MME SEE CONGRESS 2023

5th Metallurgical & Materials Engineering
Congress of South-East Europe
Trebinje, Bosnia and Herzegovina
7-10th June 2023

CONGRESS PROCEEDINGS

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The Association of Metallurgical Engineers of Serbia

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The Faculty of Metallurgy at the University of Zagreb in Sisak, Croatia;
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The Faculty of metallurgy and technology at the University of Podgorica, Montenegro.

CONGRESS PROCEEDINGS - MME SEE 2023
5th Metallurgical & Materials Engineering Congress of South-East Europe

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PREFACE

On behalf of the Scientific and Organizing Committee, it is a great honor and pleasure to wish all the participants a warm welcome to the Fifth Metallurgical & Materials Engineering Congress of South-East Europe (MME SEE 2023) which is being held in Trebinje, Bosnia and Hercegovina, 07 - 10 June 2023.

The MME SEE 2023 is a biannual meeting of scientists, professionals, and specialists working in the fields of metallurgical and materials engineering. The aim of the Congress is to present current research results related to processing/structure/property relationships, advances in processing, characterization, and applications of modern materials. Congress encompasses a wide range of related topics and presents the current views from both academia and industry: Future of metals/materials industry in South-East European countries; Raw materials; New industrial achievements, developments and trends in metals/materials; Ferrous and nonferrous metals production; Metal forming, casting, refractories and powder metallurgy; New and advanced ceramics, polymers, and composites; Characterization and structure of materials; Recycling and waste minimization; Corrosion, coating, and protection of materials; Process control and modeling; Nanotechnology; Sustainable development; Welding; Environmental protection; Education; Accreditation & certification.

The editors hope that Congress will stimulate new ideas and improve knowledge in the field of metallurgical and materials engineering. The Congress has been organized by the Association of Metallurgical Engineers of Serbia, with the co-organization of the Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade, Serbia, Faculty of Technology and Metallurgy, University of Belgrade, Serbia, Faculty of Technology, University of Banja Luka, Bosnia and Herzegovina; the Faculty of Metallurgy, University of Zagreb, Sisak, Croatia; the Faculty of Natural Sciences and Engineering, University of Ljubljana, Slovenia; and the Faculty of Metallurgy and technology, University of Podgorica, Montenegro.

Financial support from the Ministry of Science, Technological Development and Innovation of the Republic of Serbia to researchers from Serbia for attending the congress is gratefully acknowledged. The support of the sponsors and their willingness to cooperate have been of great importance for the success of MME SEE 2023. The Organizing Committee would like to extend their appreciation and gratitude to all sponsors and friends of the conference for their donations and support.

We would like to thank all the authors who have contributed to this book of abstracts and also the members of the scientific and organizing committees, reviewers, speakers, chairpersons, and all the conference participants for their support of MME SEE 2023. Sincere thanks to all the people who have contributed to the successful organization of MME SEE 2023.

On behalf of the 5th MME SEE Scientific and Organizing Committee

Miroslav Sokić, PhD

DEVELOPMENT OF SUSTAINABLE METHOD FOR METAL RECOVERY FROM OLD FLOTATION TAILINGS (MAJDANPEK, SERBIA) USING *Aspergillus niger* FUNGUS

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The accumulation of mine tailings in the mining area of Majdanpek represents a serious environmental problem. A strong impetus for the development of sustainable methods for the recovery of metals from mine tailings is given by the importance of heavy metal recovery and the economic benefits of precious and base metals. Currently, researchers are working to develop metals recovery from tailings through bioleaching, which is a more sustainable method compared to traditional metallurgical methods. Biotechnologies for leaching heavy metals from old flotation tailings (Majdanpek, Serbia) with fungus *Aspergillus niger* (*A. niger*) are discussed in this paper. The subject of this research is iron ions, since the tailings are the richest in this metal. With a simple bioleaching process, which is based on the cultivation of *A. niger* in the basic growth medium and then challenging it with tailings, 5% iron was released in 35 days. The transformation of mine tailings morphology was also confirmed by optical microscopy. Processes by which *A. niger* extracts metals from tailings include acidolysis, complexation, alkaloysis and biosorption. Also, in addition to metabolites, these fungi produce spores and mycelium that can destroy material and transform it to the soil. Given that Serbia is abundant with agricultural waste, as well as waste from the food industry, in future studies cheap waste should be used as an energy input source for *A. niger* and transformation of tailings.

Keywords: bioleaching, flotation tailings, *Aspergillus niger*, sustainable development, Majdanpek.

Introduction

Mining tailings are one of the main sources of heavy metal pollution in the environment [1]. The processing of mine tailings is necessary for environmental protection, but also for the potential recovery of residual valuable metals [2].

Currently, methods used to remediate metal-contaminated soils and tailings include excavation, soil washing, plant extraction, plant stabilization, bioremediation, electrokinetic remediation, and biochemical fixation/stabilization [3].

In recent years, microbiological leaching, also known as bioleaching, has attracted more and more attention for the separation of metals from solid waste because it is simple and environmentally friendly, and cost-effective [4, 5]. Bioleaching is based on the ability of microorganisms to separate metals from otherwise insoluble solid compounds by producing organic or inorganic acids [4, 5].

In addition to bacteria bioleaching, filamentous fungi are also studied [6]. Filamentous fungi are used in bioleaching because they are able to secrete organic acids and facilitate the dissolution of metal ions from the solution phase, but also to produce mycelium that also destroys inorganic substances [6, 7].

Filamentous fungi can be cultured either under water (in shake flasks or stirred reactors) or on the surface of a solid substrate. Most of the fungi bioleaching was done in shake bottles. For example, shake-flask bioleaching of oxidized residues for the removal of Cu, Fe, Zn, and Ni was studied using *Aspergillus niger* [8, 9]. Also, three new strains of *Mucor hiemalis* were shown to exhibit multi-metal resistance, hyperaccumulation and inducibility, intracellular fixation and mercury deposition as nanospheres in sporangiospores [10, 11].

The microbiome, germinated spores, and dead insoluble cell walls of these strains simultaneously removed >81-99% of used Al, Cd, Co, Cr, Cu, Hg, Ni, Pb, U, and Zn continued to enrich valuable Ag, Au and Ti from water, all within 48 hours [10, 11].

The most studied in the field of bioleaching is the genus *Aspergillus*, which has a high removal capacity and tolerance to multiple metals. For example, *A. niger* achieved 100% removal of Cd and Pb contaminated wastewater in six days, and only 65% was achieved using *Rhizopus oligosporium* [6, 7]. *Aspergillus* species are known to be tolerant of various metals such as Al, Cd, Co, Cr, Cu, Fe, Mn, Mg, Ni, Pb, Zn and U [6, 7]. Researchers were demonstrating microbial biotechnology to simultaneously biomine and remediate 12 ionic metals, overcoming the toxicity of polymetallic metals to microbes. [12].

In this study, mine tailings from Majdanpek (Serbia) were subjected to bioleaching with the help of *A. niger.* The concentration of the metal, in this case iron, in the solution was monitored by atomic absorption spectroscopy (AAS). The transformations of tailings achieved by bioleaching were investigated using optical microscopy.

Materials and methods

Tailings sample preparation

Flotation tailings samples were collected at several locations at the flotation tailings dump of the Majdanpek copper mine (RTB Bor, Serbia). They were previously ground, and the chemical and mineral compositions of the selected samples were determined [13].

Bioleaching experiment and analytic methods

A. niger (ATCC 12066) was cultured on dextrose agar at 30 °C for 7 days in an incubator. Mature spores were suspended in saline. The spore suspension was diluted and the spore density standardized to 21 MecFar. Bioleaching experiments were performed using a batch method using a 500 mL Erlenmeyer flask, in which sterile Tryptone Soya Broth medium (autoclaved at 121 °C for 15 min) was added and then spores were sown. After 3 days of incubation at 30 ^{o}C of *A. niger*, sterilized tailings were added, and metal concentration was monitored for 35 days.

Control experiments were performed using freshly deionized distilled water. All experiments were performed in triplicate

Atomic absorption spectroscopy device (PerkinElmer, Inc, Norwalk, CT, USA) is used here to analyze the metal concentration before and after the bioleaching process.

The micromorphology of tailings was examined before and after the action of fungi, using optical microscopy (INNOVATEST microscope).

Results and discussion

Mine tailings from the location of Majdanpek (Serbia) basically consist of quartz, carbonate and sulfide minerals [13]. The sulfide component is mostly pyrite with an iron concentration of 10% [13]. After 35 days of bioleaching, about 5.3 ± 0.2% of iron was extracted from the tailings.

Preliminary optical microscopy studies of the mine tailings before and after the bioleaching experiment are shown in Figure 1. The images clearly show that decomposition of the carbonate and sulfide components is occurring. The brightest component is pyrite with chalcopyrite inclusions (Figure 1a). After bioleaching, it can be clearly seen in Figure 1b that the largest components have been decomposed.

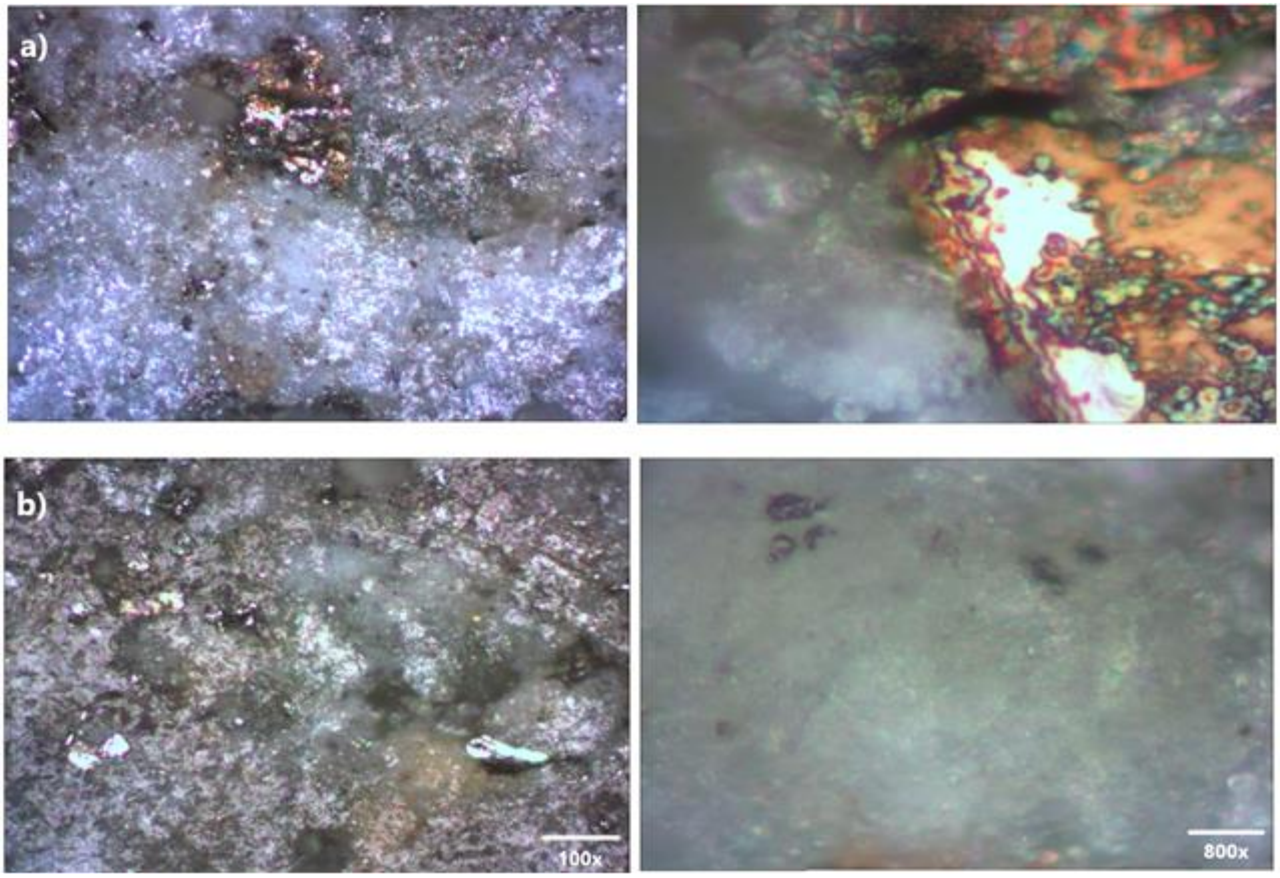

Figure 1 Micromorphological analysis of mine tailings particles before (a) and after (b) bioleaching under different magnification (left 100x and right 800x)

The mechanism of metal leaching from mining tailings using *Aspergillus niger* is shown in Figure 2. The multiple ways in which fungi extract metals from solid substrates are influenced by abiotic and biotic stressors. These fungi, notably by excreting organic acids, dissolve carbon compounds on metals. (Figure 2, a, b) [6]. The main mechanisms by which organic acids interact with metals are acidolysis, complexolysis, bioaccumulation, and chelation [6]. These mechanisms have been observed in previous studies in the species *Penicillium* and *Aspergillus* [6, 14].

Also, *A. niger* possesses hyphae and spores that mechanically destroy inorganic material and allow metabolites to continue the extraction reaction.

It has also been reported that some filamentous fungi have a primary resistance to metals due to their cell walls, regardless of their ability to produce organic acids. As can be seen in the Figure 2, fungal cell walls are composed of phosphates, polysaccharides, and lipids, but also contain enzymes, pigments, and other metabolites associated with the cell walls.

All these components work together to mobilize and immobilize metals from mine tailings. So that in addition to mechanical attack and bioleaching, the processes of biosorption, bioaccumulation and biomineralization of metals by the cells of microorganisms occurs.

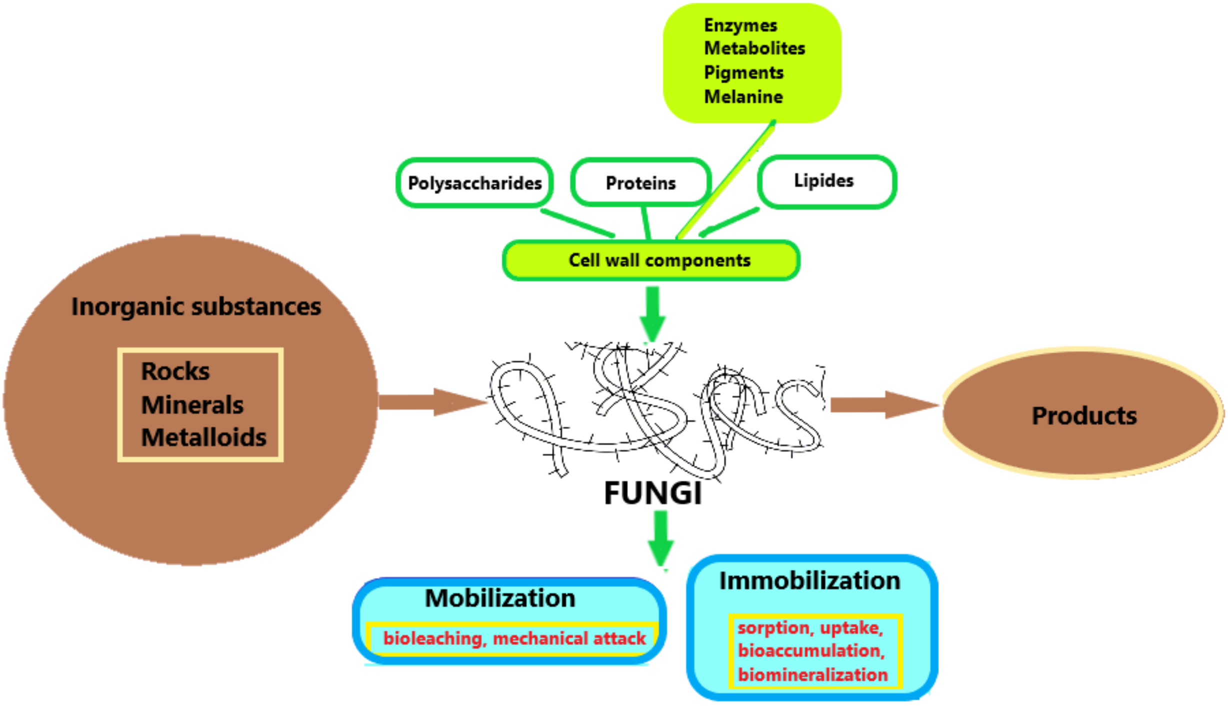

Figure 2 The scheme of interaction of filamentous fungi with inorganic substances includes bioprocesses such as mobilization (bioleaching and mechanical attack) and immobilization (biosorption, bioaccumulation and biomineralization)

This finding is in agreement with earlier research on bioleaching of mine tailings with *A. niger*. Metal mine tailings include inorganic debris and are low in nitrogen and phosphorus, which makes them more difficult to restore and clean up [15]. The primary research objective of Mulligan *et al*. (2004) [16] was to create a practical and affordable method for the microbial recovery of metals from low-grade oxide ores. This study used *A. niger* to demonstrate the viability of metal recovery from mining waste. Ability *A. niger* to produce a range of organic acids useful for metal solubilization was promising. The highest solubility levels for copper, zinc and nickel were 68%, 46% and 34%. In addition to these metals, only 7% of iron was removed.

One-step bioleaching was used in a study to extract Ni, Mo, W, and Fe from other waste metal sources using the fungus *A. niger*. Gluconic acid was generated using pulp as a nutritional substrate, and the 3% (w/v) wasted catalyst was completely leached of W, Mo, Fe, Al, and Ni [17].

In addition, Seh-Bardan *et al.* (2012) [18] used *Aspergillus fumigatus* in the bioleaching of mine tailings, and citric acid concentrations were highest when they initially separated the metabolic byproduct of microbial growth from microbial biomass. Pb, As, Fe, Mn, and Zn had respective extraction capacities of 56%, 62%, 58%, 100%, and 54% in the one-step bioleaching process using 1% tailings, whereas Pb, As, Fe, Mn, and Zn had respective extraction capacities of 88%, 32%, 45%, 58.4%, and 31.3% in the two-step bioleaching process using 1% tailings.

Conclusion

This work demonstrated that heavy metals from mine tailings can be mobilized by leaching with filamentous fungi *A. niger*. The primary mechanisms through which metabolites of *A. niger* interact with metals are acidolysis, complexolysis, bioaccumulation and chelation.

Micromorphological analysis indicates that the appearance of mine tailings particles was changed by the action of the fungus during the bioleaching process. Analytical methods confirmed the increase in metal concentration in leached polymetallic extract.

Future experiments should be done with agriculture and urban waste as an energy source for *A. niger*, the process being economically and ecologically more profitable.

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