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ASSESSMENT OF UREASE ACTIVITY AND BIOMINERALIZATION CAPACITY OF COAL-DERIVED MICROORGANISMS IN MICROBIALLY INDUCED CALCIUM CARBONATE PRECIPITATION (MICP)

Microbially induced calcium carbonate precipitation (MICP) has emerged as a promising, environmentally friendly technology for soil stabilization, dust suppression, and material strengthening. In this study, microbial communities isolated from coal samples collected at the Kiyakty coal deposit (Ulytau region, Kazakhstan) were investigated for their urease activity and biomineralization potential.

A total of 34 bacterial isolates were obtained and subjected to morphological, physiological, and biochemical characterization. Based on preliminary screening, 12 isolates with the highest adaptive and metabolic potential were selected for further investigation. Urease activity was quantified using a microplate colorimetric method based on the indophenol reaction. In addition, the effects of key environmental factors, including pH and calcium chloride concentration, on microbial activity and mineralization efficiency were evaluated.

The selected isolates exhibited high urease activity and demonstrated the ability to induce calcium carbonate precipitation under laboratory conditions. Furthermore, their capacity for biofilm formation, redox activity, and oxidative enzyme production was confirmed, indicating a strong potential for surface colonization and mineral nucleation.

Mineralized products were characterized using a complex of analytical approach, and the degree of mineralization was quantitatively assessed. The results indicate that coal-derived microorganisms possess significant potential for application in microbially induced calcium carbonate precipitation (MICP)-based technologies, particularly for dust suppression and stabilization of coal substrates.

This study provides a scientific basis for the development of sustainable biotechnological solutions in mining and environmental engineering.

Keywords: microbially induced calcium carbonate precipitation (MICP), urease activity, coal microorganisms, biomineralization, urease-producing bacteria.

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Кальций карбонатының (MICP) микробиологиялық тұндыруында көмір кен орны микроорганизмдерінің уреаза белсенділігі мен биоминерализация қабілетін бағалау

Микробиологиялық индукцияланған кальций карбонатының тұндыруы (MICP) топырақты тұрақтандыру, шаңды басу және материалдарды қатайту үшін перспективалы экологиялық таза технологияға айналды. Бұл зерттеуде Қиякты көмір кен орнынан (Ұлытау облысы, Қазақстан) жиналған көмір үлгілерінен бөлінген микробтық қауымдастықтар олардың уреаздық белсенділігі мен биоминерализация қабілеті тұрғысынан зерттелді.

Барлығы морфологиялық, физиологиялық және биохимиялық сипаттамаларын зерттеуге 34 бактериялық изолят алынды. Алдын ала скрининг нәтижесінде бейімделу және метаболикалық әлеуеті жоғары 12 изолят іріктеліп, әрі қарай терең зерттеуге таңдалды. Штаммдардың

уреазалық белсенділігі микропланшеттік колориметриялық әдіс (индофенол реакциясы) арқылы анықталды. Сонымен қатар қоршаған ортаның негізгі факторларының, атап айтқанда pH мәні мен кальций хлориді концентрациясының микроорганизмдер белсенділігіне және минералдану тиімділігіне әсері зерттелді.

Зерттелген изоляттар жоғары уреазалық белсенділік көрсетіп, зертханалық жағдайда кальций карбонатының түзілуін индукциялау қабілетін дәлелдеді. Сонымен қатар олардың биоқабықша (биопленка) түзу қабілеті, редокс-белсенділігі және тотығу ферменттерін синтездеуі анықталды, бұл олардың бетке адгезиялану және минерал нуклеация орталықтарын қалыптастыру қабілетінің жоғары екенін көрсетеді.

Минералданған өнімдер кешенді әдістер арқылы сипатталып, минералдану дәрежесі сандық тұрғыда бағаланды. Алынған нәтижелер көмірден бөлініп алынған микроорганизмдердің микробты-индукцияланған кальций карбонатының тұнуы (MICP) негізіндегі технологияларда, әсіресе шаң басу және көмір субстраттарын тұрақтандыруда жоғары әлеуетке ие екенін көрсетті.

Бұл зерттеу тау-кен өндірісі мен экологиялық инженерия салаларында тұрақты биотехнологиялық шешімдерді әзірлеуге ғылыми негіз қалайды.

Түйін сөздер: микробты-индукцияланған кальций карбонатының тұнуы (MICP), уреазалық белсенділік, көмір микроорганизмдері, биоминералдану, уреаза түзуші бактериялар.

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Оценка уреазной активности и способности к биоминерализации микроорганизмов, полученных из угля, при микробиологическом осаждении карбоната кальция (MICP)

Микробиологически индуцированное осаждение карбоната кальция (MICP) стало многообещающей экологически чистой технологией для стабилизации почвы, пылеподавления и упрочнения материалов. В этом исследовании микробные сообщества, выделенные из образцов угля, собранных на угольном месторождении Киякты (Улытауская область, Казахстан), были исследованы на предмет их уреазной активности и способности к биоминерализации.

Всего было получено 34 бактериальных изолята, которые были подвергнуты морфологической, физиологической и биохимической характеристике. На основе предварительного скрининга были отобраны 12 изолятов, обладающих наибольшим адаптационным и метаболическим потенциалом, для последующего детального исследования. Уреазную активность штаммов определяли с использованием микропланшетного колориметрического метода (индофенольная реакция). Дополнительно изучено влияние ключевых факторов среды, включая pH и концентрацию хлорида кальция, на уровень микробной активности и эффективность процессов минерализации. Отобранные изоляты продемонстрировали высокую уреазную активность и способность индуцировать осаждение карбоната кальция в лабораторных условиях. Также была подтверждена их способность к образованию биопленок, проявлению редокс-активности и синтезу окислительных ферментов, что свидетельствует о высоком потенциале к колонизации поверхности и формированию центров нуклеации минералов.

Минерализованные продукты были охарактеризованы с применением комплекса аналитических методов, а степень минерализации количественно оценена. Полученные результаты показывают, что микроорганизмы, выделенные из угольной среды, обладают значительным потенциалом для применения в технологиях, основанных на микробно-индуцированном осаждении карбоната кальция (MICP), в частности для задач пылеподавления и стабилизации угольных субстратов.

Проведённое исследование формирует научную основу для разработки экологически устойчивых биотехнологических решений в горнодобывающей промышленности и экологической инженерии.

Ключевые слова: микробно-индуцированное осаждение карбоната кальция (MICP), уреазная активность, угольные микроорганизмы, биоминерализация, уреазопродуцирующие бактерии.

Introduction

Coal mining and handling processes are associated with significant environmental challenges, including dust generation, surface instability, and degradation of surrounding ecosystems. Conventional methods for dust suppression and stabilization often rely on chemical treatments, which may have adverse environmental impacts. Therefore, the development of sustainable and eco-friendly technologies remains a critical research priority.

Microbially induced calcium carbonate precipitation (MICP) has emerged as a sustainable and efficient strategy for enhancing the physicomechanical properties of porous media and mitigating dust emissions. This process is predominantly mediated by urease-producing microorganisms that catalyze the hydrolysis of urea, leading to an increase in pH and the subsequent formation of calcium carbonate ($CaCO_3$). The resulting biomineralization promotes interparticle bonding, decreases porosity, and contributes to the overall stabilization of the material structure.

While MICP has been extensively investigated in soils and construction-related materials, its application in coal systems remains comparatively underexplored. Coal constitutes a highly heterogeneous substrate, characterized by complex organic–mineral composition, variable pore architecture, and diverse surface functional groups, all of which can significantly influence microbial colonization and mineral precipitation dynamics.

In this regard, the isolation and detailed characterization of indigenous microbial communities from coal environments are critically important. Native microorganisms are likely to possess adaptive traits that enable enhanced survival, metabolic performance, and biomineralization efficiency under such специфические условия. Moreover, comprehensive evaluation of their physiological and biochemical properties–including urease activity, biofilm-forming capacity, and tolerance to environmental factors–is essential for the effective optimization and implementation of MICP-based technologies in coal-related applications.

The aim of this study was to isolate urease-producing bacteria from coal samples, evaluate their physiological and biochemical characteristics, and assess their biomineralization potential under varying environmental conditions. Special attention was given to factors influencing MICP efficiency, including pH and calcium ion concentration. The findings contribute to the development of innovative biotechnological solutions for dust suppression and stabilization in coal mining systems.

The map of the joint occurrence of VOSviewer keywords (Web of Science; «microbial-induced carbonate precipitation» and «ureolytic bacteria isolated from coal») shows and demonstrates that the modern international body of research is structured around several interrelated scientific areas (Fig. 1). The size of the nodes on the map reflects the frequency of use of key terms in the scientific literature the lines of communication are their common occurrence, and the color clustering indicates the thematic areas of research.

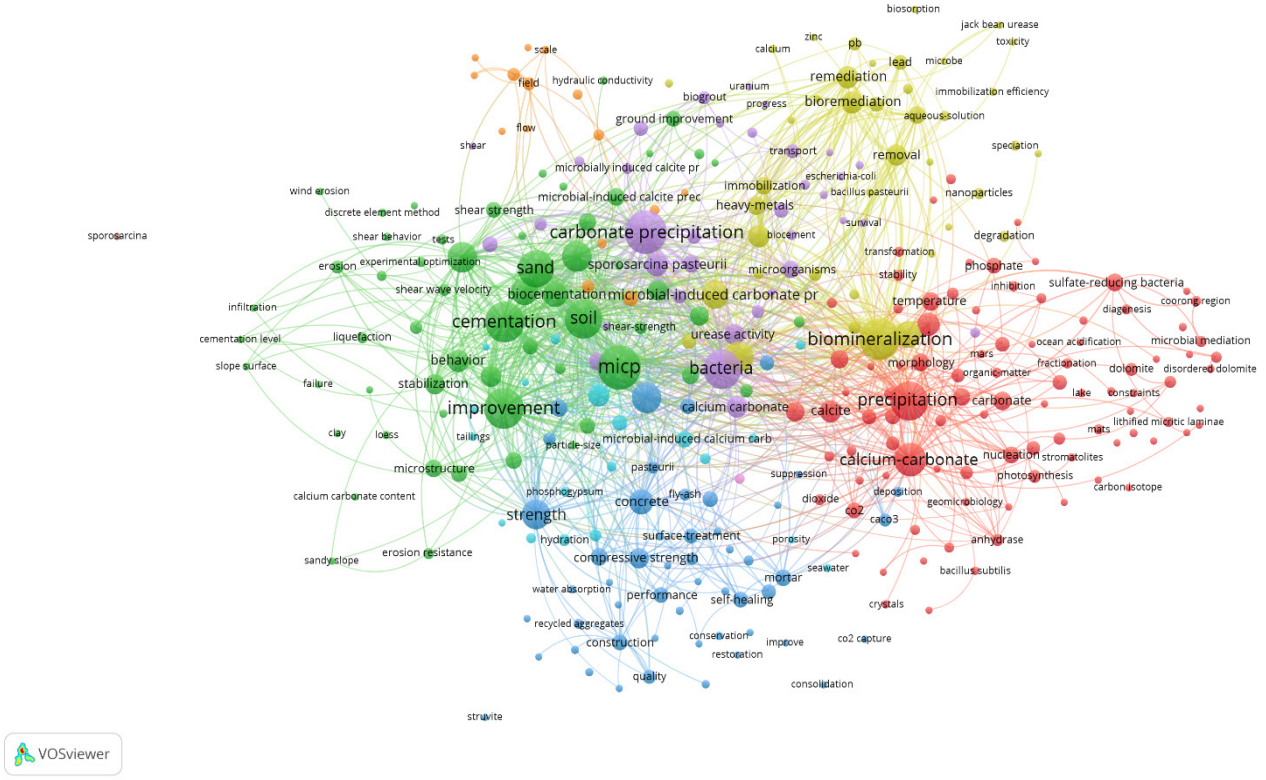
The keyword co-occurrence analysis reveals that microbially induced calcium carbonate precipitation (MICP) occupies a central position, linking key concepts such as soil, sand, cementation, calcium carbonate, bacteria, and biomineralization. The growing body of research underscores a clear emphasis on microbial-mediated mineralization as an effective strategy for strengthening porous materials. In coal-based systems, this approach is particularly significant, as the precipitation of carbonate minerals contributes to surface stabilization and mitigation of dust dispersion.

The thematic clustering of studies reveals several key research directions. The geotechnical domain (e.g., soil stabilization, cementation, strength enhancement, erosion resistance) highlights the application of MICP in improving the mechanical integrity of granular media, which can be directly translated to coal dust consolidation. The biomineralization-focused cluster (e.g., calcium carbonate formation, crystal growth, morphology) addresses the fundamental processes governing mineral nucleation and development, which ultimately control the efficiency and persistence of particle bonding. The environmental cluster (e.g., bioremediation, heavy metal immobilization) reflects the dual functionality of MICP in both dust suppression and contaminant stabilization. Furthermore, the construction-oriented cluster (e.g., concrete, self-healing materials, compressive strength) demonstrates the adaptability of MICP principles to engineered systems.

Microbiological components, particularly urease activity and model organisms such as *Sporosarcina pasteurii*, emphasize the central role of ureolytic bacteria in facilitating carbonate precipitation via urea hydrolysis. These microorganisms drive the biochemical processes that lead to the formation of stable mineral phases, thereby underpinning the effectiveness of MICP-based technologies. The resulting $CaCO_3$ acts as a natural cementing agent, binding particles and reducing their mobility.

Figure 1

Co-occurrence network of keywords generated in VOSviewer based on publications retrieved using the queries "microbially induced carbonate precipitation" and "ureolytic bacteria isolated from coal". Node size reflects keyword frequency, links indicate co-occurrence relationships, and colors represent thematic clusters



Overall, the bibliometric analysis demonstrates that MICP research is highly interdisciplinary, integrating microbiology, geotechnical engineering, environmental science, and materials science. In the context of coal systems, the integration of biomineralization processes with dust suppression technologies represents a promising and sustainable approach. However, further research is required to optimize microbial performance, mineralization conditions, and large-scale implementation strategies.

Materials and methods

Coal Sampling and Characterization

Coal samples were collected from the Kiyakty coal mine located in the Ulytau region, Karagandy areacп, Kazakhstan (48°74′79.53″N, 67°24′72.74″E), following established sampling procedures described by Dai et al. The samples were immediately sealed in sterile polyethylene bags and stored at 4 °C to minimize contamination and oxidative alterations. Prior to analysis, the coal was air-dried, ground, and sieved to obtain particles smaller than 0.2 mm. Proximate and ultimate analyses were conducted in accordance with ASTM standards (ASTM D3172-74 and ASTM D5373). Elemental composition (C, H, N, S) was determined using a Vario EL cube elemental analyzer (Elementar Analysensysteme GmbH, Germany). Oxygen content was calculated by difference, as commonly applied in coal characterization studies (Dai et al., 2012).

Preparation of Coal Suspensions and Microbial Enrichment. For microbial enrichment, 10 g of each coal sample was suspended in 100 mL of sterile distilled water in Erlenmeyer flasks. The suspensions were incubated on a rotary shaker at 150 rpm and 30 °C for 2 h to detach microorganisms associated with the coal matrix. Aliquots of the suspension were inoculated onto Luria–Bertani (LB) medium to obtain microbial cultures, following previously described enrichment protocols (Fan et al., 2020).

Isolation of Urease-Producing Bacteria

Urease-positive bacteria were isolated using a serial dilution technique. Coal suspensions were diluted up to 10^{-5}–10^{-7} and plated onto selective urea agar as well as LB agar plates. The cultures were incubated at 30 °C for 24–48 h. Distinct colonies

were purified by repeated streaking to obtain axenic cultures.

Morphological and Cultural Characterization

Pure isolates were maintained on nutrient agar at 30 °C and subcultured every 7–10 days. Colony morphology was evaluated after incubation (24–48 h at 28–37 °C), including size (mm), shape, pigmentation, surface texture (smooth/rough), elevation (flat/convex/crateriform), margin (entire/undulate/filamentous), and consistency (mucoid/dry/pasty), following standard microbiological guidelines (Cappuccino et al., 2014).

Microscopic Characterization. Cell morphology was examined using Gram staining performed on 24 h cultures. Slides were heat-fixed and stained according to the standard Gram method. Microscopic observations were carried out under oil immersion (×1000 magnification) (Madigan, M. et al., 2018). Cell shape, arrangement, and Gram reaction were recorded. Motility was assessed using the hanging drop method and semi-solid agar (0.3–0.5%). Spore formation was evaluated using the Schaeffer–Fulton staining method, with endospores confirmed microscopically.

Physiological and Biochemical Characterization.

Growth characteristics were evaluated under different environmental conditions: Temperature range: 25 °C, 35 °C, and 50 °C, pH range: 4–9 (interval of 1 pH unit), NaCl tolerance: 2%, 3%, and 4%. Growth was monitored visually and quantified by measuring optical density at 600 nm (OD_{600}).

Biochemical assays included: Catalase activity: assessed by the addition of 3% H_2O_2; bubble formation indicated a positive result. Oxidase test: color change within 30 s indicated positivity. Urease activity: evaluated using Christensen's urea medium; a pink color change indicated urea hydrolysis. Substrate hydrolysis: starch (Lugol's iodine) hydrolysis was tested (MacFaddin et al., 2000).

Biofilm Formation Assay (Congo Red Method)

Extracellular polymeric substance (EPS) production was assessed using Congo Red agar supplemented with sucrose (36 g/L) and Congo Red dye (0.8 g/L). Plates were incubated at 30 °C for 24–48 h. The formation of dark red or black colonies was interpreted as positive biofilm formation.

Oxidative Enzyme Activity (Remazol Brilliant Blue R Assay)

To evaluate oxidative enzyme activity, isolates were cultured on media containing Remazol Brilliant Blue R (100 mg/L). Plates were incubated at 30 °C for 3–5 days. Zones of decolorization or clear halos observed around microbial colonies were interpreted as evidence of oxidative enzyme production, including enzymes such as laccases and peroxidases, which are involved in the degradation of complex organic compounds.

Assessment of Redox Activity and Adsorption Capacity (Methylene Blue Assay)

The redox behavior and adsorption potential of the isolates were investigated using a methylene blue–enriched agar system. Nutrient agar was sterilized and allowed to cool to 45–50 °C, after which a sterile methylene blue solution was added to achieve a final concentration of 0.01–0.02%. The medium was homogenized and dispensed into sterile Petri dishes. Variations in dye intensity, as well as the formation of decolorized zones in the vicinity of colonies, were used as qualitative indicators of microbial redox activity and adsorption efficiency.

Microplate-Based Colorimetric Determination of Urease Activity (Indophenol Method)

The ureolytic activity of bacterial cultures was quantified using a microplate-based indophenol assay, which enables the detection of ammonia released during enzymatic urea hydrolysis. This method relies on the quantification of ammonia released during urea hydrolysis by urease-producing microorganisms. The liberated ammonia reacts with phenolic compounds and hypochlorite under alkaline conditions to form indophenol blue, which is measured spectrophotometrically. Absorbance was recorded using a microplate reader, allowing high-throughput and sensitive detection of urease activity (Yañez-Yazlle et al., 2014).

Effect of Initial pH

The initial pH is a critical parameter influencing microbial growth and biomineralization processes. Previous studies have demonstrated that pH significantly affects both the kinetics of urease activity and the morphology of precipitated calcium carbonate (Wang et al., 2009). Taking into account the specific conditions of microbial systems associated with coal, urease activity was estimated in the pH range from 5 to 11 in increments of one unit. The pH of the medium was adjusted using NaOH solution. The effect of pH on enzymatic activity and metabolic performance of the isolates was systematically assessed.

Effect of Calcium Chloride Concentration

Calcium ions play a fundamental role in microbially induced mineral precipitation by serving as the primary source of Ca^{2+} required for carbonate formation. Since the stoichiometric relationship between urea hydrolysis and carbonate ion produc-

tion is approximately 1:1, calcium chloride concentrations were set to match the corresponding urea concentrations (0; 0.1 M; 0.2 M; 0.5 M; 1.0 M; and 2.0 M). Urease activity was measured under varying $CaCl_2$ concentrations to evaluate the influence of calcium availability on the metabolic activity of urease-producing bacteria.

Characterization of Biomineralization and Precipitation Processes

Based on experimentally determined optimal conditions (urea concentration, $CaCl_2$ concentration, and pH) corresponding to maximum urease activity, bacterial suspensions were inoculated into the culture medium at a final concentration of 1% (v/v). Cultivation was carried out in a shaking incubator at 30 °C and 150 rpm for periods of 3, 6, 9, 12, 15, 18, and 21 days. At each time point, mineralized products were collected for further physicochemical characterization (Fujita et al., 2017). All experiments were performed in triplicate to ensure reproducibility and statistical reliability.

Preparation of Mineralized Products

At the end of the incubation period, the culture medium was centrifuged at 8000 rpm for 10 minutes to separate solid precipitates. The supernatant was carefully removed. To eliminate residual organic impurities, the precipitates were washed three times with distilled water followed by absolute ethanol. The cleaned samples were then dried at 105 °C for 24 hours to obtain purified mineralized products suitable for subsequent analysis.

Yield and Degree of Mineralization

The quantity of the mineralized product was determined using an acid dissolution method. The degree of mineralization was calculated as the molar ratio of precipitated calcium carbonate to the initial amount of calcium chloride added prior to cultivation. This parameter was used as a quantitative indicator of biomineralization efficiency exhibited by urease-producing microorganisms (Seifan et al., 2017).

Statistical analysis and image processing

The experimental exposures were organized using a completely randomized design with three or five repetitions (depending on the test performed). The FIJI open source image processing software was used for data collection and image processing. It was assumed that all measured data have a normal distribution with uniform errors of variance. Unless otherwise specified, the values obtained are presented as the average value ± standard deviation. The statistical significance of the observed differences was determined using a one-factor analysis of variance followed by the Tukey test. Probability levels of 0.05 or less were considered statistically significant.

Results and discussion

Collection and characterization (proximal, marginal, morphological and spectroscopic) of coal and coal dust samples from the Kiyakty coal mine (Ulytau region, Kazakhstan).

Technical characteristics of Kiyakty coal samples (hard coal). To assess the technical characteristics of the coal samples, a comprehensive analysis of the proximal and final parameters was carried out, the results of which are presented in Table 1.

Table 1

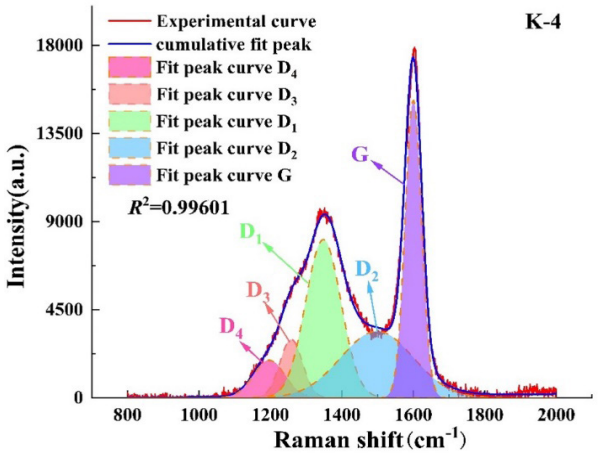
Results of proximal and elemental analyses of Kiyakty coal samples

Proximate analysis	Moisture, %	Ash, %	Volatile matter, %	
	7.62	10.58	34.01	
Ultimate analysis	C, %	H, %	N, %	S, %
	45.10	2.987	1.947	1.25

Proximal analysis of Kiyakty coal: humidity 7.62% – corresponds to moderately dried brown/slightly metamorphosed coal. The value is not critical and does not have a significant negative effect on the calorific value. The ash content of 10.58% characterizes the average content of mineral impurities. This indicates a moderate mineralization of coal, which is important when evaluating technological processes (gasification, bio-leaching, MICP modifications). The yield of volatile substances of 34.01% is a fairly high indicator typical for coals of medium degree of metamorphism. Indicates a significant content of aliphatic structures and functional groups. According to the set of parameters, coal belongs to coals of medium degree of carbonification (sub-bituminous / low-grade coal).

Elemental (ultimate) analysis: C (45.10%) – the relatively low carbon content confirms the average degree of metamorphism. For high-grade coals, this indicator usually exceeds 70%, H (2.987%) – indicates the preservation of aliphatic chains and functional groups, N(1.947%) – is quite typical for humic coals, S (1.25%) – moderate content. Both organic and pyrite forms of sulfur are possible. The C/H ratio indicates a moderate aromatization of the organic mass.

Figure 2
Raman spectroscopy results



The main bands are distinguished in the spectrum (800-2000 cm^{-1}): the G band (~1580-1600 cm^{-1}) is associated with fluctuations in sp2-hybridized carbon (aromatic structures, graphite-like domains). D1 (~1350 cm^{-1}) is a defective band reflecting disturbances in the crystalline structure of aromatic layers. D2 (~1620 cm^{-1}) is associated with surface defects and edge structures of aromatic clusters. D3 (~1500 cm^{-1}) reflects amorphous carbon and organic structures with low order. D4 (~1200 cm^{-1}) is associated with polyconjugated structures and functional groups. Kiyakty coal: refers to coals of medium degree of metamorphism, has moderate ash content, is characterized by a high proportion of volatile substances, has an amorphous-defective carbon structure, and demonstrates a low degree of graphitization according to Raman spectrum. Structural characteristics are favorable for: biomodification (MICP), microbiological oxidation, sorption processes, surface activation.

Isolation, identification and characterization of microbial strains from coal samples collected at the Kiyakty coal mine (Ulytau region, Kazakhstan).

During the microbiological study of the coal medium, 34 bacterial isolates were isolated, for which a comprehensive study of morphological, cultural, physiological and biochemical properties was carried out. The primary screening included the analysis of colony morphology, cell structure, growth characteristics on various nutrient media, as well as the determination of key biochemical characteristics and enzymatic activity.

Based on the results obtained, 12 of the most promising isolates with pronounced physiological and biochemical properties and adaptive potential were selected, which allowed them to be recommended for further experimental research and biotechnological testing.

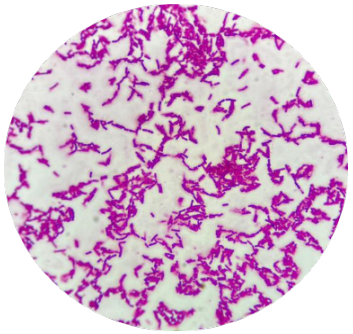
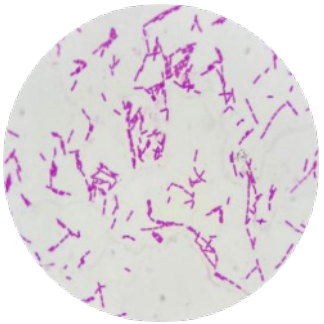
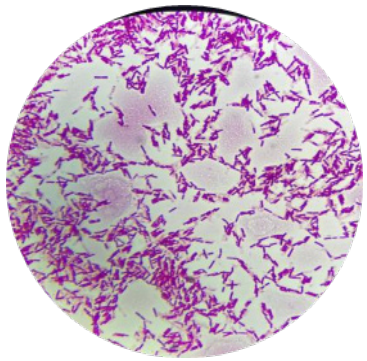
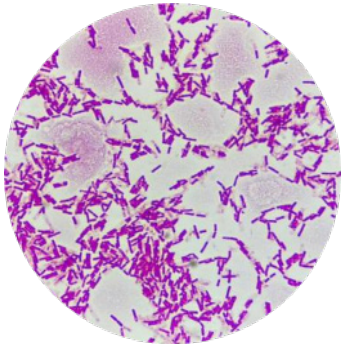
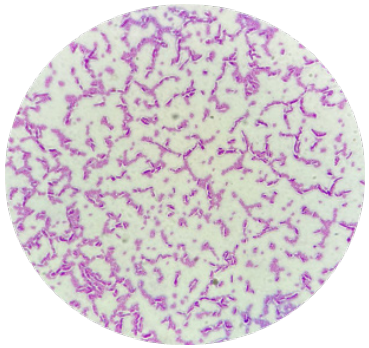
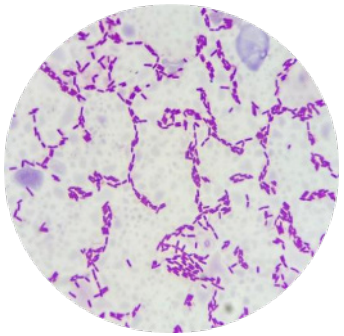
A reference strain from the international collection of microorganisms Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures – Sporosarcina pasteurii DSM 33 was also used as a control microorganism. This strain was obtained from the DSMZ collection (Deutsche Sammlung von Mikroorganismen und Zellkulturen) and is widely used in scientific research as a model urease-positive microorganism used to study the processes of microbially induced carbonate mineralization (MICP) (Wang et al., 2009). The use of the reference strain made it possible to conduct a comparative assessment of the activity of isolates isolated from the coal medium, as well as to confirm their biomineralization potential and prospects for further research in the field of biotechnological suppression of dust formation and stabilization of coal substrates.

An analysis of the morphological and cultural characteristics of microorganisms isolated from the carbon medium showed their pronounced diversity in terms of macro- and micromorphological features. Most isolates (B1–B3, K1–K4, L1, K5S) are gram-negative rod-shaped or oval-shaped bacteria, which is typical for microorganisms adapted to the extreme conditions of coal ecosystems. Colonies are characterized by various morphologies: from smooth, rounded and transparent to zigzag-shaped, with wavy edges and varying degrees of pigmentation (colorless, whitish, yellowish).

The presence of colonies with a zigzag edge and a heterogeneous structure (B2, K2, L1, K2S, K4S, K8S) may indicate high metabolic activity and the ability to form exopolysaccharides, which is an important factor in the formation of biofilms and fixation on solid substrates, including coal. Gram-negative isolates (K2S, K4S, K8S), which exhibit morphological characteristics comparable to those of the reference strain *Sporosarcina pasteurii*, warrant particular attention. Such similarity may suggest their potential capacity for urease production and involvement in biomineralization processes, including microbially induced calcium carbonate precipitation.

The reference strain *Sporosarcina pasteurii* DSM 33 displays morphological traits consistent with its taxonomic identity, including Gram-positive, rod-shaped cells forming smooth, convex colonies with a creamy-white appearance. These features validate the appropriateness of the cultivation conditions and support the use of this strain as a reliable benchmark for comparative analysis.

Table 2
Morphology-cultural properties of isolated bacteria

Strain	Micromorphology	Morphology-cultural properties	Strain	Micromorphology	Morphology-cultural properties
B1		Gram-negative round-shaped bacteria. In a dense nutrient medium, they form colonies with a pale transparent smooth edge.	K1		Gram-negative, rod-shaped, erect rod-shaped. In a dense nutrient medium, they form colonies with a whitish coating and a smooth edge.
B2		Gram-negative, short-oval bacteria. In a dense nutrient medium, they form colonies of a whitish color with a zigzag edge resembling algae.	K2		Gram-negative rod-shaped bacteria with a long-curved shape. In a dense nutrient medium, they form a colony with a zigzag edge of rounded shape.
B3		Gram-negative, long rod-shaped, erect rod-shaped. In a dense nutrient medium, they form colorless colonies with a yellowish smooth edge.	K3		Gram-negative, short sticks. In a dense nutrient medium, they form yellow colonies with a straight edge of a round shape.

Continuation of the table

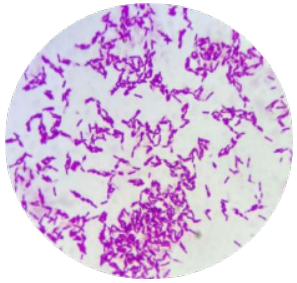
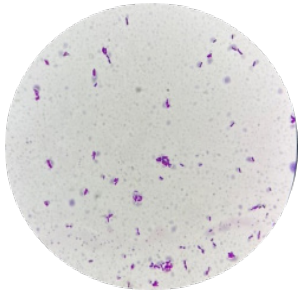
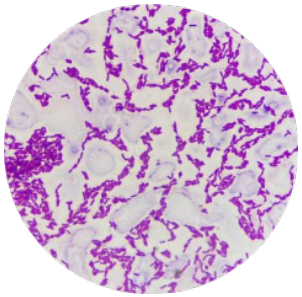
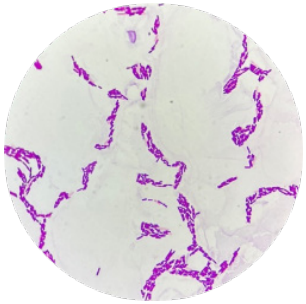
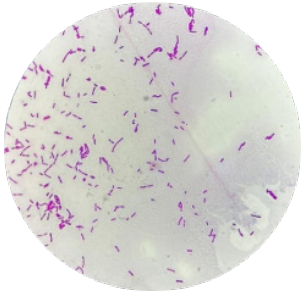
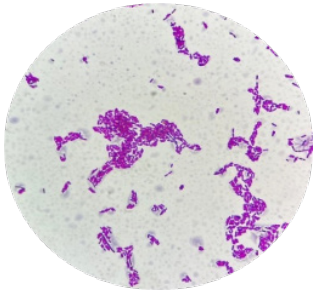
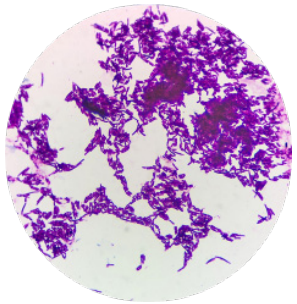
Strain	Micromorphology	Morphology-cultural properties	Strain	Micromorphology	Morphology-cultural properties
K4		Gram-negative rod-shaped bacteria of the direct form. In a dense nutrient medium, they form convex colonies with a smooth oval-shaped edge with a zigzag edge of yellowish-white color.	K4S		Gram-negative, short, oval-shaped bacteria. In a dense nutrient medium, they form colonies of a whitish color with a zigzag edge resembling algae.
L1		A gram-negative rod-shaped bacterium, an erect, curved rod is also visible. In a dense nutrient medium, they form zigzag collonies with a smooth rounded edge.	K5S		Gram-negative, long, oval-shaped bacteria. In a dense nutrient medium, they form colorless colonies with rounded edges.
K2S		Gram-negative, long, oval-shaped bacteria. In a dense nutrient medium, they are round, colorless and whitish, forming colonies with a zigzag edge.	K8S		Gram-negative, short-oval bacteria. In a dense nutrient medium, it forms whitish colonies with a zigzag edge.
Sporosarcina pasteurii		When cultivated on standard nutrient media, colonies of the strain are characterized by a rounded shape, smooth or slightly wavy edges and a smooth surface. Colonies have a convex profile, dense consistency, and creamy-white or light beige coloration. The surface of the colonies is matte or slightly shiny, the structure is uniform, without pronounced pigmentation and zonal staining. The cells are represented by gram-positive rod-shaped bacteria. The cells are arranged singly, in pairs, or form short chains. The average cell size is about 1.0–1.5 × 2.0–3.0 microns.			

Table 3
Physiology-biochemical properties of isolated bacteria

№	Strain	Gram coloring	Starch hydroly-sis	Indole test	Biofilm forma-tion (Congo red dye coloriza-tion)	Oxidase test	pH 4	pH 7	pH 9	25°C	35°C	50°C	NaCl 2%	NaCl 3%	NaCl 4%
1	*B1*	-	+	-	+	+	+	+++	+++	+++	++	++	+	+	++
2	*B2*	-	+	-	+	+	+	++	+++	+++	++	++	-	-	-
3	*B3*	-	+	-	+	+	+	+++	+++	+	+++	++	+++	+++	++
4	*K1*	-	+	-	-	+	+	+++	+++	+++	+++	+	+++	++	+++
5	*K2*	-	+	-	+	+	++	++	+++	+++	+++	+	+++	+++	++
6	*K3*	-	+	-	+	+	+	+++	+++	+++	++	++	+++	+++	+++
7	*K4*	-	-	-	+	+	++	+++	+++	+++	+++	++	+++	+++	+++
8	*L1*	-	-	-	-	+	++	+	+++	+	+++	++	++	++	+++
9	*K2S*	-	+	-	-	+	+	+++	+++	++	+++	++	-	-	-
10	*K4S*	-	-	-	+	+	++	++	+++	++	+++	+	+++	+++	++
11	*K5S*	-	+	-	-	+	+	++	+++	++	+++	++	+++	++	+++
12	*K8S*	-	+	-	+	+	++	++	+++	+	+++	+	+++	+++	++
13	*Sporosarcina pasteurii*	+	+	-	+/-	-	++	++	+++	+++	+++	++	+++	+++	++

Note: "+" low activity or growth, "++" – moderate, "+++" – intense growth or pronounced enzymatic activity; "–" indicates the absence of the studied trait, +/- Positive / moderately positive; on medium with Congo red, colonies can turn dark red or red-brown color, which indicates the synthesis of extracellular polysaccharide matrix and the ability to form biofilms (Reference strain Sporosarcina pasteurii

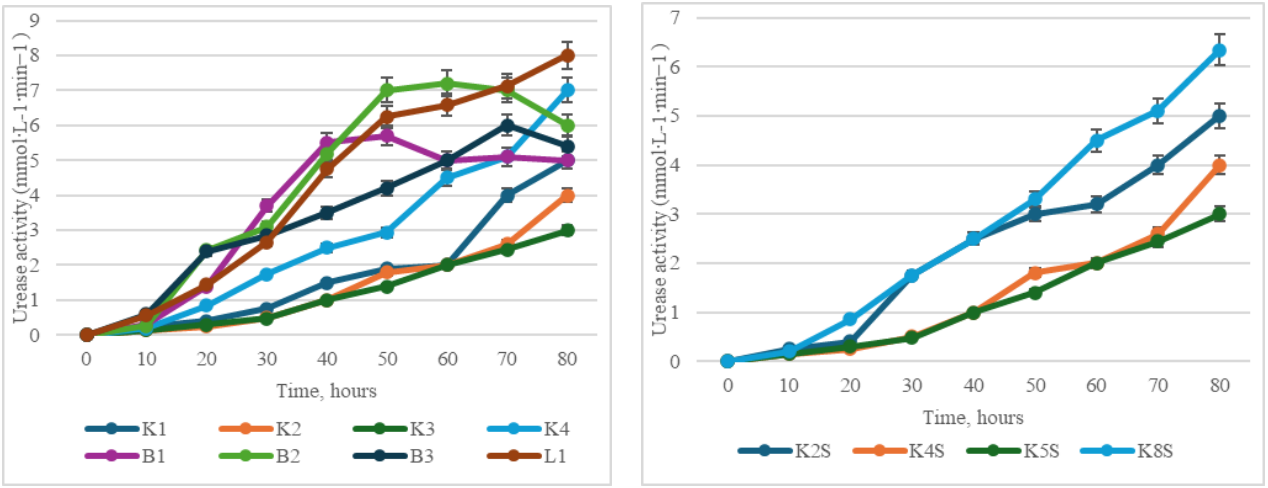
The results of the physiological and biochemical analysis showed (Table 3) that most of the studied isolates have a wide range of adaptive capabilities. Almost all strains exhibit a positive reaction to starch hydrolysis, which indicates the presence of amylolytic activity and the ability to use complex carbohydrates as a carbon source. The indole test for all strains was negative, indicating a lack of tryptophanase and a limited ability to decompose tryptophan.

A significant part of the isolates demonstrate the ability to form biofilms (Congo red dye colorization), which is confirmed by a positive or moderately positive reaction. This is an important property for the fixation of microorganisms on the surface of coal particles and the formation of stable microbial communities involved in the processes of biocementation and suppression of dust formation (Turu & Canlı, 2025). The oxidase test showed predominantly positive results, indicating the presence of cytochrome c oxidase and aerobic respiration in most isolates. Analysis of growth at various pH values showed that most strains develop optimally under neutral and slightly alkaline conditions (pH 7-9), where intensive growth is observed (+++). This is consistent with the mechanism of microbially induced carbonate mineralization, accompanied by an increase in the pH of the medium. The temperature range of growth indicates the mesophilic nature of most isolates (optimum 25-35 °C), while individual strains remain active at elevated temperatures (50 °C), which indicates their thermal tolerance. Resistance to salt stress (NaCl 2-4%) varies, but a number of strains (K2, K3, K4, K4S, K8S) demonstrate a high level of tolerance, which is an important factor when used in real conditions of coal mines, where fluctuations in the ionic composition of the medium are possible.

Screening of urease-producing strains of microorganisms in coal in terms of their survival and mineralization activity.

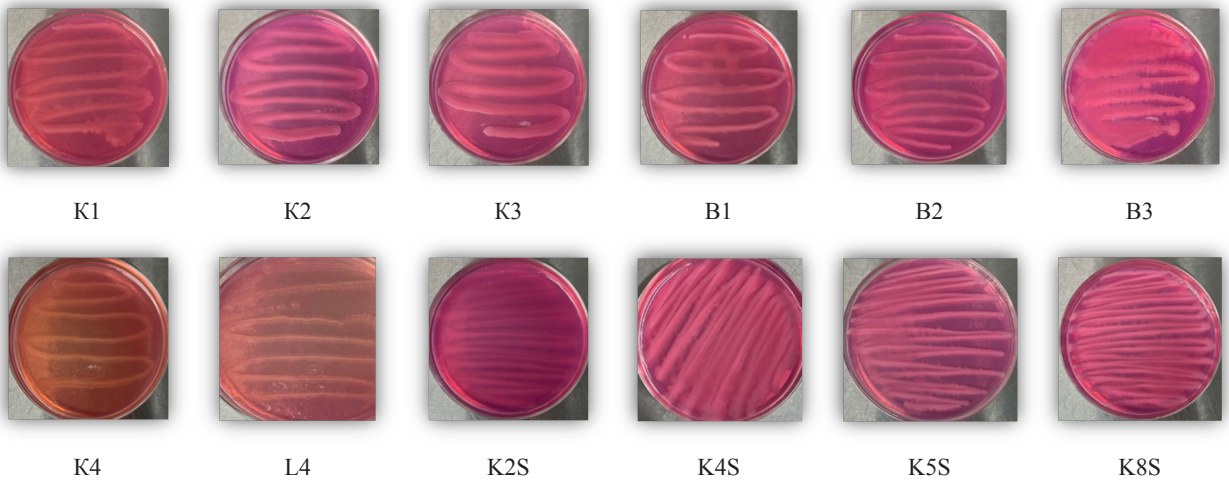
During the screening of urease-producing microorganisms isolated from a carbon medium, a quantitative assessment of urease activity was performed using a microplate method (Seifan, et al., 2020). This approach made it possible to trace the dynamics of enzymatic activity over time and identify the most promising strains in terms of their mineralization potential (Zúñiga-Barra et al., 2023). Urease activity is a key factor in microbially induced carbonate mineralization (MICP), since it is this enzyme that catalyzes the hydrolysis of urea to form ammonia and hydroxide ions, which leads to an increase in the pH of the medium and the subsequent formation of carbonate ions. In the presence of calcium ions, this leads to precipitation of calcium carbonate ($CaCO_3$). Thus, the higher the specific urease activity of the strain, the more effective the biomineralization process is.

Figure 4
Urease activity of isolated strains



An analysis of the temporal dynamics of urease activity (0-80 h) showed that all the studied strains are characterized by a lag phase in the first 10-20 hours, after which an active increase in enzymatic activity is observed. The highest values of urease activity were demonstrated by strain L1, in which activity reaches maximum values by the 80th hour (~8.0 mmol·l^{-1}·min^{-1}). This indicates a high intensity of urea hydrolysis and, consequently, a significant potential for the formation of calcium carbonate. Strains B2 and K4 also show a high level of activity, reaching peak values in the range of 6.0–7.0 mmol·l^{-1}·min^{-1}. At the same time, the B2 strain is characterized by reaching a maximum by 60 hours, followed by a slight decrease in activity, which may indicate depletion of the substrate or accumulation of inhibitory products. Strains B1 and B3 show moderately high activity (up to ~5.0–6.0 mmol·l^{-1}·min^{-1}), which also indicates their potential for use in MICP processes. In turn, strains K1, K2, and K3 are characterized by lower urease activity (within 3.0–5.0 mmol·l^{-1}·min^{-1}), which may limit their effectiveness in biomineralization processes, but they may be of interest from the point of view of resistance to stressful conditions.

Figure 5
Urease activity of isolated strains



There is a marked differentiation in the level of urease activity among gram-negative strains (K2S, K4S, K5S, K8S). The highest level of activity is demonstrated by the K8S strain, which reaches a value of about 6.3 mmol·l^{-1}·min^{-1} by the 80th hour. This result is comparable to the activity of highly effective strains from the first group and indicates its significant biotechnological potential. The K2S strain shows a steady increase in activity with a level of about 5.0 mmol·l^{-1}·min^{-1}, which also indicates its promise. K4S and K5S are characterized by more moderate activity (up to 3.0–4.0 mmol·l^{-1}·min^{-1}), however, they demonstrate stable growth dynamics, which may be important for long-term biomineralization processes.

The results obtained indicate that the urease activity varies significantly between strains, which allows their selection depending on the biotechnological tasks. Strains with high urease activity (L1, B2, K4, K8S) are the most promising for microbially induced carbonate mineralization processes, as they provide intensive $CaCO_3$ formation. This is especially important in the development of technologies for biocementation, stabilization of coal substrates and suppression of dust formation.

The use of the microplate method makes it possible to accurately quantify the enzymatic activity and optimize the process conditions (concentration of urea, calcium ions, pH of the medium), which, in turn, helps to reduce reagent costs and increase the efficiency of biomineralization (Zhu, et al., 2019). Thus, the screening made it possible to identify strains with a high level of urease activity and confirm their potential for use in MICP technologies aimed at environmentally friendly stabilization of coal materials and reducing the negative impact of dust emissions.

Determination of the urease activity of strains in relation to the decomposition of urea and the formation of calcium carbonate precipitate.

In continuation of the screening (Section 3.3) aimed at detecting urease-producing microorganisms, an in-depth assessment of the urease activity of the selected strains was performed from the perspective of their ability to microbially induced carbonate mineralization (MICP) (Zhang, et al., 2019). This process is considered as a key mechanism of biocementation, ensuring the formation of a carbonate matrix (Achal et al., 2016) and the stabilization of carbon substrates (Ramachandran, et al., 2020).

Urease activity is a determining factor in the effectiveness of MICP, since urease catalyzes the hydrolysis of urea to form ammonia and carbon dioxide:

$$CO(NH_2)_2 + H_2O \rightarrow 2NH_3 + CO_2$$

Subsequently, ammonia interacts with water, increasing the pH of the medium due to the formation of hydroxide ions (OH^-), which leads to a shift in the carbonate equilibrium towards the formation of carbonate ions (CO_3^{2-}). In the presence of calcium ions (Ca^{2+}) calcium carbonate precipitates:

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3\downarrow$$

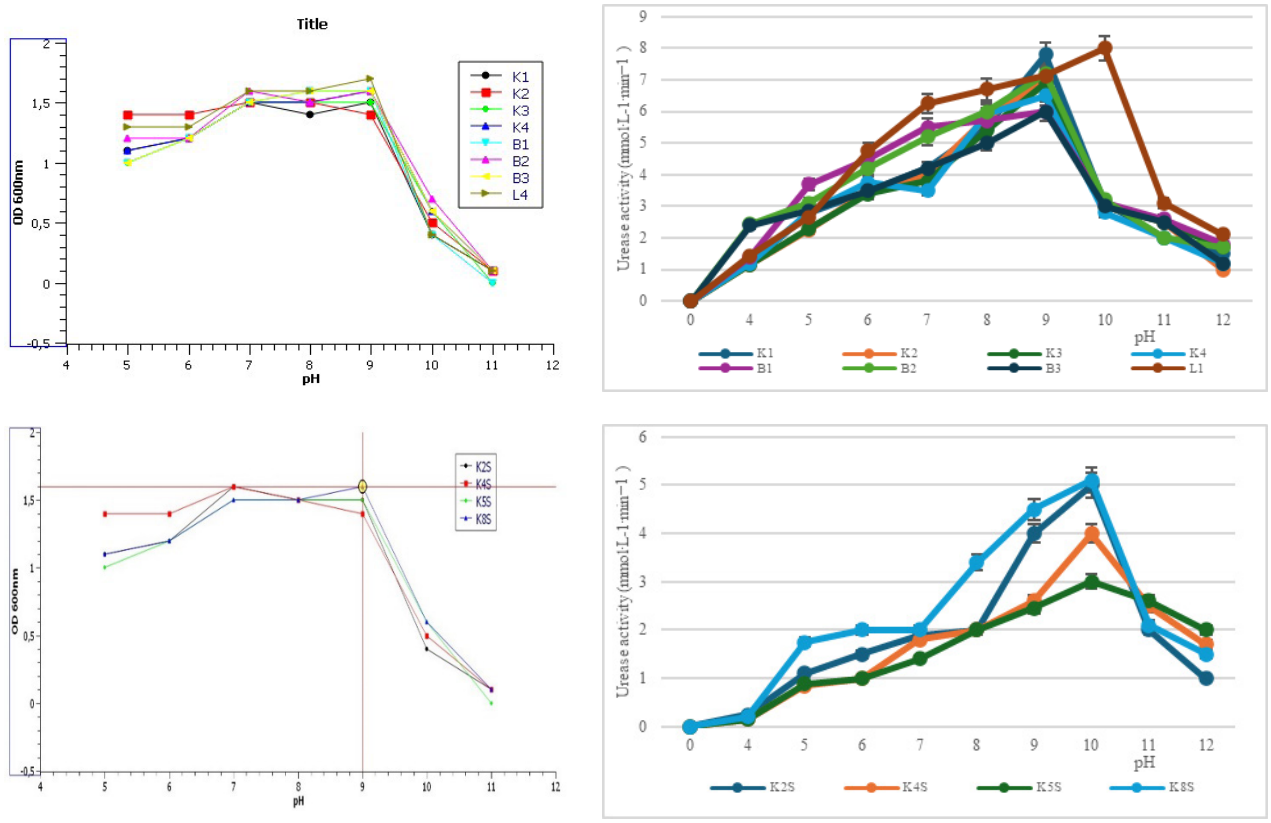
Thus, the intensity of urease activity directly determines the rate of increase in the pH of the medium and, consequently, the efficiency of $CaCO_3$ deposition.

Influence of pH of the medium on urease activity and mineralization.

The results presented in Table 3 show that most of the studied strains exhibit maximum growth and enzymatic activity under neutral and slightly alkaline conditions (pH 7-9). This corresponds to the optimal MICP flow conditions, under which the maximum accumulation of carbonate ions is observed. At acidic values (pH 4), the growth of microorganisms and urease activity are significantly reduced, which limits the process of biomineralization. At the same time, the ability of a number of strains to remain active in a wide pH range indicates their high adaptability to the conditions of coal mines.

Figure 6

Effect of pH of the medium on urease activity and mineralization



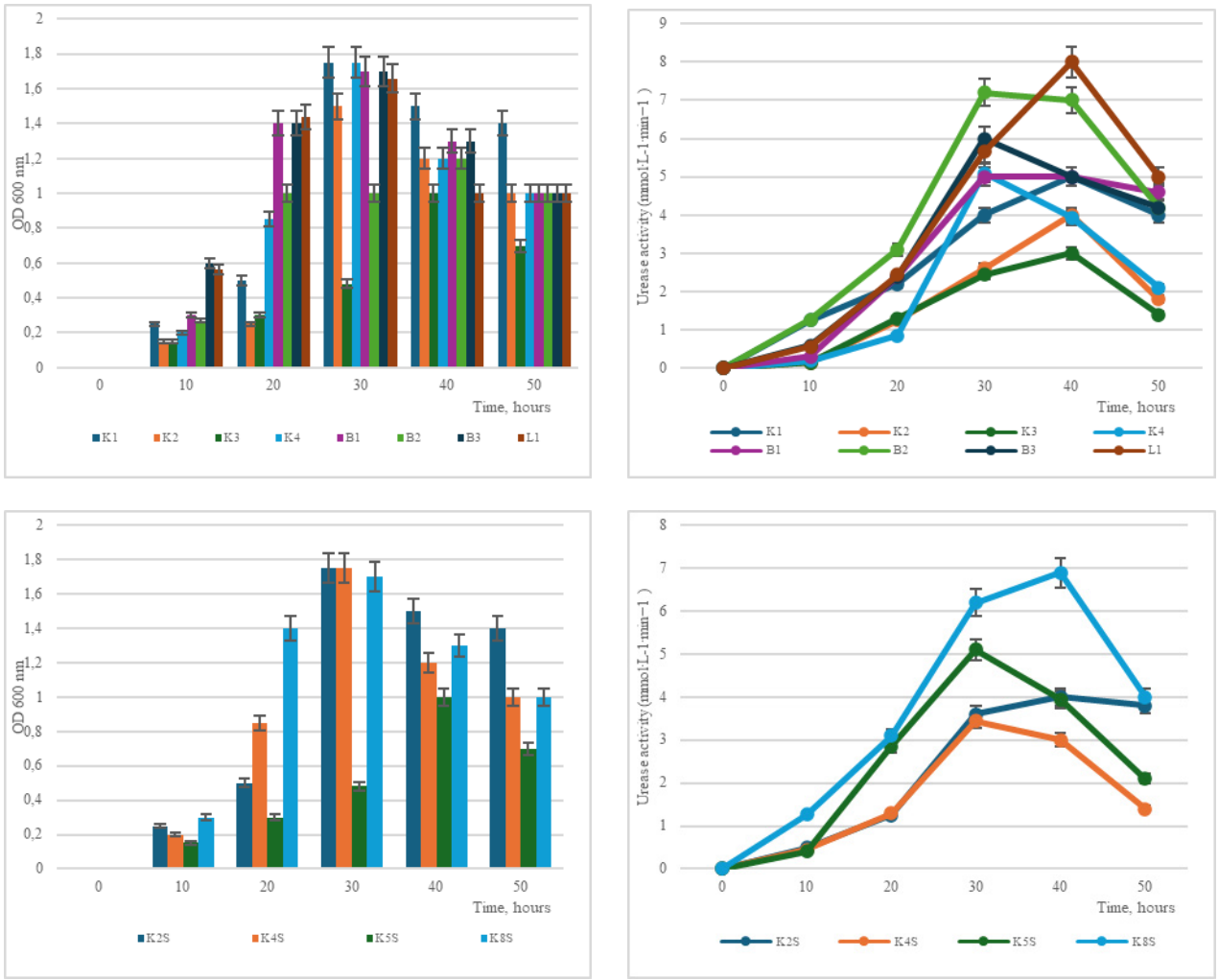
The results show that bacterial growth is strongly influenced by pH, with optimal growth occurring in the pH range of 7-9. This range corresponds to the most favorable conditions for urease activity and subsequent precipitation of calcium carbonate during the MICP process. At acidic pH values (5-6), bacterial growth slows down moderately, while highly alkaline conditions (pH ≥10) significantly inhibit the activity of microorganisms. Thus, maintaining conditions close to neutral or slightly alkaline is crucial for effective biomineralization and stabilization of coal dust.

Temperature dependence of urease activity.

The analysis of temperature parameters showed that most strains are mesophilic, with optimum activity in the range of 25-35°C. When the temperature rises to 50 °C, some of the isolates retain their enzymatic activity, which indicates their thermal tolerance and expands the possibilities of practical application in various climatic conditions.

Figure 7

Dynamics of growth (OD_{600}) and urease activity of strains as an indicator of their potential in microbial-induced carbonate mineralization (MICP) processes



The combined analysis of growth kinetics (OD_{600}) and urease activity for all investigated strains, including both primary isolates (K1–K4, B1–B3, L1) and Gram-positive derivatives (K2S, K4S, K5S, K8S), provides a comprehensive understanding of their biomineralization potential within microbially induced carbonate precipitation (MICP) systems.

Across all strains, a consistent growth pattern was observed, characterized by an active exponential phase occurring between 20 and 40 hours of cultivation. During this interval, most isolates reached maximum biomass levels ($OD_{600} \approx 1.5–1.8$), indicating favorable adaptation to the experimental conditions and efficient substrate utilization. Notably, strains K8S and K2S demonstrated the highest optical density values among Gram-positive isolates, reflecting enhanced metabolic activity and biomass accumulation. In contrast, K5S exhibited comparatively moderate growth, suggesting potential sensitivity to environmental conditions or metabolic limitations.

Urease activity profiles closely followed biomass development, with peak enzymatic activity observed within the same 30–40 hour timeframe corresponding to the late exponential and early stationary growth phases. Among the tested strains, B2, L1, and K8S exhibited the highest urease activity (up to ~7–8 $mmol \cdot L^{-1} \cdot min^{-1}$), indicating a strong capacity for urea hydrolysis. Strains K2S, K1, K4, and B1–B3 showed moderate activity (approximately 4–6 $mmol \cdot L^{-1} \cdot min^{-1}$), whereas K2, K3, and K4S were characterized by comparatively lower enzymatic performance.

From the perspective of MICP, the observed synchronization between biomass accumulation and urease activity is of critical importance. Urease catalyzes the hydrolysis of urea, leading to the formation of ammonia and carbon dioxide, which subsequently increases the pH of the medium and promotes the generation of carbonate ions. In the presence of calcium ions, this process drives the precipitation of calcium carbonate ($CaCO_3$), forming a mineral matrix that contributes to particle binding and structural stabilization. Therefore, strains exhibiting both high biomass density and elevated urease activity are considered the most effective contributors to biomineralization.

A decline in urease activity was observed in most strains after 40–50 hours, despite relatively stable biomass levels. This phenomenon can be attributed to substrate depletion, accumulation of inhibitory metabolites (e.g., ammonia), or physiological shifts associated with the stationary phase. However, strains K8S and K2S maintained comparatively higher enzymatic activity at later stages, indicating improved resilience and sustained metabolic functionality under changing environmental conditions.

Importantly, the integration of growth and enzymatic parameters allows for the identification of the most promising candidates for practical MICP applications. Strains such as B2, L1, K8S, and K2S combine rapid biomass accumulation with high urease activity, making them particularly suitable for biotechnological processes including biocementation, coal particle stabilization, and dust suppression. The ability of these microorganisms to effectively precipitate $CACO_3$ contributes to the formation of a cementing mineral structure, reducing particle dispersion and increasing mechanical stability.

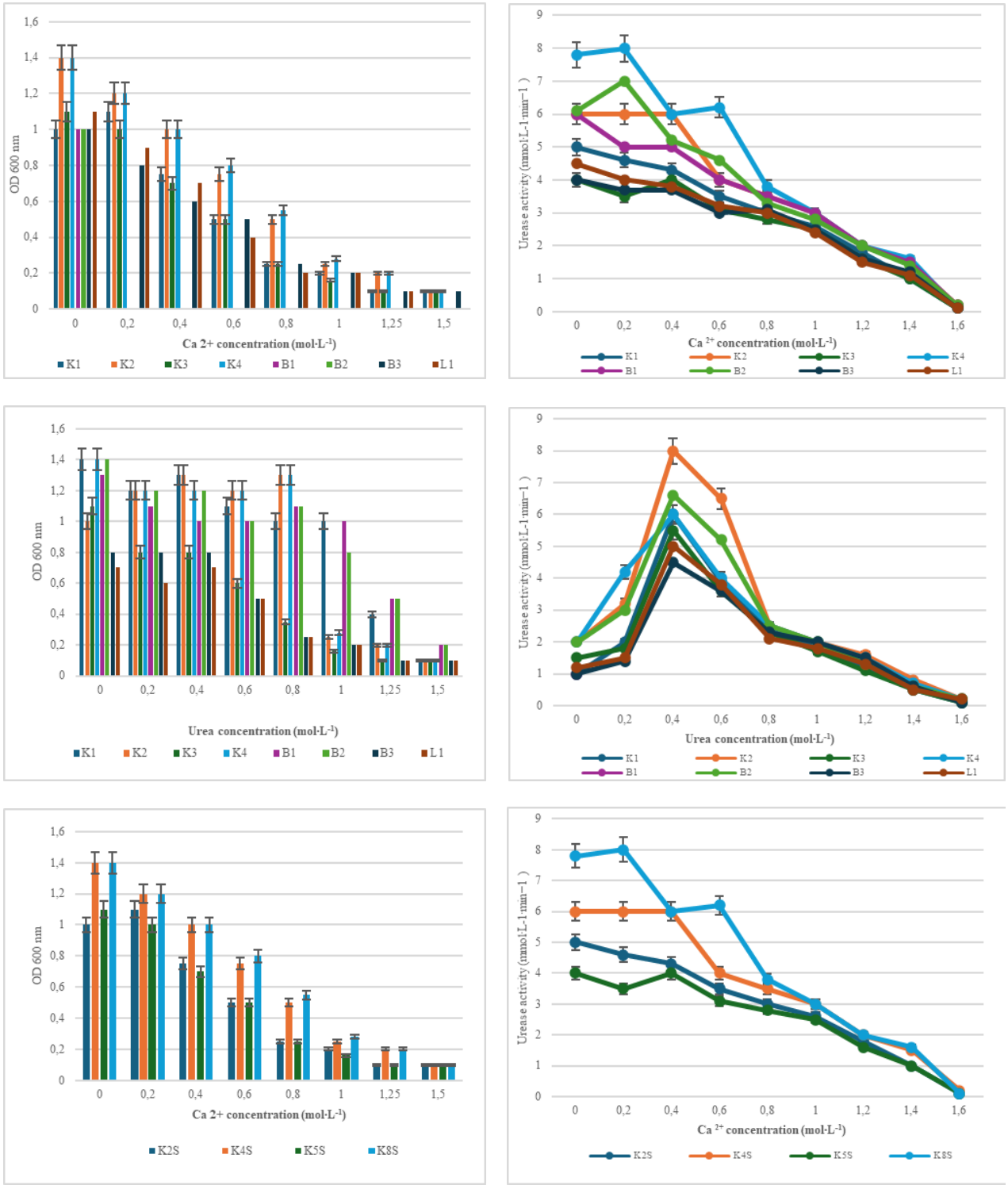
Overall, the results demonstrate that both the intensity and timing of microbial activity are critical factors governing MICP efficiency. Optimization of cultivation conditions, particularly within the 30–40 hour window, is essential for maximizing calcium carbonate deposition and improving the performance of MICP-based technologies in coal-related applications.

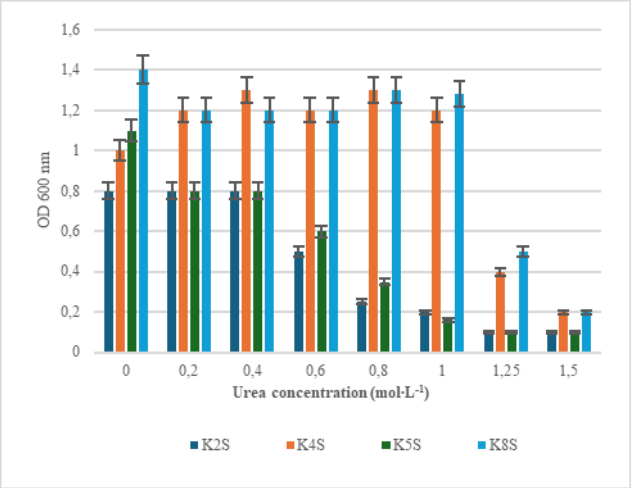
Influence of the concentration of substrate (urea) and calcium ions

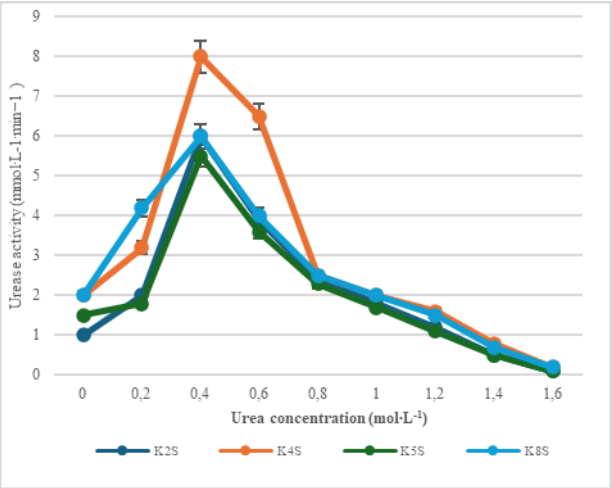
The effectiveness of the MICP process is determined not only by the activity of urease, but also by the availability of the substrate (urea) and calcium ions. With an increase in urea concentration, there is an increase in urease activity to a certain optimum, after which an inhibitory effect associated with the accumulation of ammonia is possible.

Similarly, an increase in the concentration of Ca^{2+} contributes to an increase in the yield of $CaCO_3$, however, at excessive concentrations, inhibition of cellular activity is possible. Thus, for each strain there is an optimal ratio of *"urease – urea – Ca^{2+}"*, which determines the maximum mineralization efficiency.

Figure 8
Effect of the concentration of substrate (urea) and calcium ions







The effect of substrate (urea) concentration on the physiological activity of urease-producing microorganisms is a key factor (Mugwar et al., 2016) determining the effectiveness of the microbially induced carbonate mineralization (MICP) process (Gupta et al., 2017).

As shown in Figure 8, an increase in urea concentration is accompanied by an increase in optical density (OD600), which indicates active proliferation of bacterial cells. At the same time, there is an increase in urease activity, which indicates an increase in the enzymatic hydrolysis of urea.

However, at high substrate concentrations (≥1.5–2.0 mol/L), there is a decrease in both bacterial growth and urease activity. This effect is probably related to the accumulation of ammonia (NH_3/NH_4^+) formed as a result of urea hydrolysis, which leads to inhibition of cellular activity and enzymatic systems. Thus, the optimal range of urea concentrations (0.5–1.0 mol/L) is revealed, at which the maximum efficiency of biocatalysis is achieved.

The next critical factor in the MICP process is the concentration of calcium ions involved in the formation of calcium carbonate ($CaCO_3$). An increase in the concentration of Ca^{2+} leads to an increase in the yield of carbonate sediment, which indicates an intensification of biomineralization processes (Muhammad et al., 2016). This is due to the increased availability of calcium cations necessary for the formation of the $CaCO_3$ crystal structure (Zhuang et al., 2018).

However, at excessively high concentrations of Ca^{2+}, there is a decrease in the efficiency of the process (Vijay et al., 2017). The probable cause is osmotic stress and the toxic effect of calcium ions on bacterial cells, which leads to a decrease in their metabolic activity. Therefore, there is an optimal range of Ca^{2+} concentrations (Al-Salloum et al., 2017), providing a balance between the deposition rate and the viability of microorganisms. Combining the data obtained, it can be concluded that the effectiveness of the MICP process is determined by the synergistic interaction of three key parameters: urease activity, urea concentration, and calcium ion content. The maximum degree of mineralization is achieved with the optimal ratio of these factors. Urease catalyzes the hydrolysis of urea to form carbonate ions (CO_3^{2-}), which in the presence of Ca^{2+} form a precipitate $CaCO_3$.

Conclusion

This study demonstrates that coal-derived microbial communities from the Kiyakty coal mine represent a promising source of urease-producing bacteria with significant potential for microbially induced carbonate precipitation (MICP). Comprehensive physicochemical characterization confirmed that the coal substrate possesses moderate metamorphism, relatively high volatile content, and a structurally defective amorphous carbon matrix, which together create favorable conditions for microbial colonization, biomodification, and mineralization processes.

A total of 34 bacterial isolates were obtained, among which 12 strains exhibited pronounced physiological and biochemical properties, including amylolytic activity, biofilm formation, oxidase positivity, and tolerance to a wide range of environmental conditions (pH 4–9, 25–50 °C, and 2–4% NaCl). These adaptive characteristics play a crucial role in ensuring microbial survival and functional

performance within structurally complex and heterogeneous coal matrices, thereby supporting their potential application in large-scale biotechnological systems.

Urease activity screening using microplate-based assays revealed considerable variability among the tested isolates. The most active strains (L1, B2, K4, and K8S) exhibited elevated enzymatic rates, reaching approximately 7–8 mmol·L^{-1}·min^{-1}, indicating a strong capacity for rapid urea hydrolysis and subsequent calcium carbonate formation. Analysis of growth dynamics and enzyme production demonstrated that peak biomineralization efficiency occurs during the exponential and early stationary growth phases (approximately 30–40 hours), underscoring the importance of precise process timing for optimizing MICP performance.

Environmental factors were found to exert a significant influence on both microbial activity and mineral precipitation. Optimal urease activity and cell growth were observed under neutral to mildly alkaline conditions (pH 7–9), which are favorable for carbonate ion generation. Likewise, moderate temperatures in the range of 25–35 °C supported mesophilic metabolism, although certain isolates displayed tolerance to elevated temperatures, broadening their potential application range. The study further identified optimal concentrations of urea (0.5–1.0 M) and calcium ions, while higher levels led to inhibitory effects, likely due to ammonia accumulation and increased osmotic stress, ultimately reducing both microbial activity and precipitation efficiency.

Notably, the combined assessment of microbial growth behavior, urease activity, and environmental resilience enabled the identification of the most promising candidates for MICP-based applications. In particular, the gram-negative isolates K8S and K2S demonstrated a favorable balance of high enzymatic activity and robustness under variable conditions, highlighting their suitability for applications such as biocementation and coal dust suppression.

Overall, the findings demonstrate that indigenous microbial communities associated with coal are capable of effectively inducing calcium carbonate precipitation and can contribute significantly to the stabilization and consolidation of coal substrates. The formation of almond carbonate promotes particle aggregation, reduces dust dispersion, and increases the mechanical stability of the coal surface. This highlights the potential of MICP as an environmentally friendly and sustainable approach for mitigating dust emissions and improving the geotechnical properties of coal-related materials. Future research should focus on scaling up the process, optimizing microbial consortia, and evaluating long-term performance under field conditions to enable practical implementation of MICP-based technologies in the coal industry.

Conflict of interest

All the authors have read and are familiar with the content of the article and have no conflict of interest.

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References

Achal, V., & Mukherjee, A. (2015). A review of microbial precipitation for sustainable construction. *Construction and Building Materials, 93*, 1224–1235. https://doi.org/10.1016/j.conbuildmat.2015.04.051

Achal, V., Mukherjee, A., & Zhang, Q. (2016). Unearthing ecological wisdom from natural habitats and its ramifications on development of biocement and sustainable cities. *Landscape and Urban Planning, 155*, 61–68. https://doi.org/10.1016/j.landurbplan.2016.04.013

Al-Salloum, Y., Hadi, S., Abbas, H., Almusallam, T., & Moslem, M. A. (2017). Bio-induction and bioremediation of cementitious composites using microbial mineral precipitation – A review. *Construction and Building Materials, 154*, 857–876. https://doi.org/10.1016/j.conbuildmat.2017.07.203

Aziz, Z. A. A., Mohd-Nasir, H., Ahmad, A., Mohd Setapar, S. H., Peng, W. L., Chuo, S. C., Khatoon, A., Umar, K., Yaqoob, A. A., & Mohamad Ibrahim, M. N. (2019). Role of nanotechnology for design and development of cosmeceutical: Application in makeup and skin care. *Frontiers in Chemistry, 7*, 739. https://doi.org/10.3389/fchem.2019.00739

Cappuccino, J. G., & Sherman, N. (2014). *Microbiology: A laboratory manual* (10th ed.). Pearson.

Dai, S., Zhao, L., Ward, C. R., French, D., Graham, I. T., Li, X., & Wang, X. (2012). Petrology, mineralogy, and geochemistry of the Ge-rich coal from the Wulantuga Ge ore deposit, Inner Mongolia, China: New data and genetic implications. *International Journal of Coal Geology, 90–91*, 72–99. https://doi.org/10.1016/j.coal.2011.11.002

Fan, Y. (2020). Urease producing microorganisms for coal dust suppression isolated from coal: Characterization and comparative study. *Advanced Powder Technology, 31*(9), 4095–4106. https://doi.org/10.1016/j.apt.2020.08.014

Fujita, M., Nakashima, K., Achal, V., & Kawasaki, S. (2017). Whole-cell evaluation of urease activity of *Pararhodobacter* sp. isolated from peripheral beachrock. *Biochemical Engineering Journal, 124*, 1–7. https://doi.org/10.1016/j.bej.2017.04.004

Gupta, S., Pang, S. D., & Kua, H. W. (2017). Autonomous healing in concrete by bio-based healing agents – A review. *Construction and Building Materials, 146*, 419–428. https://doi.org/10.1016/j.conbuildmat.2017.04.111

MacFaddin, J. F. (2000). *Biochemical tests for identification of medical bacteria* (3rd ed.). Lippincott Williams & Wilkins.

Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2018). *Brock biology of microorganisms* (15th ed.). Pearson.

Muhammad, N. Z., Shafaghat, A., Keyvanfar, A., Abd. Majid, M. Z., Ghoshal, S. K., Mohammadyan Yasouj, S. E., Ganiyu, A. A., Samadi Kouchaksaraei, M., Kamyab, H., Taheri, M. M., et al. (2016). Tests and methods of evaluating the self-healing efficiency of concrete: A review. *Construction and Building Materials, 112*, 1123–1132. https://doi.org/10.1016/j.conbuildmat.2016.03.017

Mugwar, A. J., & Harbottle, M. J. (2016). Toxicity effects on metal sequestration by microbially-induced carbonate precipitation. *Journal of Hazardous Materials, 314*, 237–248. https://doi.org/10.1016/j.jhazmat.2016.04.039

Ramachandran, A. L., Polat, P., Mukherjee, A., & Dhami, N. K. (2020). Understanding and creating biocementing beachrocks via biostimulation of indigenous microbial communities. *Applied Microbiology and Biotechnology, 104*, 3655–3673. https://doi.org/10.1007/s00253-020-10474-6

Seifan, M., Samani, A. K., & Berenjian, A. (2017). New insights into the role of pH and aeration in the bacterial production of calcium carbonate ($CaCO_3$). *Applied Microbiology and Biotechnology, 101*, 3131–3142. https://doi.org/10.1007/s00253-017-8109-8

Seifan, M., Sarabadani, Z., & Berenjian, A. (2020). Microbially induced calcium carbonate precipitation to design a new type of bio self-healing dental composite. *Applied Microbiology and Biotechnology, 104*, 2029–2037. https://doi.org/10.1007/s00253-019-10345-9

Sporosarcina pasteurii (Miquel 1889) Yoon et al. (2001). (n.d.). DSMZ–German Collection of Microorganisms and Cell Cultures. https://www.dsmz.de/collection/catalogue/details/culture/dsm-33

Vijay, K., Murmu, M., & Deo, S. V. (2017). Bacteria based self-healing concrete – A review. *Construction and Building Materials, 152*, 1008–1014. https://doi.org/10.1016/j.conbuildmat.2017.07.040

Wang, R. X. (2009). *Study on biomineralization process of calcium carbonate mediated by microbe and its application in restoration of cement-based materials defects* (Doctoral dissertation, Southeast University).

Wong, L. S. (2015). Microbial cementation of ureolytic bacteria from the genus *Bacillus*: A review of the bacterial application on cement-based materials for cleaner production. *Journal of Cleaner Production, 93*, 5–17. https://doi.org/10.1016/j.jclepro.2015.01.019

Yañez-Yazlle, M. F., Romano-Armada, N., Rajal, V. B., & Irazusta, V. P. (2021). Amelioration of saline stress on chia (*Salvia hispanica* L.) seedlings inoculated with halotolerant plant growth-promoting bacteria isolated from hypersaline environments. *Frontiers in Agronomy, 3*. https://doi.org/10.3389/fagro.2021.655978

Yaqoob, A. A., Ibrahim, M. N. M., Ahmad, A., & Reddy, A. V. B. (2021). Toxicology and environmental application of carbon nanocomposite. In M. Jawaid, A. Ahmad, N. Ismail, & M. Rafatullah (Eds.), *Environmental remediation through carbon based nano composites* (pp. 1–18). Springer. https://doi.org/10.1007/978-981-15-6699-8_1

Zhang, J., Xie, L., Huang, X., Liang, Z., Liu, B., Han, N., Xing, F., & Deng, X. (2019). Enhanced calcite precipitation for crack healing by bacteria isolated under low-nitrogen conditions. *Applied Microbiology and Biotechnology, 103*, 7971–7982. https://doi.org/10.1007/s00253-019-10066-z

Zhu, X., Wang, J., De Belie, N., & Boon, N. (2019). Complementing urea hydrolysis and nitrate reduction for improved microbially induced calcium carbonate precipitation. *Applied Microbiology and Biotechnology, 103*, 8825–8838. https://doi.org/10.1007/s00253-019-10128-2

Zúñiga-Barra, H., Ortega-Martínez, E., Toledo-Alarcón, J., Torres-Aravena, Á., Jorquera, L., Rivas, M., & Jeison, D. (2023). Potential use of microbially induced calcite precipitation for the biocementation of mine tailings. *Minerals, 13*, 506. https://doi.org/10.3390/min13040506

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