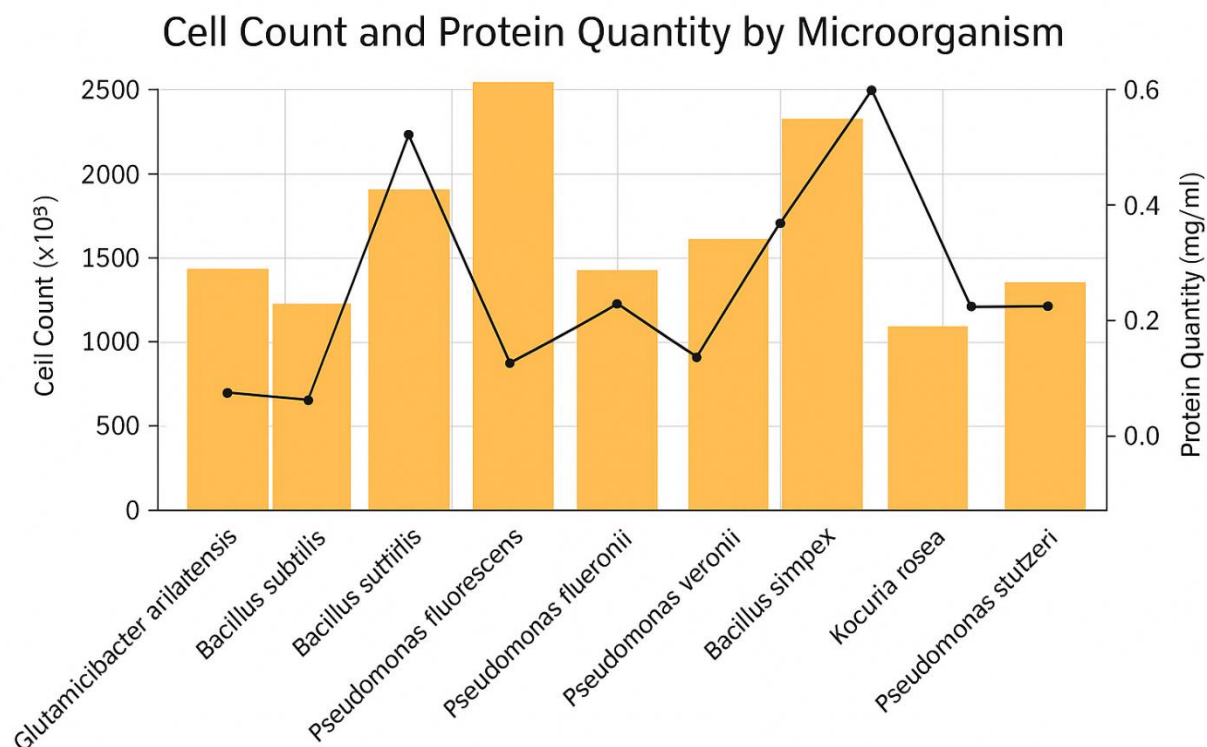
Soil samples exhibited greater microbial diversity. Predominant species included *Pseudomonas fluorescens*, *Pseudomonas stutzeri*, *Pseudomonas koreensis*, *Acinetobacter vivianii*, *Mycobacterium avium*, *Kocuria rosea*, *Bacillus simplex*, and *Pseudarthrobacter oxydans*. These species were primarily distributed in soils along the coal transportation routes and waste accumulation areas [10].

One of the most significant observations in this study was the isolation of *Glutamicibacter arilaitensis* from two distinct and independent water sources. This finding indicates the high ecological adaptability of this strain and its ability to maintain metabolic activity in various polluted environments.

Originally, *G. arilaitensis* was first isolated from cheese products in France, where it demonstrated the capacity to survive under extreme conditions, particularly in acidic or saline environments [11]. Our investigations revealed that this strain also thrives in contaminated water systems, suggesting its potential application as a promising agent for industrial wastewater bioremediation.

In the subsequent stage of the experiments, the enzymatic activity, coal growth capability, and potential bioremediation properties of these strains were analysed.

The experiments on the physiological and biochemical activity of the strains grown in coal-containing nutrient media were conducted using the nine previously isolated and identified strains. They were cultivated in specially prepared mineral medium (with 1% coal powder as the carbon source) in 100 mL Erlenmeyer flasks, incubated at 37°C with continuous shaking at 160 rpm for 3–5 days. The initial pH of the nutrient medium was 7.04. The results obtained at the end of the experiment, including changes in measured parameters and cell counts, are presented in the figure below.



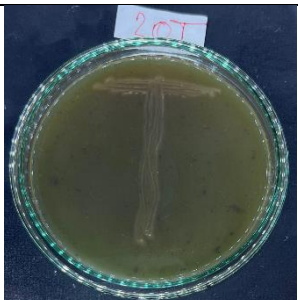
Analysis of Diagram Data.

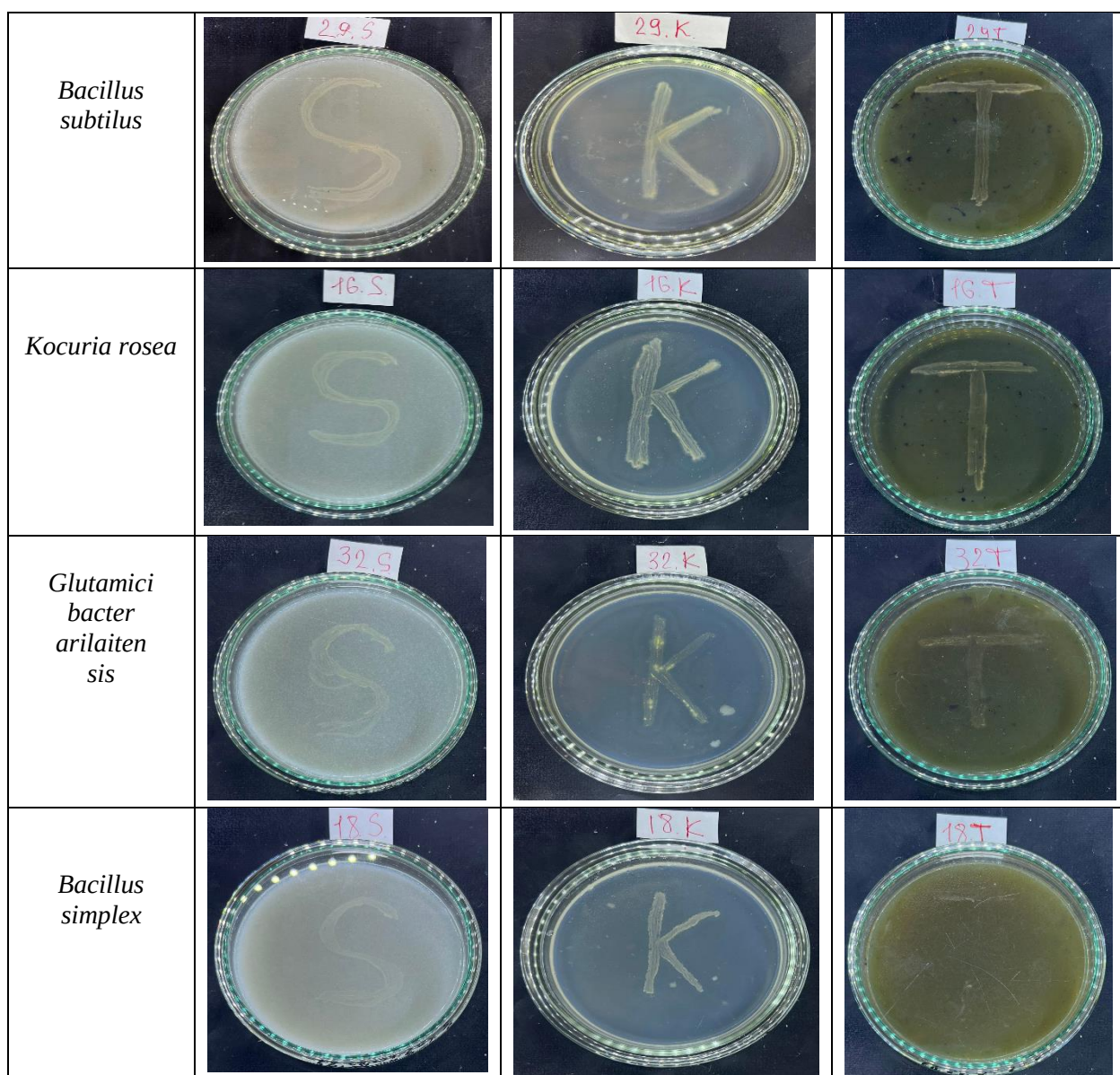
The summary diagram of the experimental results demonstrates that among the strains cultivated in coal-based nutrient medium, *Pseudomonas fluorescens* and *Bacillus simplex* exhibited the highest cell growth. Specifically, the cell density reached $2,800 \times 10^3$ CFU/mL for *Pseudomonas fluorescens* and $2,700 \times 10^3$ CFU/mL for *Bacillus simplex*. These values confirm the high efficiency of these strains in utilizing coal substrate and their rapid adaptation to this carbon source. Additionally, the *Kocuria rosea* strain accumulated the highest protein content, measuring 1.10 mg/mL, indicating a high level of metabolic activity. This parameter suggests that this strain may play a significant role in biosynthetic processes and nutrient

cycling.

For all strains, the pH values of the medium remained stable within a range of 6.85 to 7.12 under incubation conditions, maintaining near-neutral levels throughout the experiment. The absence of drastic pH fluctuations further indicates the adaptability of these strains to various biochemical reactions. Overall, the data confirm that the indigenous strains demonstrate effective growth and pronounced metabolic activity in coal-based media, highlighting their considerable potential for application in bioconversion processes.

The enzymatic potential of the strains was evaluated using Raymond's mineral nutrient medium supplemented with starch, cellulose, and tannin substrates.

Bacterial Species.	Cellulose MKC	Starch	Tannin
<i>Pseudomonas fluorescens</i>			



Enzymatic Activity Evaluation.

Visual assessment based on the hydrolysis zone diameters (mm) around microbial colonies on various substrates revealed the following results:

- In the starch-containing medium, the most active strains were: *Glutamicibacter arilaitensis* > *Pseudomonas fluorescens* > *Kocuria rosea* > *Bacillus simplex* > *Bacillus subtilis*.
- In the tannin medium, the highly active strains were: *Kocuria rosea* > *Bacillus subtilis* >

Glutamicibacter arilaitensis.

- In the cellulose medium, the strains with notable enzymatic activity were: *Bacillus simplex* > *Bacillus subtilis*.

These findings confirm that the active strains possess the capability to hydrolyse natural biopolymers such as starch, tannin, and cellulose, indicating their potential role in carbon cycling and the natural transformation of organic matter in the biosphere. The obtained results demonstrate the high bioremediation potential of the tested strains.

Hydrolysis Zone Diameter Table

Identified species	Hydrolysis zone diameter (mm)
<i>Pseudomonas fluorescens</i>	8
<i>Bacillus subtilis</i>	9
<i>Kocuria rosea</i>	7
<i>Glutamicibacter arilaitensis</i>	9

The table summarizes the starch-hydrolysis performance of five indigenous bacterial strains isolated from the Shargun coal-mine environment. For each species, the diameter of the clear (amylolytic) halo formed on starch-supplemented agar is reported in millimetres (mm). *Bacillus simplex* produced the widest hydrolysis zone (10 mm), indicating the highest extracellular amylase activity among the tested isolates. *Bacillus subtilis* and *Glutamicibacter arilaitensis* showed intermediate halo sizes (9 mm each), followed by *Pseudomonas fluorescens* (8 mm). *Kocuria rosea* exhibited the smallest clearance zone (7 mm), suggesting comparatively lower amylase production under the assay conditions. Overall, the data highlight notable interspecific variability in starch-degrading capacity, with *B. simplex* emerging as the most efficient amylolytic strain in this screening.

In summary, *Bacillus simplex*, *Bacillus subtilis*, and *Pseudomonas fluorescens* are promising candidates for use as bioremediation agents due to their high growth rates, efficient protein production, and broad enzymatic spectra. Notably, *Bacillus simplex* distinguished itself by maintaining stable pH levels, exhibiting high cell density, and demonstrating strong cellulolytic and amylolytic enzyme activities. This species could be regarded as an important microbiological resource for the future biotransformation of coal waste and the biological restoration of environmentally polluted sites.

DISCUSSION

According to the results obtained in the course of this study, the high level of industrial pollution in the “Shargun Coal Mine” area has resulted in considerable variation in the taxonomic composition, metabolic profiles, physiological traits, and biochemical activities of local microorganisms, with the isolated strains belonging to diverse genera and species.

Among the isolated and identified cultures, *Bacillus simplex*, *Bacillus subtilis*, and *Pseudomonas fluorescens* were distinguished by their remarkable ecological adaptability, biosynthetic capacity, and enzymatic activity. Notably, in the mineral medium prepared with coal as the main substrate, *Bacillus simplex* and *Pseudomonas fluorescens* exhibited high cell densities (2.7 and 2.8 million CFU/mL, respectively), minimal pH variation, and a steady increase in protein concentration. These parameters confirm the strong substrate adaptation and high metabolic activity of these strains. In particular,

Bacillus simplex reached a cell density of 2.7×10^6 CFU/mL, demonstrating pronounced biochemical activity. These findings are consistent with the results reported by Zhao Y.Y. et al. (2023), who highlighted the effectiveness of *Bacillus* species in dust suppression and calcium carbonate precipitation.

In terms of enzymatic activity, *Bacillus simplex* and *Bacillus subtilis* demonstrated high enzyme production in media containing starch, tannin, and cellulose, indicating their capacity to degrade natural biopolymers such as amylose, phenolic compounds, and cellulose. This observation aligns with the bacterial enzyme mechanisms described by Unitskiy A.E. (2022) regarding lignin, cellulose degradation, and carbon cycling.

Although *Kocuria rosea* showed relatively lower cell density, it recorded the highest protein content (1.10 mg/mL), indicating an active metabolic state with pronounced enzymatic function. Furthermore, this strain exhibited notable enzymatic activity in the tannin-containing medium, reflecting its capability to produce specialised biochemical enzymes.

Glutamicibacter arilaitensis and *Pseudomonas chlororaphis* exhibited moderate values for cell count and protein content, yet stood out as effective working strains due to their ability to utilise coal as a carbon source, maintain stable pH levels, and demonstrate enzymatic activity in starch-containing media.

Overall, the microorganisms isolated from the “Shargun Coal Mine” demonstrate high ecological adaptability and biological activity. Some strains — notably *Bacillus simplex*, *Bacillus subtilis*, and *Pseudomonas fluorescens* — possess the capacity to degrade complex natural substrates such as lignin, cellulose, starch, and tannin, making them promising candidates for application in bioremediation and microbiological reclamation processes.

The obtained results are in agreement with findings from international studies by David et al. (2020) and Zhou et al. (2024), which have likewise reported the effective biodegradation capabilities of *Bacillus* and *Pseudomonas* species for metals, coal, and various industrial pollutants.

Moreover, the findings obtained in this study are consistent with research conducted by international scholars, in particular Yokimiko et al. (2017). In their study, promising microorganisms for the biological degradation of coal were isolated and their biochemical activity towards low-rank coal was

investigated. The strains were identified based on 16S rRNA gene sequencing. Among the 45 isolated microbial strains, four active isolates belonging to the genera *Cupriavidus* sp., *Pseudomonas* sp., and *Alcaligenes* sp. were studied in detail. These strains increased the pH of the medium, which was interpreted as a physiological response to the acidic nature of coal. The degree of coal decomposition was evaluated by measuring the UV absorbance coefficient (at 450 nm), which indicated the efficiency of biological solubilisation. Furthermore, laccase-like enzymatic activity was detected in these microorganisms, which is important for the breakdown of aromatic polymeric structures such as those found in coal. This study demonstrates the potential for converting coal through sustainable biological processes instead of chemical combustion, and suggests that such isolates can serve as key catalysts for industrial-scale detoxification of coal waste, conversion into biochemically and biotechnologically valuable products, or the development of environmentally safe fuel alternatives [12].

In addition, the cultures investigated in this research may have practical significance for dust suppression at coal production facilities. It is likely that bacteria can remain active at various coal-to-soil ratios in airborne dust. This is important because the ascent of soil dust particles, their suspension in the atmosphere, and the increased quantities facilitate bacterial binding and a cementing effect, which in turn can enhance the overall efficiency of dust suppression measures in the future.

In particular, the widespread occurrence of these bacteria in polluted environments, their adaptation and growth in coal-containing media, their ability to lower the pH of the medium, produce proteins and other metabolites, and their demonstrated enzymatic activity all align with their capacity to modify the coal surface through the action of microbiological metabolites. This indicates that these strains can be regarded as an alternative method for microbial dust suppression and as an environmentally safe and economically viable approach. Furthermore, their practical implementation in coal mines — including under the specific conditions found in Uzbekistan — appears to be a feasible prospect.

CONCLUSION

The research conducted on indigenous microorganisms isolated from the “Shargun Coal Mine” area demonstrated their high degree of adaptation to environmental pollutants under industrial conditions. Among these, *Bacillus simplex*,

Bacillus subtilis, and *Pseudomonas fluorescens* were identified as exhibiting outstanding growth performance, protein synthesis, and enzymatic activity in coal-based nutrient media.

The isolated strains showed the ability to hydrolyse various complex organic compounds — including starch, cellulose, and tannin — thereby highlighting their potential for broad application in bioremediation processes. Notably, the maintenance of stable pH levels in the coal substrate throughout the experiments indicates the strains’ resilience to acidic and stress conditions.

Based on the results obtained, these microorganisms may be effectively employed in the future as bioindicators and bio-cleaning (bioremediation) agents for different contaminated industrial zones, particularly in coal mining regions. This approach holds particular significance as a cost-effective, environmentally safe, and biologically active strategy for ecosystem restoration.

Future work will continue to focus on developing bio-preparations based on the most promising strains — especially *Bacillus simplex* and *Pseudomonas fluorescens* — assessing their effectiveness under field conditions, and exploring their application in coal waste processing, specifically for the microbial production of humic acids.

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