Eng. Sci., 2025, **36**, 1579

Engineered Science
DOI: https://dx.doi.org/10.30919/es1579

Microbiological Extraction of Copper and Zinc from Metallurgical Waste

Alfira Sabitova,[1] Asylan Ualikhanov,[1,*] Alexey Klivenko,[1] Kabysheva Zhanar,[1,*] Aitkaliyeva Gulzat[2] and Kassymova Zhanar[1]

Abstract

The accumulation of metal-containing waste, particularly lead and copper slags, presents a pressing environmental and economic issue in Kazakhstan. This study investigates bioleaching as a sustainable alternative to conventional extraction methods for recovering valuable metals from industrial waste. Metal-tolerant microorganisms, including *Acidithiobacillus ferrooxidans*, *Nitrosomonas europaea*, and micromycetes such as *Aspergillus niger* and *Aspergillus flavus*, were isolated and characterized to enhance bioleaching efficiency. Experiments using heap and tank leaching under controlled conditions examined key factors such as temperature, pH, particle size, and organic content. Bioleaching showed superior performance over sulfuric acid leaching, achieving recovery rates of 4.32 kg/t for copper and 14.91 kg/t for zinc. Optimal conditions were identified at +30 to +35 °C, pH 7–9, and particle sizes of 0.5–1.0 mm. Microscopic analysis confirmed that micromycetes contributed to metal dissolution by stabilizing pH and supporting microbial activity. The findings demonstrate that bioleaching is an effective, eco-friendly method that reduces the need for harsh chemicals while improving resource efficiency. The study supports further development of bioleaching technologies for industrial-scale applications.

Keywords: Bioleaching; Metal extraction; Industrial waste; Micromycetes; Sustainable extraction.

Received: 11 April 2025; Revised: 08 May 2025; Accepted: 20 May 2025.
Article type: Research article.

1. Introduction

The growing global demand for critical metals, coupled with the depletion of high-grade ore deposits, has intensified the search for sustainable and efficient metal recovery technologies. Among the most promising alternatives is bioleaching an environmentally friendly method that utilizes microorganisms to extract valuable metals from low-grade ores and industrial waste.[1,2] This technology has proven effective for processing a wide range of metallurgical by-products, including copper and lead slags, steel slags, and electronic waste, offering a viable alternative to conventional hydrometallurgical and pyrometallurgical methods.[3] Recent research has confirmed the potential of bioleaching for the recovery of metals such as copper, zinc, iron, chromium, and vanadium.[4] Classical bioleaching agents like *Acidithiobacillus ferrooxidans*, known for oxidizing iron and sulfur compounds, have been extensively studied for their role in enhancing metal solubilization from solid matrices.[5] Other acidophilic bacteria, such as *A. thiooxidans* and *A. ferridurans*, have shown success in the treatment of steel slags, often combined with selective precipitation techniques to optimize metal recovery. In more complex systems, mixed microbial consortia including *Leptospirillum spp.*, *Sulfobacillus spp.*, *Ferroplasma spp.*, and *Acidithiobacillus spp.* have achieved high recovery rates of copper, zinc, and even gold from pyritic tailings and non-ferrous slags.[6]

The present study builds on these advancements by focusing on the bioleaching of lead slag for the recovery of copper and zinc, introducing a broader microbial consortium that includes not only acidophilic bacteria but also nitrifying bacteria (*Nitrosomonas europaea*) and filamentous fungi (*Aspergillus niger* and *Aspergillus flavus*).[1,3,7,8] This represents a notable departure from previous studies that primarily relied on iron- and sulfur-oxidizing bacteria. The

---

[1] *Department of Chemistry and Ecology, Shakarim University, 20 A Glinka Street, Semey, 071412, Kazakhstan*
[2] *Satbayev University, 22 Satbayev str., Almaty, 050000, Kazakhstan*
**Email:* a.a.ualikhanov@gmail.com (A. Ualikhanov); rosekingroserei@gmail.com (K. Zhanar)

inclusion of *N. europaea*, typically associated with nitrogen cycling, adds a novel dimension to the process, as its metabolic by-products can influence pH dynamics and indirectly affect metal solubility. Likewise, the use of fungal strains is particularly significant due to their ability to secrete organic acids, facilitating metal dissolution even under neutral to mildly alkaline conditions.[9–13] While *Acidithiobacillus* species remain dominant in bioleaching literature, the combination of fungi and nitrifying bacteria explored in this study offers a unique and innovative approach. For instance, *A. ferrooxidans* is commonly employed in copper slag leaching, while *A. thiooxidans* and *A. ferridurans* are favored for their selectivity in treating steel slags. Studies on printed circuit boards (PCBs) also highlight the use of biogenic sulfuric acid and ferric iron for cost-effective metal recovery.[14–17] However, few investigations have examined the synergistic role of micromycetes, which, in this study, were found to stabilize pH and promote microbial activity during metal extraction.[18]

Another key distinction of the current study is its operational pH. Whereas most bioleaching studies report optimal metal recovery at highly acidic conditions (pH 1.8–2.5), the present research achieved superior results in the neutral to mildly alkaline range (pH 7–9). This atypical pH preference suggests that certain microbial consortia may function effectively in less corrosive environments, potentially reducing infrastructure costs and environmental risks.[17–25] By evaluating the bioleaching potential of an expanded microbial spectrum, this study advances current understanding of microbial metal recovery and its application to industrial waste treatment.[16,22–24] The inclusion of *Nitrosomonas europaea* and filamentous fungi underscores the importance of microbial diversity in optimizing metal recovery processes, particularly from complex matrices such as lead slag.[25–28] Future research should aim to scale this approach for industrial applications and explore its integration into circular economy frameworks for metal recycling.[29–31]

2. Materials and methods

2.1 Materials

Technological waste samples were collected from the former Shymkent Lead Plant in October 2024. Two distinct types of waste were identified: lead slag and copper slag. Prior to experimentation, the samples were manually ground using a porcelain mortar and sieved through a laboratory mesh with a cell size of 0.1 cm to obtain uniform particle sizes. Iron-oxidizing bacteria (*Acidithiobacillus ferrooxidans*), nitrifying bacteria (*Nitrosomonas europaea*), and micromycetes (*Aspergillus niger* and *Aspergillus flavus*) were isolated from the waste materials under laboratory conditions through incubation in selective nutrient media, as described in Section 2.2. All chemical reagents used in this study including ammonium sulfate ($(NH_4)_2SO_4$), potassium hydrophosphate (K_2HPO_4), magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), iron sulfate ($FeSO_4$), calcium carbonate ($CaCO_3$), sodium nitrate ($NaNO_3$), and potassium chloride (KCl) were of analytical grade and employed without further purification.

2.2 Methods

2.2.1 Microorganisms cultivation

To isolate and identify microorganisms suitable for the bioleaching process, selective nutrient media were employed to promote the targeted growth of different microbial groups. Incubation was performed at 25 °C for 7 days, with biomass growth monitored every 24 hours. Iron-oxidizing bacteria (*Acidithiobacillus ferrooxidans*) were cultivated in an acidic medium (pH 2.0) containing: $(NH_4)_2SO_4$ – 2.0 g/L, K_2HPO_4 – 1.0 g/L, $MgSO_4·7H_2O$ – 0.5 g/L, NaCl – 0.2 g/L, and $FeSO_4·7H_2O$ – 44.2 g/L. Nitrifying bacteria (*Nitrosomonas europaea*) were grown on Vinogradsky medium comprising glucose – 20.0 g/L, K_2HPO_4 – 1.0 g/L, $MgSO_4·7H_2O$ – 0.5 g/L, $CaCO_3$ – 20.0 g/L, yeast extract – 10.0 g/L, and a microelement solution – 1 mL. Micromycetes (*Aspergillus niger* and *Aspergillus flavus*) were cultivated on both liquid and solid Czapek media containing: sucrose – 30.0 g/L, $NaNO_3$ – 2.0 g/L, K_2HPO_4 – 1.0 g/L, $MgSO_4·7H_2O$ – 0.5 g/L, KCl – 0.5 g/L, and $FeSO_4·7H_2O$ – 44.2 mg/L. Fungal cultures were incubated at 28–30 °C for 5–7 days under aeration.

2.2.2 Polymerase chain reaction (PCR) analysis

Polymerase chain reaction (PCR) analysis was employed to determine the species composition of the isolated microorganisms. Genomic DNA was extracted using the PureLink™ Genomic DNA Kit (Invitrogen, Carlsbad, USA) and the GeneJET Genomic DNA Purification Kit. DNA concentration was measured with a Qubit® 2.0 fluorimeter using the Qubit™ dsDNA HS Assay Kit (Life Technologies, Oregon, USA). DNA quality was assessed via electrophoresis. Universal primers were used to amplify bacterial 16S rRNA, while ITS primers were used for fungal identification: ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTATTGATATGC-3′). Fig. 1 *Phylogenetic tree of* Aspergillus niger *strain AS1 based on ITS region sequences.* The tree was constructed using the Neighbor-Joining method. Reference sequences were obtained from GenBank and annotated with their accession numbers. The position of the isolated strain AS1 is marked with a black dot (●). The evolutionary relationships indicate high similarity between

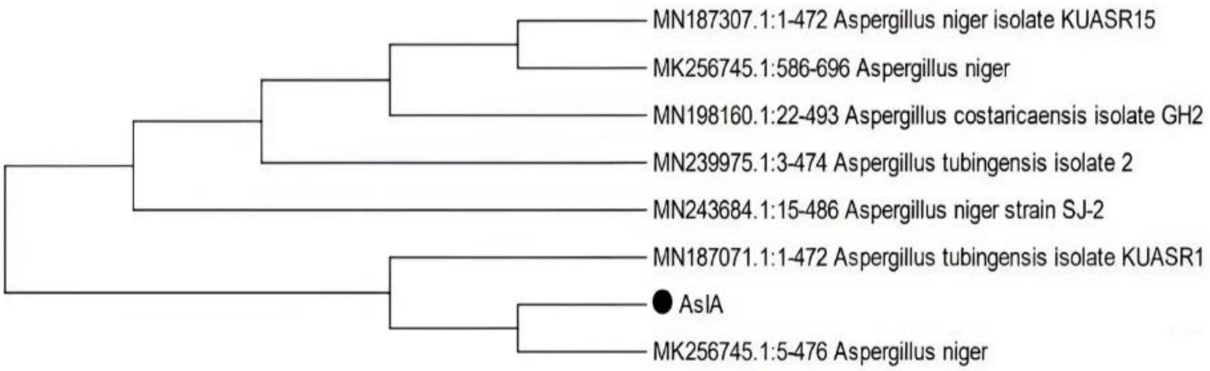

Fig. 1: Phylogenetic tree of Aspergillus niger AS1.

Fig. 2: Setups for bioleaching in laboratory conditions: A. Laboratory setup simulating heap leaching ; B. Laboratory setup simulating tank leaching.

AS1 and other *A. niger* and *Aspergillus* spp. strains, confirming its taxonomic identity.

2.2.3 Method of bioleaching

The bioleaching process was simulated using two approaches: heap bioleaching and tank bioleaching. Heap bioleaching was performed in 200 mL plastic percolators, where the leaching solution percolated through a stationary layer of solid material, simulating natural leaching conditions (Fig. 2A). In contrast, tank bioleaching was conducted in 250 mL glass conical flasks, maintaining a solid-to-liquid (S:L) ratio of 1:5 (Fig. 2B). Both experiments were carried out for 12 days at ambient temperatures ranging from 22 to 24 °C. The study utilized particles with a size fraction of 0.05–0.1 cm. For comparison, a control experiment using conventional sulfuric acid leaching (H_2SO_4 at 3 g/L) was included.

The study included four experimental variants: (1) bioleaching with *Acidithiobacillus ferrooxidans* cultures, (2) bioleaching with *Nitrosomonas europaea* cultures, (3) bioleaching using a consortium of micromycetes (*Aspergillus niger* and *Aspergillus flavus*), and (4) a control leaching experiment using sulfuric acid. The pH of the leachate was measured using a Hanna HI-2211 pH meter. Biomass growth was assessed via membrane filtration. The concentration of Fe^{2+} ions was determined by complexometric titration using disodium EDTA, with sulfosalicylic acid serving as the colorimetric indicator. Microbial counts were estimated by the serial dilution method.

2.2.4 Method of metal concentration control

Metal concentrations in the leachate were measured using the ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) method on an EXPEC 6500 spectrometer (FPI, China). The instrument was operated under the following conditions: pump speed – 50 rpm, observation mode – dual view, RF power – 1150 W, and analysis time – 15 seconds.

2.2.5 Statistical analysis of data

Statistical analysis was performed using Microsoft Excel. The arithmetic mean was calculated for each experimental group based on the number of replicates and the group as a whole. The standard deviation (ΔX) of the results was determined using the following Eq. (1):

$$\Delta X = \frac{t_{Pf} \cdot S}{\sqrt{n}} \qquad (1)$$

where t_{Pf} – coefficient, S - the absolute standard deviation of the measured value, calculated for a sample of n data points, n – number of samples.

3. Results and discussion

3.1 Identification of microorganisms promising for bioleaching

Microbiological analysis of technogenic waste from the lead smelter revealed the absence of *Acidithiobacillus ferrooxidans* in all surface samples, suggesting that oxidative microbial activity had ceased likely due to the neutral or slightly alkaline pH of the environment. However, aqueous waste samples contained Thiobacillus thiooxidans, and small quantities of T. thiocyanooxidans were detected, likely associated with the presence of organic carbon compounds such as xanthates and cyanides. In copper slag deposits, no microorganisms were identified in the upper layers (0 - 20 cm), but microbial populations were detected at depths of 20 - 60 cm, with concentrations ranging from 10 to 10^2 cells/g. These subsurface isolates were found to be adapted to low temperatures (5 - 15 °C) and high concentrations of molybdenum ions (>13.0 mg/L). Additionally, thiobacteria closely related to Thiobacillus thiooxidans and Acidithiobacillus ferrooxidans were isolated from nearby mine waters. In total, 56 isolates of autotrophic microorganisms and 42 isolates of micromycetes with potential bioleaching activity were successfully cultured from the technogenic waste samples.

3.2 Screening and selection of active strains

As a result of screening for growth on waste-containing media, the following promising strains were identified: *Acidithiobacillus ferrooxidans* ShU1, a Gram-negative, rod-shaped bacterium (0.3–0.4 × 0.7–1.7 μm), exhibits motility via a polar flagellum. Its optimal growth occurs at pH 0.5–9.0 and 28–35 °C. This strain oxidizes Fe^{2+}, elemental sulfur, and sulfur compounds. SEM images of the samples are presented on Fig. 3.

Fig. 3 presents scanning electron microscopy (SEM) images of *Acidithiobacillus ferrooxidans* strain ShU1 at different magnifications. Image A (left) shows a densely populated microbial community at 5,500× magnification, while Image B (right) provides a high-resolution view of a single bacterium at 30,000× magnification. In both images, multiple rod-shaped cells are observed, consistent with the known morphology of *A. ferrooxidans*, a Gram-negative, acidophilic, chemolithoautotrophic bacterium commonly involved in bioleaching and iron/sulfur oxidation processes.[32,33] The cells exhibit a length of approximately 1.36–1.38 μm and a diameter of 0.45–0.48 μm, which aligns well with previously reported dimensions of *A. ferrooxidans*, typically ranging from 1.0–2.5 μm in length and 0.3–0.6 μm in width.[34] The differences in scale and morphology between images A and B highlight the dynamic structural features of *A. ferrooxidans* in varying environmental or growth conditions. In the context of bioleaching, such structural traits are critical, as they influence microbial adhesion to mineral surfaces, colonization efficiency, and metal solubilization performance.[35] These SEM images confirm the successful isolation and characterization of *A. ferrooxidans* ShU1 from technogenic environments, with morphological attributes suitable for metal bioleaching applications. The rod-like form, size consistency, and smooth cell surface morphology reflect well-documented traits of this species and support its role in biohydrometallurgical processes.

Fig. 4 presents microscopic and SEM images of the filamentous micromycete *Aspergillus niger* strain AS1, highlighting its morphological structures and surface characteristics. Image A (left) is a light microscopy image captured at 250× magnification. It clearly shows typical features of *A. niger*, including long, septate hyphae and darkly pigmented, globular conidial heads formed on conidiophores. The branching conidiophores with radiating phialides terminating in chains of dark conidia (asexual spores) are characteristic of this species.[36] The bluish coloration may result from staining (*e.g.*, lactophenol cotton blue) commonly used for fungal visualization, enhancing contrast between the hyphae and conidial structures. Image B (right) shows a SEM image at 200× magnification, revealing the ultrastructural surface details of *A. niger* colonies. The hyphal structures are visible as tubular filaments, while conidial heads appear as densely packed, globular aggregates with rough surface textures. These surface features are consistent with the formation of mature conidia and indicate the organism's ability to form spores under solid-state culture conditions. SEM analysis also confirms robust hyphal development and sporulation, both essential traits for bioleaching efficiency due to high metabolic activity and secretion of organic acids. The ability of *Aspergillus niger* to form extensive mycelial networks and produce significant quantities of organic acids (especially citric, oxalic, and gluconic acids) makes it particularly attractive for biohydrometallurgical applications, including bioleaching of heavy metals from industrial wasteand slag.[37] These acids play a key role in metalsolubilization by chelating metal ions or lowering the pH of the environment, thereby enhancing leaching kinetics. The

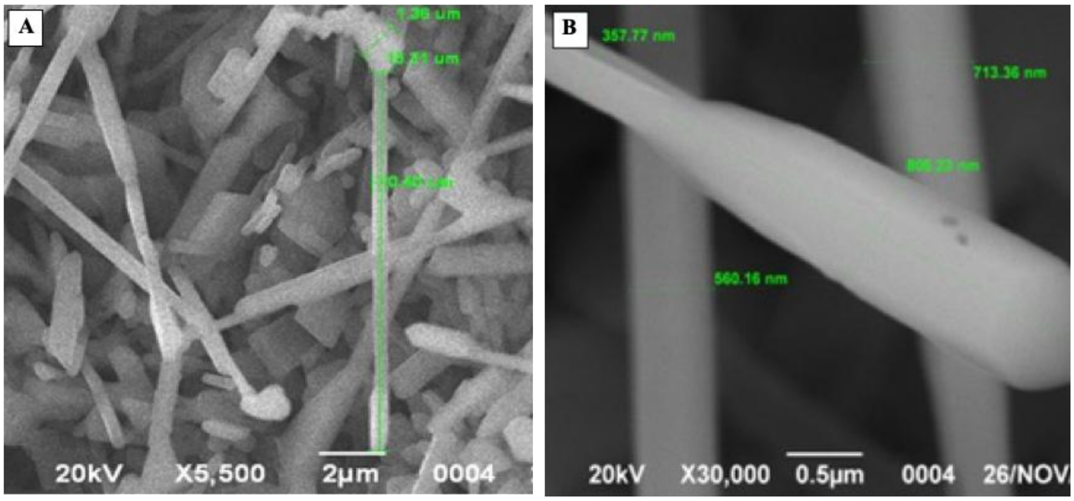

Fig 3: SEM images of *Acidithiobacillus ferrooxidans* ShU1.

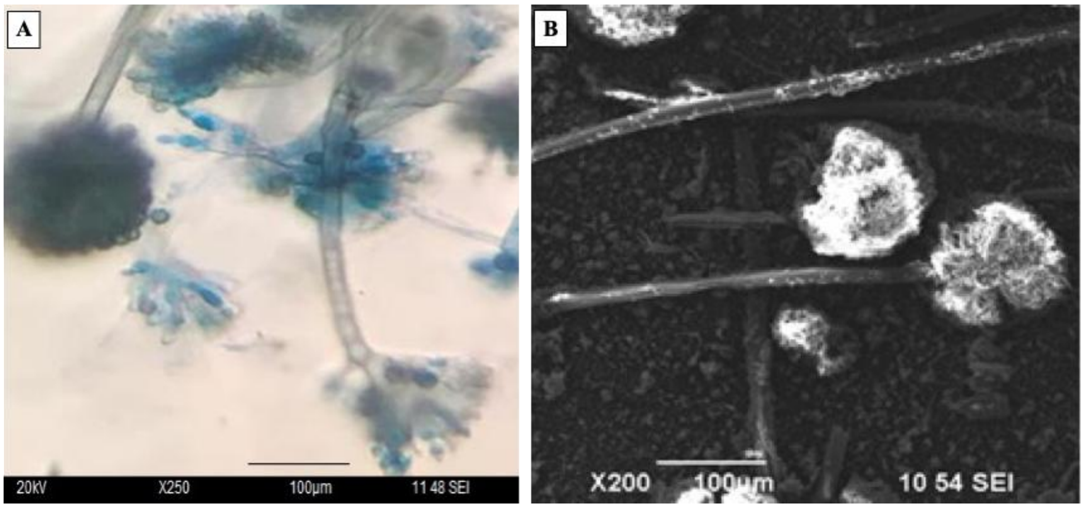

Fig. 4: Microscopic images of the micromycete *Aspergillus niger* AS1.

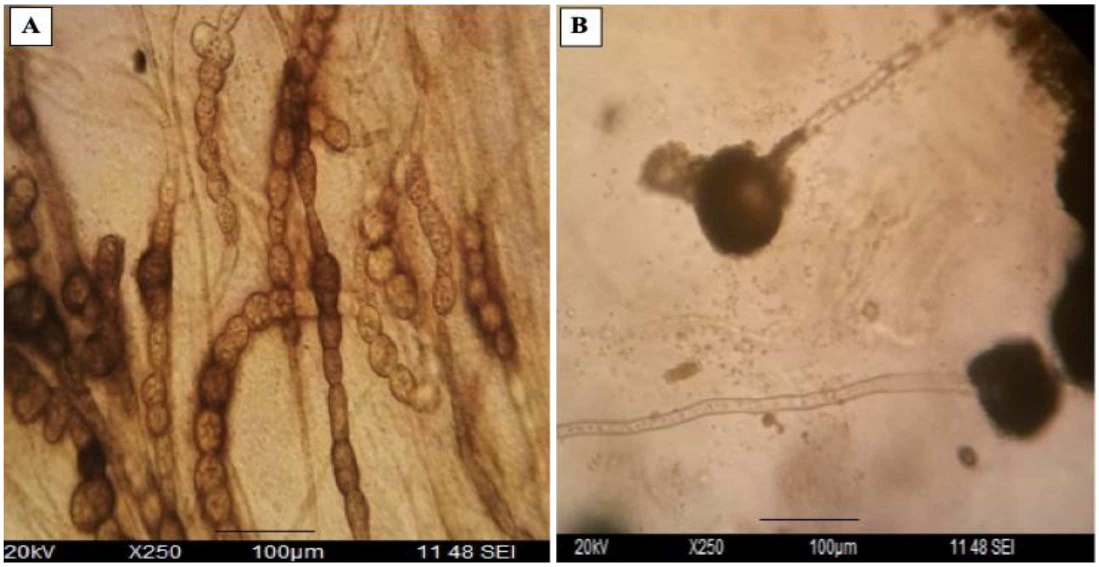

Fig. 5: General appearance of colonies and conidia of *Aspergillus flavus* AISK.

distinct morphological integrity and sporulation observed in *A. niger* AS1 suggest that this strain is well-suited for use in bioleaching processes under variable environmental conditions. Its filamentous growth form enables extensive colonization of solid substrates, while its resistance to metal toxicity allows it to survive in metal-rich waste environments.[38]

Fig. 5 presents light microscopic images of the filamentous fungus *Aspergillus flavus* strain AISK, captured at 250× magnification. The figure illustrates key morphological features characteristic of the species, including hyphal networks, conidiophores, and chains of conidia, essential for fungal identification and biofunctional assessments. Fig. 5A displays elongated, septate hyphae with branching conidiophores terminating in chains of conidia. The conidia are globose to subglobose in shape, arranged in loose, dry chains, and vary in pigmentation from light to dark brown. These are consistent with descriptions of *A. flavus*, whose conidia typically measure 3.5 - 5.0 µm in diameter and exhibit echinulate (roughened) surfaces under higher magnification.[39] The distinct separation between spore chains and hyphal structures supports the species' well-known ability for prolific asexual sporulation. Morphological integrity in sporulation is an important indicator of fungal viability and potential for environmental colonization, including in bioleaching systems. Fig. 5B shows a broader view of the fungal colony structure, with visible vesicles at the tips of conidiophores where conidial formation initiates. Spherical terminal vesicles, a hallmark of *A. flavus*, support biseriate conidial development involving both metulae and phialides. The image also shows developing conidial heads, loosely radiating from the vesicle another taxonomic trait differentiating *A. flavus* from close relatives like *A. niger* or *A. oryzae*. Aerial hyphae in the image appear transparent and thin-walled, likely representing younger mycelium. While it is infamous for aflatoxin production in food spoilage,[40-42] non-toxigenic strains such as AISK are increasingly studied for their ability to participate in bioleaching and biosorption processes. *A. flavus* is known to produce a range of organic acids such as oxalic, gluconic, and citric acids that facilitate the solubilization of heavy metals.[43,44] Its robust sporulation and resistance to extreme conditions, including high metal concentrations, make it a promising candidate for mycogenic metal recovery from industrial slags, mine tailings, and contaminated soils.

3.3 The influence of abiotic and biotic factors on the efficiency of bioleaching

The waste leaching solutions had a pH of 7–9, which could inhibit microbial growth and activity. To evaluate the influence of different factors on bioleaching efficiency, the following conditions were examined: temperature, light exposure, and organic compound concentration.

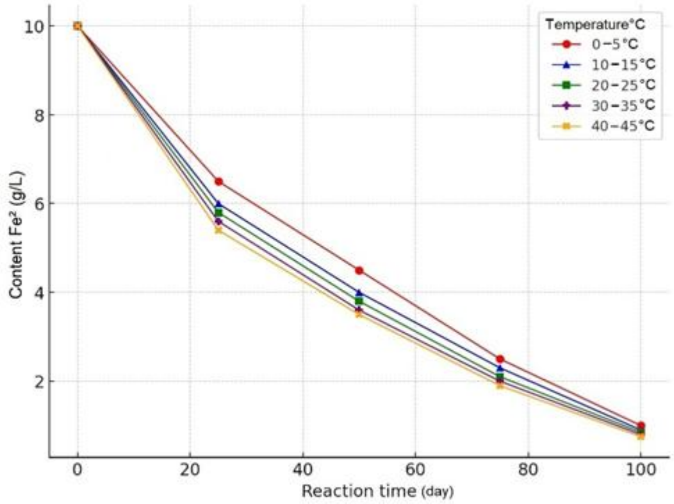

Fig. 6: Effect of temperature conditions on the rate of oxidation of divalent iron by a composition of thiobacteria.

3.3.1 Temperature conditions

Temperature is a key factor for bioleaching processes. As can be seen from Fig. 6 at 30-35°C the oxidation rate of Fe^{2+} reached 8.9 ± 0.2 g/L per day. When at 15°C the process speed decreased by 2 times. However, at 5.5°C, growth inhibition was observed by 9 times.

Fig. 6 illustrates the effect of varying temperature conditions on the oxidation rate of ferrous iron (Fe^{2+}) by a microbial consortium of thiobacteria over a 100-day reaction period. The data demonstrate a clear trend of decreasing Fe^{2+} concentration over time across all temperature conditions, indicating ongoing biological oxidation activity. The initial concentration of Fe^{2+} was approximately 10 g/L in all variants. The most rapid decrease in Fe^{2+} was observed at optimal temperature ranges of 30 – 35 °C and 40 – 45 °C, where Fe^{2+} levels dropped to below 1 g/L by day 100. In contrast, lower temperatures (0 – 5 °C and 10 – 15 °C) showed a slower rate of iron oxidation, with residual Fe^{2+} concentrations remaining higher throughout the experiment. This confirms that temperature is a critical factor influencing the metabolic activity of iron-oxidizing bacteria. The data suggest that microbial oxidation of Fe^{2+} is most efficient within the 30 – 45 °C range, consistent with earlier studies indicating that *A. ferrooxidans* thrives optimally around 35 °C.[45] This increased efficiency may be attributed to enhanced enzymatic activity and cell growth under warmer conditions, facilitating faster electron transfer and biooxidation kinetics.[46,47] This result has significant implications for bioleaching and bioremediation strategies involving iron-oxidizing consortia, especially in climates with variable seasonal temperatures. Operating within the identified optimal range can maximize Fe^{2+} oxidation and thus enhance metal recovery processes or environmental detoxification involving iron cycling.

3.3.2 The influence of lighting

Lighting is a crucial factor in bacterial growth, influencing their metabolism, reproduction, and survival. Typically most bacteria thrive in darkness.[25] Certain species respond to light exposure differently. For example, phototrophic bacteria, such as cyanobacteria, use light for energy through photosynthesis, while excessive exposure to UV or blue light can inhibit growth or even cause DNA damage in non-phototrophic bacteria. In our case (Fig. 7) direct sunlight caused inhibition of bacterial activity due to ultraviolet radiation. Artificial lighting had no noticeable effect. The same result was observed.[28] Protecting bacterial cultures from UV exposure and using controlled lighting conditions can prevent potential bacterial inhibition and maintain leaching efficiency.

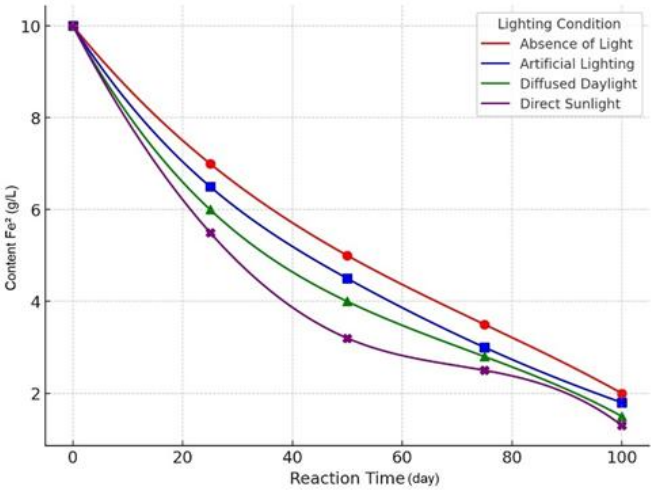

Fig. 7: Effect of light source on the dynamics of biological oxidation of divalent iron by a composition of thiobacteria.

3.3.3 The influence of organic impurities

As can be seen from Fig. 8 increasing the organic concentration from 0.1% to 10% slowed down the bioleaching process. Organic impurities can significantly affect bacterial activity by serving as nutrient sources or inhibitors, depending on their composition and concentration. Readily biodegradable organic compounds, such as sugars and amino acids, can enhance bacterial growth and metabolism, promoting biofilm formation and enzymatic activity. Conversely, complex or toxic organic pollutants, such as hydrocarbons or phenolic compounds, may inhibit bacterial functions or require specialized microbial communities for degradation. The presence of organic impurities can also influence microbial diversity and interactions, impacting overall ecosystem stability and bioremediation processes.[25] As can be seen from Fig. 8 some organic compounds can serve as energy sources for heterotrophic bacteria, potentially enhancing bioleaching under certain conditions. However, excess organic matter can lead to unwanted microbial competition, which can negatively impact the process.

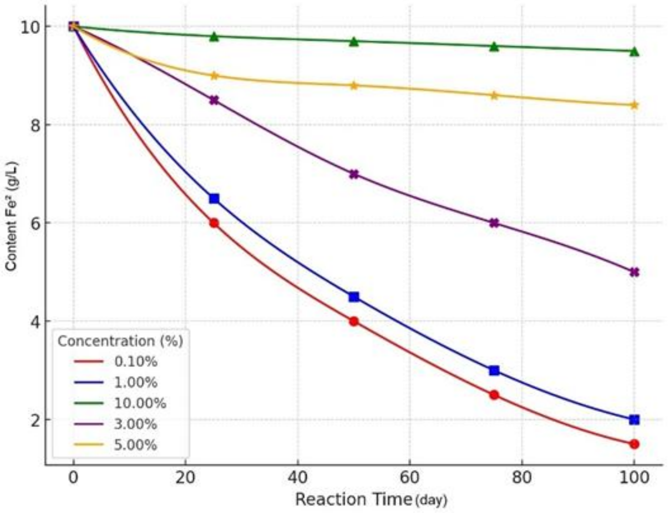

Fig. 8: Effect of organic impurities on the rate of oxidation of divalent iron by a composition of thiobacteria.

3.3.4 Fractional composition of waste

The optimal particle size is 0.5–1.0 mm (ensures maximum extraction of metals). Particles <0.5 mm – reduced leaching efficiency due to poor diffusion of solutions. Further studies confirm that ultrafine particles can lead to passivation effects due to silica and jarosite precipitation, which further reduces leaching efficiency. Controlled milling and optimization of particle size distribution are key to maximizing metal recovery.

3.3.5 Comparison of bacterial-chemical and sulfuric acid leaching

The results showed that bioleaching using thiobacteria demonstrated higher efficiency of copper and zinc extraction compared to the sulfuric acid method. The average metal extraction rates for each method are shown in Table 1.

Data analysis shows that bioleaching provides 24% more copper and 34% more zinc than sulfuric acid leaching (Fig. 9). This method allows for more efficient processing of metal-containing waste, increasing the degree of its utilization and reducing the use of aggressive chemical reagents. Recent studies have also demonstrated successful bioleaching of nickel, cobalt, and rare earth elements from low-grade ores and waste. The integration of bioleaching with hydrometallurgical methods such as iron leaching has shown promising results for recovering valuable metals from complex waste streams.[15,16,18] Expanding the application of bioleaching to a wider range of industrial wastes can make a significant contribution to sustainable metal recovery, reducing environmental pollution and minimising reliance on traditional mining methods.

4. Conclusion

The conducted studies confirmed the high efficiency of bioleaching for extracting copper (4.32 kg/t) and zinc (14.91 kg/t) from lead smelting waste, surpassing conventional acid leaching by 33% and 26%, respectively. It is important to emphasize that the high process efficiency at pH 7–9 was primarily ensured by the activity of micromycetes (Aspergillus niger and A. flavus), as evidenced by fungal mycelium development and the secretion of organic acids (citrates, oxalates) that stabilized the pH of the medium. The participation of Acidithiobacillus ferrooxidans under such alkaline conditions requires further investigation, though their contribution during initial process stages or in local microzones with reduced pH cannot be ruled out. The process exhibited pronounced temperature dependence: decreasing the temperature to +15 °C reduced efficiency by half, while at +5.5 °C, a ninefold decline in productivity was observed. The introduction of micromycetes stabilized the process in the presence of organic impurities due to the buffering properties of fungal metabolites and reduced medium toxicity, as confirmed by the development of associated microflora (*Candida spp.*). The obtained results demonstrate the promise of a combined microbial consortium for processing industrial waste in a neutral-to-alkaline pH range. Further research should focus on: A detailed study of the individual contributions of consortium components, Process parameter optimization for industrial scaling, Assessing the long-term stability of the system. The developed approach significantly reduces the use of aggressive chemical reagents and offers an environmentally safe alternative for the disposal of metal-containing waste.

Table 1: Analysis of metal content in waste.

Leaching method	Cu recovery (kg/t)	Zn recovery (kg/t)
Bioleaching (heap)	4.32 ±0.15	14.91 ±0.15
Bioleaching (vat)	3.11 ±0.15	12.60 ±0.15
Sulfuric acid leaching (heap)	3.24 ±0.15	11.81 ±0.15
Sulfuric acid leaching (vat)	2.73 ±0.15	6.37 ±0.15

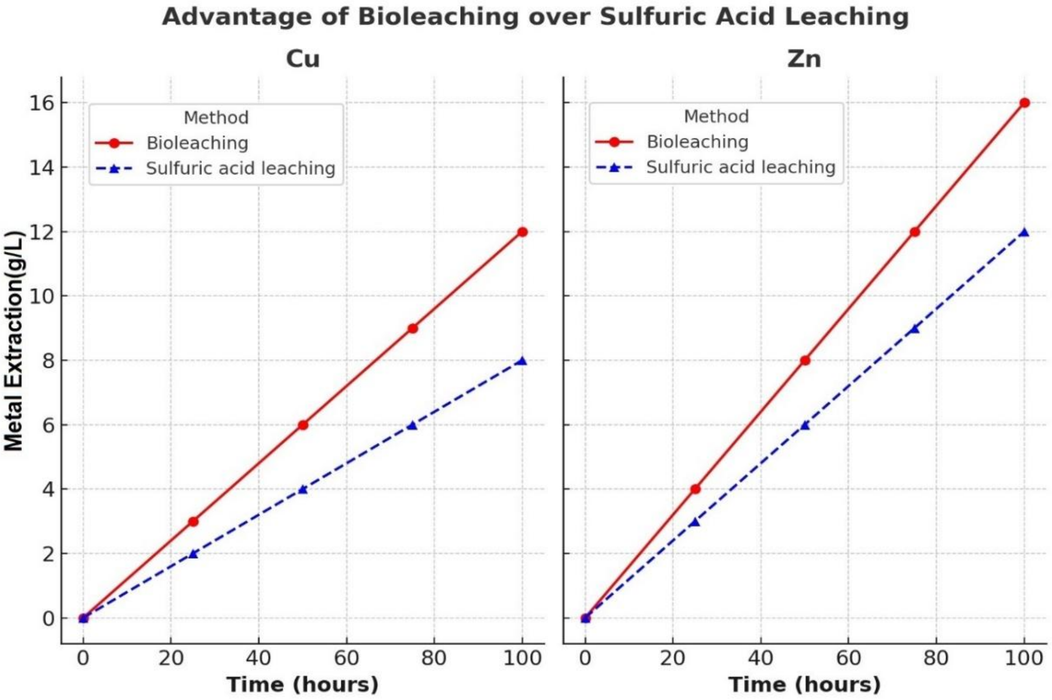

Fig. 9: Advantage of bioleaching over sulfur acid leaching.

Acknowledgments

This research is funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. BR24993178 Development of innovative technology for processing secondary resources - waste from metallurgical production, by-products of oil refining and oil production).

Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

References

[1] B. Mikoda, A. Potysz, E. Kmiecik, Bacterial leaching of critical metal values from Polish copper metallurgical slags using Acidithiobacillus thiooxidans, *Journal of Environmental Management*, 2019, **236**, 436-445, doi: 10.1016/j.jenvman.2019.02.032.

[2] A. Ghavidel, S. Naji Rad, H. Ali Alikhani, M. Sharari, A. Ghanbari, Bioleaching of heavy metals from sewage sludge, direct action of Acidithiobacillus ferrooxidans or only the impact of pH? *Journal of Material Cycles and Waste Management*, 2018, **20**, 1179-1187, doi: 10.1007/s10163-017-0680-7.

[3] M. Kunarbekova, Y. Yeszhan, S. Zharylkan, M. Alipuly, U. Zhantikeyev, A. Beisebayeva, K. Kudaibergenov, K. Rysbekov, Z. Toktarbay, S. Azat, The state of the art of the mining and metallurgical industry in Kazakhstan and future perspectives: a systematic review, *ES Materials & Manufacturing*, 2024, **25**, 1219, doi: 10.30919/esmm1219.

[4] S. Feng, Q. Li, Z. Huang, Y. Tong, H. Yang, Specific mechanism of extracellular polymeric substances from Acidithiobacillus caldus in bioleaching of copper-bearing sulfide ore, *Extremophiles*, 2020, **22**, 851-863, doi: 10.1007/s00792-018-1042-7.

[5] L. Castro, H. Gomez-Alvarez, M. Carmona, F. Gonzalez, J. A. Muñoz, Effect of biosurfactants on REE extraction from monazite using Burkholderia thailandensis, *Hydrometallurgy*, 2023, **222**, 106-178, doi: 10.1016/j.hydromet.2023.106178.

[6] S. Yin, L. Wang, E. Kabwe, H. Chen, R. Yang, Q. An, A. Wu, Copper bioleaching in China: A review and prospects, *Minerals*, 2018, **8**, 32, doi: 10.3390/min8020032.

[7] K. Akimzhanova, A. Sabitova, B. Mussabayeva, Z. Kairbekov, B. Bayakhmetova, J. Proch, Chemical composition and physicochemical properties of natural therapeutic mud of Kazakhstan salt lakes: a review, *Environmental Geochemistry and Health*, 2024, **46**, 43, doi: 10.1007/s10653-023-01813-3.

[8] P. Kinnunen, J. Mäkinen, M. Salo, R. Soth, K. Komnitsas, Efficiency of chemical and biological leaching of copper slag for the recovery of metals and valorisation of the leach residue as raw material in cement production, *Minerals*, 2020, **10**, 654, doi: 10.3390/min10080654.

[9] A. Zhubanova, N. Akimbekov, K. Tastambek, G. Abdieva, P. Ualieva, A. Malik, N. Altynbay, X. Qiao, Whey to ethanol: unlocking the potential of cheese whey for sustainable bioethanol production, *Engineered Science*, 2023, **27**, 998, doi: 10.30919/es998.

[10] H. I. Gomes, V. Funari, W. M. Mayes, M. Rogerson, T. J. Prior, Recovery of Al, Cr and V from steel slag by bioleaching: Batch and column experiments, *Journal of Environmental Management*, 2018, **222**, 30-36, doi: 10.1016/j.jenvman.2018.05.056.

[11] C. Zhang, B. Hu, H. Wang, M. Wang, X. Wang, Recovery of valuable metals from copper slag, *Mining, Metallurgy & Exploration*, 2020, **37**, 1241-1251, doi: 10.1007/s42461-020-00224-7.

[12] A. Ydyrys, G. Askerbay, Z. Ashirova, J. Jielile, M. Ilesbek, Green synthesis of silver nanoparticles using plant leaf extraction andphytochemical profile and biological potential of Artemisia schrenkiana, *International Journal of Biology and Chemistry*, 2024, **17**, 167-178, doi: 10.26577/ijbch2024v17.i2.14.

[13] K. Akimzhanova, A. Sabitova, B. Kh, Mussabayeva, B. Bayakhmetova, Inorganic composition and physico-chemical properties of the peloid of the salt lake moiyldy (Kazakhstan) As a natural source of biologically active substances, *Chemical Engineering Transactions*, 2023, **103**, 433-438, doi: 10.3303/CET23103073.

[14] N. Fomchenko, M. Muravyov, Sequential bioleaching of pyritic tailings and ferric leaching of nonferrous slags as a method for metal recovery from mining and metallurgical wastes, *Minerals*, 2020, **10**, 1097, doi: 10.3390/min10121097.

[15] K. Kremser, S. Thallner, S. Spiess, J. Kucera, T. Vaculovic, D. Všianský, M. Haberbauer, G. M. Guebitz, Bioleaching and selective precipitation for metal recovery from basic oxygen furnace slag, *Processes*, 2022, **10**, 576, doi: 10.3390/pr10030576.

[16] D. Rouchalova, K. Rouchalova, I. Janakova, V. Cablik, S. Janstova, Bioleaching of iron, copper, lead, and zinc from the sludge mining sediment at different particle sizes, pH, and pulp density using acidithiobacillus ferrooxidans, *Minerals*, 2020, **10**, 1013, doi: 10.3390/min10111013.

[17] N. Nurgaliyev, A. Akimzhanov, A. Klivenko, A. Sabitova, K. Astemessova, G. Turlybekova, S. Nyssanbayeva, R. Kurmangaliyev, Mathematical model of biomethane yield at anaerobic methane fermentation of organic wastes: influence of various parameters and factors, *ES Energy & Environment*, 2024, **26**, 1280, doi: 10.30919/esee1280.

[18] H. K. Park, J. Y. Lee, S. W. Cho, J. S. Kim, Overview on the

technologies for extraction of rare earth metals, *Journal of the Korean Institute of Resources Recycling*, 2012, **21**, 74-83, doi: 10.7844/kirr.2012.21.3.074.

[19] A. Jordens, Y. P. Cheng, K. E. Waters, A review of the beneficiation of rare earth element bearing minerals, *Minerals Engineering*, 2013, **41**, 97-114, doi: 10.1016/j.mineng.2012.10.017.

[20] P. S. Ualieva, G. Z. Abdieva, N. S. Akimbekov, A. M. Malik, K. T. Tastambek, Construction and characterisation of a novel microbial consortium for animal feed enrichment, *ES Food & Agroforestry*, 2024, **17**, 1243, 10.30919/esfaf1243.

[21] K. Z. Abdiyev, M. B. Zhursumbayeva, N. Z. Seitkaliyeva, G. K. Kussainova, M. N. Mohamad Ibrahim, D. N. Shakhmetova, M. Y. Yermaganbetov, Z. Toktarbay, Synthesis and characterization of N, N-dimethylacrylamide and [(3-methacryloyl-amino)propyl] trimethylammonium chloride copolymers: kinetics, reactivity, and biocidal properties, *Engineered Science*, 2024, **30**, 1217, doi: 10.30919/es1217.

[22] T. Naseri, V. Beiki, S. M. Mousavi, S. Farnaud, A comprehensive review of bioleaching optimization by statistical approaches: recycling mechanisms, factors affecting, challenges, and sustainability, *RSC Advances*, 2023, **13**, 23570-23589, doi: 10.1039/d3ra03498d.

[23] A. Sabitova, K. Akimzhanova, B. Mussabayeva, B. Bayakhmetova, O. Lazzyat, K. Alexey, N. Nurzhan, E. Yermoldina, Physico-chemical characteristics natural mud of salt lakes of north-east Kazakhstan, *Engineered Science*, 2023, **25**, 930, doi: 10.30919/es930.

[24] A. H. Kaksonen, A. M. Lakaniemi, O. H. Tuovinen, Bioleaching of uranium ores with acid and ferrous sulfate: a review, *Journal of Cleaner Production*, 2020, **264**, 121586, doi: 10.1016/j.jclepro.2020.121586.

[25] L. Castro, M. L. Blázquez, F. González, J. A. Muñoz, Bioleaching of phosphate minerals using aspergillus Niger: recovery of copper and rare earth elements, *Metals*, 2020, **10**, 978, doi: 10.3390/met10070978.

[26] A. H. Kaksonen, X. Deng, T. Bohu, L. Zea, H. N. Khaleque, Y. Gumulya, N. J. Boxall, C. Morris, K. Y. Cheng, Prospective directions for biohydrometallurgy, *Hydrometallurgy*, 2020, **195**, 105376, doi: 10.1016/j.hydromet.2020.105376.

[27] A. Issayeva, Influence of different factors on the process of biological decontamination of pyrophoric iron, *International Journal of Education and Research*, 2014, **2**, 281-286.

[28] K. K. Keekan, J. C. Jalondhara, Abhilash, Extraction of Ce and Th from monazite using REE tolerant *Aspergillus niger*, *Mineral Processing and Extractive Metallurgy Review*, 2017, **38**, 312-320, doi: 10.1080/08827508.2017.1350956.

[29] M. Vera, A. Schippers, S. Hedrich, W. Sand, Progress in bioleaching: fundamentals and mechanisms of microbial metal sulfide oxidation - part A, *Applied Microbiology and Biotechnology*, 2022, **106**, 6933-6952, doi: 10.1007/s00253-022-12168-7.

[30] A. Issayeva, M. Syzdykova, A. Akhmet, Z. Bakhov, Z. Ospanova, B. Chingisbayev, M. Kamalova, S. Karimova, Use of *Acidithiobacillus ferrooxidans* for decontamination of explosive waste from oil refineries, *Journal of Ecological Engineering*, 2023, **24**, 19-24, doi: 10.12911/22998993/163250.

[31] A. Bachar, A. Mabrouk, R. Amrousse, S. Azat, C. Follet, C. Mercier, F. Bouchart, Properties, bioactivity and viability of the new generation of oxyfluoronitride bioglasses, *Eurasian Chemico-Technological Journal*, 2024, **26**, 43-52, doi: 10.18321/ectj1565.

[32] P. Caputo, P. Calandra, V. Loise, M. Porto, A. Le Pera, A. A. Abe, B. Teltayev, M. L. Luprano, M. Alfè, V. Gargiulo, G. Ruoppolo, C. Oliviero Rossi, Physical chemistry supports circular economy: toward a viable use of products from the pyrolysis of a refuse-derived fuel and granulated scrap tire rubber as bitumen additives, *Eurasian Chemico-Technological Journal*, 2023, **25**, 173-181, doi: 10.18321/ectj1520.

[33] K. Kabdulkarimova, S. Seilgazina, N. Omarova, Z. Kassymova, L. Ibraeva, M. Kaygusuz, Chemical analysis of filamentous algae of the Semipalaninsk Region by inductively coupled plasma mass spectrometry, *Fresenius Environmental Bulletin*, 2018, **27**, 5975-5979.

[34] D. E. Rawlings, Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates, *Microbial Cell Factories*, 2005, **4**, 13, doi: 10.1186/1475-2859-4-13.

[35] T. Rohwerder, T. Gehrke, K. Kinzler, W. Sand, Bioleaching review part A, *Applied Microbiology and Biotechnology*, 2003, **63**, 239-248, doi: 10.1007/s00253-003-1448-7.

[36] K. B. Hallberg, D. Barrie Johnson, Biodiversity of acidophilic prokaryotes, *Advances in Applied Microbiology*, Amsterdam: Elsevier, 2001, 37-84, doi: 10.1016/s0065-2164(01)49009-5.

[37] M. Vera, A. Schippers, S. Hedrich, W. Sand, Progress in bioleaching: fundamentals and mechanisms of microbial metal sulfide oxidation - part A, *Applied Microbiology and Biotechnology*, 2022, **106**, 6933-6952, doi: 10.1007/s00253-022-12168-7.

[38] E. Demirci, M. Arentshorst, B. Yilmaz, A. Swinkels, I. D. Reid, J. Visser, A. Tsang, A. F. J. Ram, Genetic characterization of mutations related to conidiophore stalk length development in *Aspergillus niger* laboratory strain N402, *Frontiers in Genetics*, 2021, **12**, 666684, doi: 10.3389/fgene.2021.666684.

[39] M. Papagianni, Advances in citric acid fermentation by *Aspergillus niger*: Biochemical aspects, membrane transport and modeling, *Biotechnology Advances*, 2007, **25**, 244-263, doi:

10.1016/j.biotechadv.2007.01.002.

[40] R. Say, A. Denizli, M. Yakup Arıca, Biosorption of cadmium(II), lead(II) and copper(II) with the filamentous fungus Phanerochaete chrysosporium, *Bioresource Technology*, 2001, **76**, 67-70, doi: 10.1016/S0960-8524(00)00071-7.

[41] M. Thathana, H. Murage, A. Abia, M. Pillay, Morphological characterization and determination of aflatoxin-production potentials of aspergillus flavus isolated from maize and soil in Kenya, *Agriculture*, 2017, **7**, 80, doi: 10.3390/agriculture7100080.

[42] S. Amaike, N. P. Keller, *Aspergillus flavus*, *Annual Review of Phytopathology*, 2011, **49**, 107-133, doi: 10.1146/annurev-phyto-072910-095221.

[43] H.-Y. Wu, F. Wang, L. Yang, L. Chen, J.-R. Tang, Y. Liu, D. Liu, Z. Toktarbay, H. Algadi, J. Deng, Carboxymethyl chitosan promotes biofilm-formation of Cryptococcus laurentii to improve biocontrol efficacy against Penicillium expansum in grapefruit, *Advanced Composites and Hybrid Materials*, 2024, **7**, 23, doi: 10.1007/s42114-023-00828-9.

[44] G. Rahman, J. Muhammad, M. Ilyas, M. Subhanullah, K. Ullah, M. Massimzhan, M. Toktar, Y. Bektay, M. Kalybekov, A. Ydyrys, Y. Zhakypbek, Phytoremediation of heavy metals from soil and their effects on plant physiology - A review, *ES Materials & Manufacturing*, 2024, **26**, 1298, doi: 10.30919/esmm1298.

[45] E. C. Giese, L. S. Santos, A. C. O. Santana, Fungal extracellular carboxylic acids associated with ore mining: a wide perspective for the future, *Bioactive Microbial Metabolites*, Amsterdam: Elsevier, 2024, 123-150, doi: 10.1016/b978-0-443-18568-7.00004-5.

[46] M. I. Adakawa, M. A. Bagwai, N. S. Harinarayana, Production of organic acids by filamentous fungi: A review, *Fungal Biotechnology*, Amsterdam: Elsevier, 2025, 37-55, doi: 10.1016/b978-0-443-13263-6.00002-x.

[47] D. B. Johnson, K. B. Hallberg, The microbiology of acidic mine waters, *Research in Microbiology*, 2003, **154**, 466-473, doi: 10.1016/s0923-2508(03)00114-1.

Open Access