



FACULTY OF EARTH AND ENVIRONMENTAL SCIENCES AND ENGINEERING
INSTITUTE OF RAW MATERIAL PREPARATION AND ENVIRONMENTAL TECHNOLOGY

SCIENTIFIC STUDENT (TDK) THESIS
**Experimental Investigation of Eco-Friendly Metal Recovery through Innovative
Bioleaching of Mine Tailings using *Acidithiobacillus ferrooxidans***

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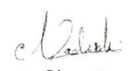
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Declaration of authenticity

The undersigned Budagodage Lisani Nimira Nadeeshani, a student of the University of Miskolc, aware of my criminal and disciplinary liability, I declare and confirm with my signature that I have prepared this thesis myself, that I have carried out the tests described in it – if not indicated separately – myself, and that I understand the presented results myself away. I used data and information only from the sources listed in the bibliography. All parts that I have taken verbatim or paraphrased from another source have been clearly marked with the source.

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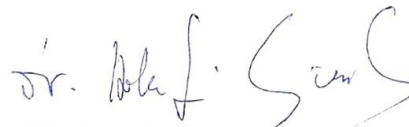
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Declaration of the supervisor

I, the undersigned Dr. Ljudmilla Bokányi [Honorary Professor], University of Miskolc, Institute of Raw Material Preparation and Environmental Technology, consider the TDK thesis suitable for submission.

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Abbreviations

OECD - Organization for Economic Cooperation and Development

Act. ferroxidans – *Acidithiobacillus ferroxidans*

REE – Rare Earth Elements

ORP – Oxidation Reduction Potential

Cu- Copper

Zn – Zinc

Pb – Lead

As – Arsenic

Au – Gold

Ag - Silver

Chapter 1: Introduction

In recent decades, the rapid escalation in resource demand, often described as the "Great Acceleration," has fueled considerable expansion in the global mining industry (Luckeneder et al., 2021). This expansion has resulted in the generation of substantial waste materials, specifically mine tailings, which are among the largest global waste streams. These tailings cover vast areas in the form of large piles, impoundments such as tailings dams, and other storage facilities (Hudson et al., 2011). Tailing disposal represents environmental and health risks, including the potential contamination of aquatic systems with heavy metals, and is often both costly and complex, requiring compliance with stringent environmental standards (Silva et al., 2020). Addressing these risks is crucial to mitigate environmental and health hazards and to capitalize on the remaining economic value within the tailings.

A circular economy approach in mining requires prioritizing tailings valorization (Kinnunen and Kaksonen, 2019). Reprocessing tailings offer benefits, such as reduced capital and operational costs since the materials have already been mined and partially processed, as well as minimizing the consumption of natural resources and waste generation. Tailings recycling also reduces human and environmental exposure to hazardous substances (Abbadi and Mucsi, 2024).

Several strategies for tailings valorization include reprocessing to recover metals, using tailings as backfill, incorporating them into construction materials, recovering energy, and carbon dioxide sequestration (Lottermoser, 2011). Recovering metals and minerals from tailings has been emphasized by the European Commission, recognizing mine waste as a potential source of secondary raw materials (Parviainen et al., 2020).

Valuable minerals and metals within tailings often exist as fine and ultrafine particles either embedded in gangue minerals or as liberated particles that are difficult to recover via flotation or other concentration techniques (Parviainen et al., 2020). Advances in separation and extraction technologies are increasingly facilitating the recovery of these materials (Tunsu et al., 2019; Sarker et al., 2022). Traditional methods for metal recovery, such as pyrometallurgical and hydrometallurgical techniques, remain widely used but involve complex control, high costs, environmental risk, and secondary pollution. In response, the bioleaching has gained attention as an efficient and sustainable alternative for metal extraction from mine tailings or low-grade ores (Bajestani et al., 2014).

Bioleaching, a more sustainable approach to metal recovery, provides a low-impact alternative to conventional methods (Spooren et al., 2020). It employs microorganisms, including bacteria and fungi, to solubilize metal compounds, making them extractable (Anjum et al., 2012). Commercially, bioleaching has been applied to metals such as copper, gold, and uranium, utilizing a variety of bacterial species (Akcil, 2004). By utilizing microbial processes to leach metals, bioleaching reduces reliance on harsh chemicals and extensive energy inputs, potentially providing an eco-friendly approach to metal recovery (Taltech 2024).

However, the heterogeneity of tailings complicates reprocessing due to variations in ore type, host rock, processing methods, and environmental influences, necessitating tailored assessments to

identify recovery potential (Surrette et al., 2024). In this study, bioleaching experiments with *Act. ferrooxidans* were conducted to assess metal recovery from tailings at a historical mine in Leoben, Austria. This preliminary study is part of a collaborative bilateral project between the University of Miskolc (project leader Dr. L. Bokányi Honorary Professor) and Montanuniversität Leoben (project leader Dr. J. Antrekowitsch Associate Professor). The project aims to determine the feasibility of bioleaching as a foundation for future experiments to enhance mineral recovery. The study was undergone by joining with the bioprocessing team of the University of Miskolc. The investigation focuses on waste sample characterization, bioleaching under varied pulp densities, determining metal recovery rates, and analyzing pH and ORP data. Through bioleaching, particularly leveraging *Act. ferrooxidans* known for their capacity to oxidize iron and sulfur compounds in acidic environments, this study seeks to identify the potential of bioleaching for eco-friendly metal recovery from Leoben mine tailings.

Chapter 2: Literature Review

2.1 Heightened resource demand and mining

The intensifying pressures on global resources are being driven by several factors, including population growth, industrialization, digitalization, increased demand from developing economies, and the transition toward climate neutrality. The OECD projects that global demand for materials will more than double by 2060, rising from today's 79 billion tons to 167 billion tons, further heightening competition for essential resources such as metals and minerals (European Commission, 2023a). Critical materials including copper, nickel, cobalt, and graphite are indispensable for green energy technologies, such as wind turbines, electric grids, and electric vehicles, with demand projected to surge by nearly 500% by 2024, which is nearly six times of current levels (IEA, 2021).

This escalating demand for raw materials mirrors the global trend of accelerated resource consumption, often referred to as the "Great Acceleration" (Steffen et al., 2015). However, the increasing demand is rapidly exhausting high-quality reserves of essential resources, such as oil, copper, and gold, as consumption rates become increasingly unsustainable (UNEP, 2023). In response, the mining industry has expanded its efforts through new projects, site enlargements, and optimized extraction techniques. While these measures are necessary to meet rising demand, mining activities come with severe environmental and health impacts, as byproducts such as mine tailings pose significant risks to ecosystems and communities (Xiaolong et al., 2021).

2.2 Mine tailings

Mine tailings are the residual byproducts remaining after ore extraction by the mining and upgrading of valuable, metal-bearing minerals through various physical separation processes (Silva et al., 2020). This material consists of a combination of finely ground pulverized rock and

processing effluents generated during ore upgrading, with its composition depending on the specific type of ore and the applied separation techniques. In the context of copper mining, tailings can account for as much as 95–99% of the total material post-crushing and grinding (Araya et al., 2020). These tailings may contain base metals such as copper, iron, zinc, and nickel at notable concentrations, typically in the range of 0.5% to 3%. Precious metals like gold and silver can also be present, alongside toxic elements including arsenic, beryllium, bismuth, and tungsten at concentrations reaching up to 100 mg/kg. Additionally, sulfur-bearing minerals such as pyrrhotite and pyrite are often found in ore tailings (Falagán et al., 2017; Mulenshi et al., 2021).

Globally, mine tailings are one of the largest waste streams, occupying vast areas on the Earth's surface in forms such as waste piles, large surface impoundments, tailings dams, and other storage facilities (Hudson et al., 2011). Since the Stone Age, it is estimated that approximately 1,150 million tons of heavy metals have been extracted worldwide (Edraki et al., 2014). Currently, the annual global production of mine tailings is estimated to exceed 20 billion tons, covering an expanse of over 200,000 km² (Abbadi and Mucsi, 2024).

Gao et al., 2021 highlighted that the environmental impact of mine tailings can directly or indirectly pose serious threats to human health (Figure 1). Over the past 2,000 years, chemical pollutants from human activities have accumulated in soils and sediments, with heavy-metal mining waste among the earliest sources of such contamination. Despite the economic gains associated with mining, the environmental risks posed by the large volumes of tailings and tailing storage sites are substantial. Tailings contain toxic substances in concentrations that can harm ecosystems and human health through several pathways, including the dispersion of dry tailings by wind, leaching of toxic elements into soil and groundwater, erosion into water systems, and subsequent bioaccumulation in the food chain.

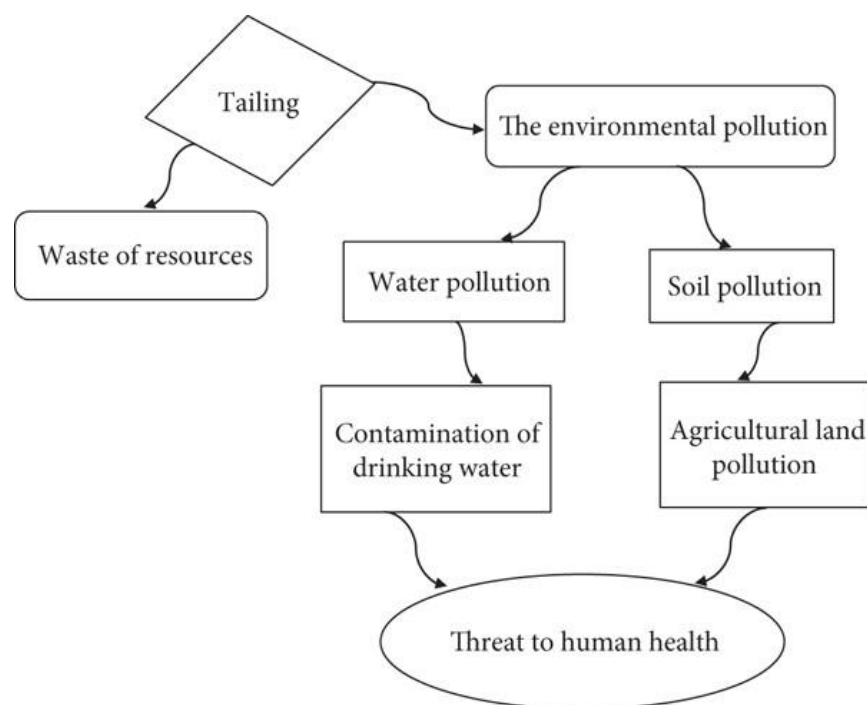


Figure 1: Environmental and health impacts of mine tailings (Gao et al., 2021)

2.2.1 European context of mine tailings as a secondary resource

The European Commission regularly revises its list of critical raw materials to align with evolving economic demands and to evaluate supply chain risks within the European Union (EU). In the latest update issued in 2023, this list includes 34 raw materials, notably copper and arsenic (European Commission, 2023a). Currently, the EU lacks active mines for rare earth elements (REEs), which presents a significant challenge to its supply chain. To address this issue, the 2023 Critical Raw Materials Act outlines strategic initiatives aimed at enhancing the collection and recycling of critical materials (European Commission, 2023b).

As part of its circular economy agenda, the EU actively promotes the reprocessing of extractive wastes. Under European Union regulations, member states are required to periodically compile inventories of closed and abandoned waste sites, especially those that currently or potentially pose environmental and health hazards (Directive 2006/21/EC, 2006). These initiatives also include evaluating the feasibility of recovering valuable materials from both operational and decommissioned mining sites. In parallel, the EU is exploring the utilization of accessible, lower-grade sources that could be processed more sustainably without the need for initiating new mining projects. One such promising resource is the mine tailings from historical mining activities, which have been identified as a potential sustainable supply of critical raw materials (Abbadi and Mucsi, 2024; Parviainen et al., 2020).

2.3 Disposal, reuse, and reprocessing of mine tailings

Globally, most mines continue to rely on conventional tailings storage facilities (TSFs) to manage tailings and process water in slurry form. However, TSFs present a risk of dam failure if water balance is not adequately managed (Edraki et al., 2014). A major incident highlighting these risks occurred in 2000 at Baia Mare (Nagybánya), Romania, when a TSF breach released approximately 100,000 cubic meters of cyanide-contaminated, metal-rich liquid waste into nearby rivers, ultimately affecting the Tisa (Tisza) and Danube (Duna) rivers (Soldán et al., 2001).

To mitigate the environmental impact of tailings, alternative disposal methods, such as paste disposal for surface deposition or underground backfilling, are increasingly under consideration (Edraki et al., 2014; Abbadi et al., 2022). Additionally, geopolymerization presents an innovative method for utilizing mine tailings (MTs) as precursors in cementitious materials or aggregates. This process involves the alkali activation of aluminosilicate-rich tailings, creating sustainable construction materials that contribute to a reduced carbon footprint (Mucsi, 2016; Xu and Van, 2000). In literature, numerous studies support mine tailings' viability in geopolymerization, demonstrating their effective use in sustainable building applications (Abbadi and Mucsi, 2024; Obenaus et al., 2020; Aseniero et al., 2019).

Among the strategies for reprocessing and reusing tailings, valorization by recovering residual metals and repurposing mineral content is a priority. This approach aligns with the United Nations Sustainable Development Goals (SDGs) by promoting sustainable resource use and reducing waste (Kinnunen and Kaksonen, 2019). Numerous studies have examined pyrometallurgical and hydrometallurgical techniques for metal recovery from mine tailings. For example, Lemos et al.

(2023) demonstrated that specialized metallurgical methods, including roasting, could recover valuable metals such as gold while reducing hazardous contaminants like arsenic and antimony in mining waste. Similarly, research by Zou et al. (2018) showed that alkaline roasting with sodium carbonate significantly improved the leaching efficiency of boron from boron-bearing tailings, raising it from 45-55% to 86.78%.

Further advancements in REE recovery have been achieved. For instance, Tunsu et al. (2019) used solvent extraction to recover rare earth elements (REEs) from tailings in New Kankberg, Sweden, and Covas, Portugal. Peelman et al. (2016) also examined REE recovery from Kiruna iron ore mine tailings in Sweden, extracting around 5300 ppm of REE-rich apatite and monazite using acidic leaching with nitric acid. Fleming et al. (2021) evaluated REE extraction through hydrometallurgical leaching using hydrochloric and nitric acid, successfully demonstrating effective REE recovery from mining tailings. These studies underscore the potential of customized extraction techniques to enhance metal recovery from mine tailings, providing a sustainable approach to reclaim valuable resources from mining byproducts.

Hydrometallurgical methods, in particular, offer advantages in recovering metals from secondary sources like mine tailings, as they are generally more energy-efficient compared to pyrometallurgical approaches. These methods lower the production costs, reduce waste generation, and limit emissions of pollutants, including SO_x, NO_x, CO₂, and CO. Additionally, hydrometallurgy enables more precise recovery of pure metals or compounds (Erust et al., 2023). Bio-hydrometallurgical methods are also promising, offering socio-economic and environmental benefits by minimizing chemical usage and reducing processing costs (Anjum et al., 2012).

2.4 Bioleaching as a promising approach

Bio-hydrometallurgical techniques utilize microorganisms for metal extraction through processes such as bioleaching, biosorption, and bio-beneficiation. This approach traces its roots back approximately 2,000 years before the scientific discovery of microbes (Anjum et al., 2012). The process of metal extraction through bacterial oxidation was initially observed in 1670 at the Rio Tinto mine in Spain. However, it was not until approximately 1900 that the practice of leaching was widely applied to piles of low-grade ore overburden, commonly referred to as dumps (Michael, 2014). The earliest recorded microbial recovery of copper dates to the copper extraction from the Falun Mine in central Sweden, which was first documented by Hallberg and Rickard in 1973 as having occurred since 1687. In the 1950s, Kennecott Copper Corporation introduced the first patented method for copper bioleaching, marking a modern application of bio-hydrometallurgy (Brierley and Brierley, 2001). There is evidence of microbial metal recovery in medieval historical Upper Hungary (Żmuda-Trzebiatowska et al., 2016).

Today, bio-hydrometallurgy is recognized as a promising interdisciplinary technology, particularly valuable for extracting metals from low-grade ores and detoxifying industrial waste byproducts. Increasingly, companies are adopting these methods to recover valuable metals from various sources, including industrial waste, sewage sludge, soil, coal, and fossil fuels (Akcil, 2004; García-Balboa et al., 2010). This growing interest highlights bio-hydrometallurgy's potential as

an environmentally and economically viable alternative in the field of metal recovery and waste management.

In bio-hydrometallurgy, bioleaching is increasingly regarded as a sustainable approach to extract metals such as gold, copper, zinc, nickel, cobalt, and uranium from sulfide ores (Schippers et al., 2014). This method utilizes microorganisms including bacteria, archaea, and fungi to effectively facilitate the extraction and recovery of metals from ores, concentrates, and even mine tailings (Schueler et al., 2024). Industrial applications often depend on oxidative bioleaching involving autotrophic microorganisms, particularly sulfur- and iron-oxidizing bacteria like *Acidithiobacillus* species (Spooren et al., 2020).

Currently, bioleaching is estimated to contribute around 15%, 13%, and 25% to the global production of copper, uranium, and gold, respectively (Akci, 2004). Tests conducted on the pyritic copper deposits of the Kure Mine demonstrated a copper recovery rate of approximately 80% when utilizing mesophilic mixed microbial cultures (Akci, 2004).

Key factors that influence bioleaching efficiency include temperature, pulp density, energy source or substrate concentration, pH, and particle size (Bajestani et al., 2014). *Act. ferrooxidans*, an acidophilic bacterium, thrive in highly acidic environments with a pH range of 1–2.5. Mass transfer, a critical component in chemical engineering, poses challenges in bioleaching as well. Factors such as contact surface area directly impact mass transfer. In bioleaching, the tension between particle surfaces within the pulp can exert pressure on microbial cell walls, potentially leading to cell damage or lysis. Reducing particle size mitigates this tension and increases the available contact surface, thereby enhancing mass transfer rates and improving bioleaching efficiency (Bajestani et al., 2014). Several studies have focused on identifying the inhibition effect of bioleaching to improve the process (Valéria et al., 2024).

2.4.1 Microorganisms for bioleaching

Autotrophic leaching primarily involves chemolithotrophic, acidophilic bacteria that utilize carbon dioxide as their carbon source and derive energy from the oxidation of ferrous iron or reduced sulfur compounds. These metabolic reactions produce ferric iron (Fe^{3+}) or sulfuric acid (H_2SO_4) as end products. Key microorganisms involved in autotrophic leaching include sulfur-oxidizing bacteria like *Act. thiooxidans*, iron- and sulfur-oxidizing bacteria such as *Act. ferrooxidans*, and iron-oxidizing bacteria like *Leptospirillum ferrooxidans*. Through sulfur and iron oxidation, these bacteria facilitate the dissolution of metal sulfides and reduce the pH in their local environment, which in turn promotes further solubilization of metal compounds (Anjum et al., 2009).

In contrast, heterotrophic leaching involves microorganisms that rely on organic carbon sources rather than obtaining energy directly from the ore. The metabolic byproducts of these organisms, primarily organic acids, can leach metals indirectly, without direct interaction with the mineral itself. This form of leaching is effective for minerals with low sulfur and sulfide content. Heterotrophic leaching organisms are typically filamentous fungi, such as *Aspergillus* and *Penicillium* species, as well as certain bacteria (Anjum et al., 2012).

The foundation of modern commercial biohydrometallurgy was established with Colmer and Hinckle's isolation and identification of *Thiobacillus ferrooxidans* from a coal mine. Further studies revealed that microbial activity was responsible for the formation of acidic drainage in coal mines. Subsequent research demonstrated that bacteria could expedite the leaching process for metal sulfides, including those containing copper and other metals. The microorganisms most frequently studied concerning commercially viable metal extraction include *Thiobacillus ferrooxidans* (now classified as *Acidithiobacillus ferrooxidans*), *Thiobacillus thiooxidans*, and *Leptospirillum ferrooxidans*. Research efforts are also exploring the application of other microorganisms, such as certain archaea and fungi, in bio-hydrometallurgical processes ((Michael, 2014). Table (1) presents the selection of organisms recognized for their role in facilitating metal leaching, along with their specific growth conditions, including metabolic type, pH, and temperature requirements.

Table 4: Possible bioleaching microbes (Nguyen et al., 2021)

Organism	Metabolism type	pH range	pH opt.	Temperature (°C)
Archaea				
<i>Acidianus brierleyi</i>	heterotroph	acidophilic	1.5–3.0	45–75
<i>Acidianus infernus</i>	heterotroph			
<i>Ferroplasma acidiphilum</i>	autotroph	1.3–2.2	1.7	15–45
<i>Metallosphaera sedula</i>	autotroph	acidophilic	n.d	Extreme thermophilic
<i>Sulfolobus acidocaldarius</i>	autotroph	0.9–5.8	2.0–3.0	55–85
<i>Sulfolobus solfataricus</i>	autotroph	n.d	n.d	Extreme thermophilic
<i>Sulfolobus thermosulfidooxidans</i>	autotroph	n.d	n.d	Extreme thermophilic
Bacteria				
<i>Acidithiobacillus caldus</i>	autotroph	n.d	n.d	45
<i>Acidithiobacillus ferrooxidans</i>	autotroph	1.4–6.0	2.4	28–35
<i>Acidithiobacillus thiooxidans</i>	autotroph	0.5–6.0	2.0–3.5	10–37
<i>Acidiphilium cryptum</i>	heterotroph	2.0–2.6	n.d	mesophilic
<i>Bacillus megaterium</i>	heterotroph	n.d	n.d	n.d
<i>Leptospirillum ferrooxidans</i>	autotroph	n.d	2.5–3.0	30
<i>Leptospirillum thermoferrooxidans</i>	autotroph	n.d	1.7–1.9	45–50
<i>Pseudomonas putida</i>	heterotroph	n.d	n.d	n.d
<i>Sulfobacillus thermosulfidooxidans</i>	autotroph	n.d	n.d	50
<i>Thiobacillus acidophilus</i>	mixotroph	1.5–6.0	3.0	25–30
<i>Thiobacillus albertis</i>	autotroph	2.0–4.5	3.5–4.0	28–30
<i>Thiobacillus thioparus</i>	autotroph	4.5–10.0	6.6–7.2	11–25
Fungi				
<i>Aspergillus awamori</i>	heterotroph	n.d	n.d	28
<i>Aspergillus fumigatus</i>	heterotroph	n.d	n.d	
<i>Aspergillus niger</i>	heterotroph	n.d	n.d	30
<i>Penicillium chrysogenum</i>	heterotroph	n.d	n.d	28
<i>Penicillium funiculosum</i>	heterotroph	n.d	n.d	
<i>Trichoderma lignorum</i>	heterotroph	n.d	n.d	24–26
Yeasts				
<i>Candida lipolytica</i>	heterotroph	n.d	n.d	30
<i>Saccharomyces cerevisiae</i>	heterotroph	n.d	n.d	28

*n.d: no data

2.4.2. Mechanisms of bioleaching

Several studies have refined the understanding of microorganism-mineral interactions in bioleaching, identifying three primary mechanisms; Indirect, Indirect contact, and Direct contact mechanism (Valéria and Ljudmilla, 2020; Crundwell, 2003).

1. Indirect Mechanism: Microorganisms oxidize ferrous ions to ferric ions in the leachate, and the ferric ions then chemically oxidize sulfide minerals, thereby leaching valuable metals from the ore.
2. Indirect Contact Mechanism: Microorganisms adhere to the mineral surface, where they secrete extracellular polymers that form complexes with ferric ions, facilitating mineral oxidation. The released ferrous ions and sulfur compounds from mineral oxidation are used for microbial growth.
3. Direct Contact Mechanism: In this process, microorganisms directly oxidize and break down minerals independently of ferric ions.

These mechanisms are widely accepted in bioleaching research and provide valuable insights into the microbial interactions that drive metal recovery from sulfide minerals (Figure 2).

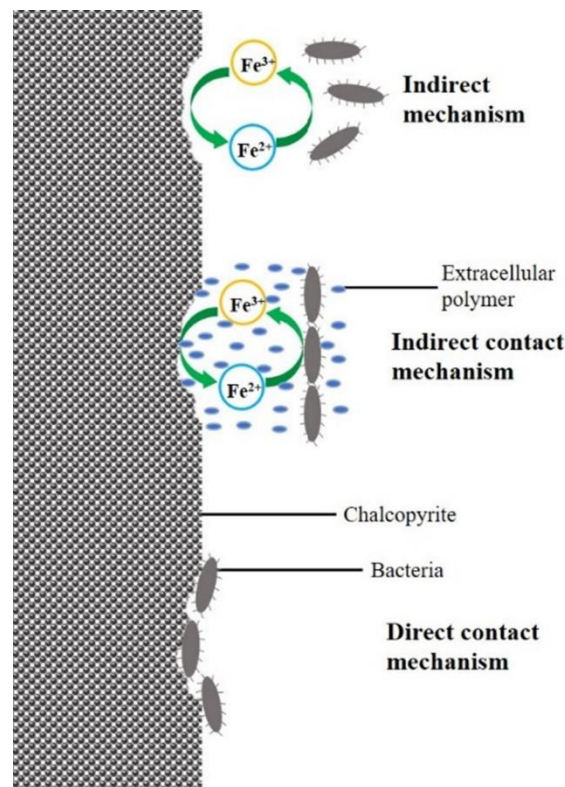


Figure 2: Mechanisms of chalcopyrite bioleaching; indirect, indirect contact, and direct contact mechanism (Tian et al., 2021)

2.4.3 Bioleaching experiments for mine tailings

Bioleaching is regarded as a reliable approach for metal extraction from mine tailings with distinct advantages over traditional hydrometallurgical methods. Microbial leaching, characterized by its process simplicity, eco-friendly nature, and low energy requirements, addresses the limitations of conventional chemical and physical extraction methods that often involve high reagent and energy consumption, as well as significant environmental pollution (Dong et al., 2023).

A study comparing bioleaching and chemical leaching for sulphidic tailings of the Finnish Hitura mine and Pyhäsalmi mine by using mixed acidophilic cultures demonstrated substantially higher metal extraction efficiencies in bioleaching than chemical leaching. This research also highlighted that chemical leaching faced limitations, including high acid consumption and poor selectivity, while bioleaching offered greater selectivity for valuable metals in tailings (Aromaa et al., 2013). Consequently, microorganisms such as metal-tolerant fungi and bacteria show potential for recovering metals as well as remediating heavy metal-contaminated tailings.

Research on the bioleaching of mining waste and mine tailings is extensive, with most experiments utilizing well-characterized iron- and sulfur-oxidizing bacteria or mixed cultures from indigenous mining environments (Nguyen et al., 2021). While bioleaching offers promising applications for the valorization of mine tailings, further advancements are necessary to improve the efficiency of heavy metal extraction from these materials (Dong et al., 2023).

Numerous studies on the bioleaching of mine tailings are summarized in Table 2, highlighting the various microorganisms used, the metals targeted, and their corresponding recovery rates. These studies also consider a range of conditions applied, including pulp densities, substrate types, pH levels, temperature, agitation speed, and residence time.

Table 5: Bioleaching efforts for mine tailings in the literature

Author	Bioleaching conditions	Microorganisms	Metal recovery rate
Park et al., 2014	- Lab-scale: 500 mL flasks - Inoculum: 10% (v/v) - PD: 0.5, 1.0, 2.0, and 4.0% (w/v) - S: 20 g/L FeSO₄·7H₂O - pH: 1.8, 2.0, and 2.2 - T: 25, 30, and 35 °C - AS: 150 rpm - RT: 25 d	<i>Acidithiobacillus ferrooxidans</i>	As 85%
Lee et al., 2015	- Lab-scale: 500 mL flasks - Inoculum: 10% (v/v) - PD: 5% (w/v) - S: 45 g/L FeSO₄·7H₂O, 1% sulfur - pH: 2.5–3.0 - T: 30 °C - AS: 200 rpm for - TR: 25 d	<i>Acidithiobacillus ferrooxidans</i> and <i>Acidithiobacillus thiooxidans</i>	- As: 72.2% - Cu: 47.1% - Mn: 99.5% - Zn: 78.4%
Ye et al., 2017	- Lab-scale: 3 L reactor - Inoculum: 10% (v/v) - PD: 10 and 20% (w/v) - pH: 2.0 - T: 30 °C - RT: 200 rpm - RT: 50 d	<i>Acidithiobacillus ferrooxidans</i>	- Fe: 71.37% - Pb: 0.82% - Zn: 97.38%
Dong et al., 2011	- Lab-scale: 250 mL flasks - Inoculum: 10% (v/v) - PD: 5% (w/v) - S: 44.2 g/L FeSO₄·7H₂O - pH: 2.0 - T: 30 °C - AS: 160 rpm - RT: 42 d	<i>Acidithiobacillus ferrooxidans</i>	- Cu: 25%
Kondrat'Eva et al., 2012	- Lab-scale: percolators - PD: 3.75: 1 to 3: 1 - pH: 1.6–1.8 - T: 20–24 °C - RT: 134 d	Indigenous culture of acidophilic chemolithotrophic microorganisms	- Cu: 46.0% - Zn: 59.5%
Mehrabani et al., 2013	- Lab-scale: 250 mL - Inoculum: 10% (v/v) - PD: 5–10% (w/v) - S: Fe (II): 8, 16 g/L - pH: 1.5–2 - T: 30 and 45 °C - AS: 150 rpm - RT: 14 d	<i>A. ferrooxidans</i> , <i>Acidithiobacillus thiooxidans</i> and <i>Leptospirillum ferrooxidans</i>	- Zn: >90%
Ilyas et al., 2013	- Lab-scale: flasks - Bioleaching medium: Supernatant - PD: 10% (w/v) - T: 30 °C - AS: 120 rpm - RT: 25 d	<i>Penicillium chrysogenum</i>	- Co: 60% - Cu: 67% - Mg: 69% - Ni: 55% - Zn: 65%

*PD: Pulp Density, S: Substrate, T: Temperature, AS: Agitation Speed, RT: Retention Time

2.4.4 Copper Recovery by Bioleaching

Copper, one of the earliest metals utilized by humans, remains essential in modern applications, including cables, electronics, electrical components, and construction, ensuring that its demand will continue to rise. Due to the extensive exploitation of primary copper resources, sustainable development now requires a focus on utilizing alternative sources such as mine tailings (Gao et al., 2021). A recent assessment of copper content in the tailings at the abandoned Musina mine in South Africa's northern Limpopo Province revealed a remaining copper reserve of approximately 8,555 tons (Singo et al., 2019).

Bio-leaching has emerged as a viable alternative due to its simplicity, efficiency, cost-effectiveness, and eco-friendly attributes, making it a promising solution for processing low-grade ores. Among copper-bearing minerals, chalcopyrite (CuFeS_2) is the most abundant, representing approximately 70% of global copper resources. Moderately thermophilic bacteria, such as *Acidithiobacillus* and *Leptospirillum ferriphilum*, hold considerable industrial potential as they enhance the dissolution kinetics of chalcopyrite, improving extraction rates (Zhao et al., 2016).

Serbian copper ore tailings underwent biological leaching using thermophilic acidophilic bacteria at 40°C, achieving a maximum copper leaching rate of 84% (Conić et al., 2020). In another study, copper ore from the Qarashoshaq deposit in northern Zhylandy, Kazakhstan, demonstrated a copper extraction rate of 95% through biological leaching compared to 66.8% via chemical leaching. These results indicate a superior leaching efficiency for copper when using biological methods over traditional chemical leaching (Zhapparet et al., 2019). Currently, biological leaching of oxide ores remains underexplored. However, studies have shown that the heterotrophic bacterium *Pseudomonas aeruginosa* has been applied in the bioleaching of copper and zinc oxide ores, achieving extraction rates of 47% for copper and 41% for zinc (Shabani et al., 2019).

Bioleaching to recover Cu presents challenges due to its refractory nature, primarily caused by passivation, which impedes the dissolution process. Addressing how to enhance Cu dissolution using moderately thermophilic bacteria has become a key research focus (Lindblom, 2019). Researchers worldwide are investigating methods to optimize bioleaching for Cu recovery, as it is known to develop a passive surface layer in high-acidic solutions, especially when solution potential exceeds a certain threshold. This passivity is a significant barrier for Cu dissolution, resulting in slow and often incomplete copper recovery. Although the exact composition of the passive layer remains uncertain, it is generally attributed to several possible compounds, including a compact sulfur layer, a metal-deficient chalcopyrite-like sulfide layer, polysulfides such as Cu_xS_y , or iron-bearing precipitates like jarosite, all of which hinder chalcopyrite's surface reactivity.

To mitigate this passivation effect, researchers suggest controlling the pulp ORP during bioleaching (Ahmadi et al., 2010). Maintaining optimal ORP levels appears essential to preventing passive layer formation, with certain studies proposing ORP thresholds that favor Cu dissolution. Lindblom (2019) found that bioleaching cultures maintaining a redox potential between 400 and 500 mV achieved more effective Cu dissolution. Additionally, Gericke et al. (2010) observed that a high redox potential facilitates pyrite bioleaching, while a lower redox potential (~400mV) is more favorable for chalcopyrite.

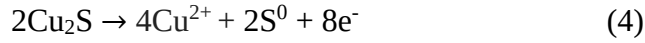
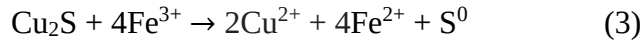
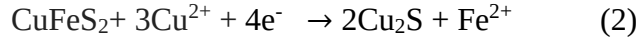
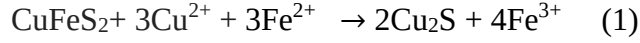
For pyrite ash and converter slag, the maximum copper recovery achieved using chemolithotrophic meso-acidophilic iron and sulfur oxidizers ranged from 95% to 100% as reported by Brar et al., (2021). Optimal process performance was observed within the following conditions: pH range of 1.75–3.0, temperature between 30–32°C, pulp density of 1.4–20% (w/v), initial Fe²⁺ concentration of 7.3–9.0 g/L, and particle size ranging from 10 to 95 µm. Further, various approaches adopted by several studies for Cu recovery on a large scale by bioleaching are reported in Table (3).

Table 6: Summary of previous studies in the literature for Cu extraction by bioleaching

Author	Bioleaching conditions	Microorganisms	Cu recovery rate
Muravyov and Fomchenko (2013)	Reactor and conical flask Fe ³⁺ (33.6 g/l) T: 40 °C, pH: 1.5, PD: 10%	<i>A. ferrooxidans</i> , <i>L. ferrooxidans</i> , <i>S. thermotolerans</i>	89.4%
Panda et al. (2015)	Erlenmeyer flasks 9 K medium Fe ²⁺ (3.0 g/l) PS: <0.1 mm, T: 35 °C, pH: 1.5, RT: 12 days, PD: 7% (w/v)	<i>A. ferrooxidans</i> , <i>L. ferrooxidans</i> , <i>A. thiooxidans</i>	96%
Tuovinen et al. (2015)	Shake Flask Mineral salt medium + S ⁰ (10 g/L) T: 27 °C, pH 2.1–2.2, AS: 150 rpm, RT: 25 days, PD: 10%, ORP: >600mV	<i>A. thiooxidans</i> , <i>L. ferriphilum</i> , <i>A. ferrooxidans</i>	69–83%
Castro et al. (2016)	Erlenmeyer flasks MAC medium + S ⁰ (1.0 g/L) + Fe ²⁺ (1.0 g/L) + K ₂ O ₆ S ₄ (3.0 g/L) + glucose (1.0 g/L) + yeast extract (1.0 g/L) T: 65 °C, pH: 2.0, AS: 120 rpm (Dark), RT: 50 Days, PD: 2%	<i>Acidianus copahuensis</i>	~100%
Kaksonen et al. (2016)	Batch Stirred Tank Mineral salts medium + S ⁰ (10 g/L) T: 27 °C, pH: 3.5, AS: 170 rpm, RT: 47 days, PD: 10%, ORP: 540–560 mV	<i>Acidithiobacillus ferrivorans</i> , <i>Alicyclobacillus tolerans</i> , <i>Al. cycloheptanicus</i> , <i>Al. herbarium</i>	73–83%
Falagan et al. (2017)	Continuous stirred Tank Basal salt medium, S: 2% (w/v) T: 45 °C, pH: 1.0 alternated aerobic/anaerobic conditions bioleaching Oxygen and nitrogen flow rate ~ 1 L/min PD: 5%,	<i>A. caldus</i> , <i>S. thermosulfidooxidans</i>	84 to 90%
Schippers (2017)	Erlenmeyer flasks PS: < 63 µm, T: 30 °C, pH: < 3.0, AS: 120 rpm, RT: 51 days, PD: 2%	<i>A. thiooxidans</i> , <i>Acidiphilium spp.</i> <i>A. ferrooxidans</i> ,	91%
Georgiev et al. (2017)	Batch and Continuous Stirrer Tank Reactor 9 K medium pH: 1.0–3.5 Aeration: air enriched with CO ₂ to 0.15%, PD: 5–20%	<i>A. ferrooxidans</i> , <i>A. caldus</i> , <i>A. thiooxidans</i> , <i>L. ferrooxidans</i> <i>S. thermosulfidooxidans</i> <i>S. metallicus</i> , <i>Metallosphaera sedula</i> , <i>Acidianus</i>	65–88%

*T: Temperature, AS: Agitation Speed, RT: Residence Time, PD: Pulp Density

To enhance copper leaching, some researchers suggest that chalcopyrite can be reduced to chalcocite (Cu_2S) under a low redox potential, after which the chalcocite can undergo rapid oxidation, facilitating copper recovery (Zhao et al., 2016). This process can be represented through the following reactions 1 to 4:

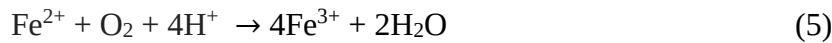


In the bioleaching process, redox potential (ORP) control can be achieved either electrochemically, by applying an external potential through a working electrode, or chemically, by introducing reducing or oxidizing agents. The electrochemical reduction approach is particularly advantageous as it minimizes solution contamination and avoids the depletion of additional agents (Fuentes-Aceituno et al., 2008). Ahmadi et al. (2010) utilized an electro-bioreactor to control ORP during chalcopyrite bioleaching, finding that maintaining a redox potential between 400 and 425 mV significantly enhances copper recovery.

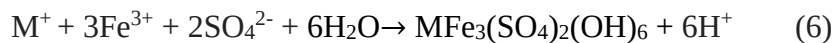
In addition, various methods have been applied to further improve leaching efficiency in chalcopyrite bioleaching systems. For instance, the addition of chloride can alter the passivation layer's composition, increasing temperature can boost copper dissolution rates, and establishing galvanic cells on the chalcopyrite surface can further enhance recovery (Tian et al., 2021).

2.4.5 Problem of jarosite formation during bioleaching

Jarosite is a predominant secondary mineral that forms in acidic supergene environments through the oxidation of metal sulfides. Its deposition during the bio-hydrometallurgical processing of sulfide ores can have significant adverse effects. Iron-oxidizing bacteria derive energy from the oxidation of various inorganic substrates, with ferrous sulfate being the most common (Daoud and Karamanev, 2006). The overall biochemical reaction for the bacterial oxidation of ferrous ions is as follows (Equation 5):



This reaction consumes hydrogen ions, initially increasing the pH of the medium. However, this pH rise is moderated by the hydrolysis of ferric ions, indicating that pH significantly influences both oxidation and hydrolysis reactions. Additionally, a competitive reaction with the hydrolysis process yields basic ferric hydroxysulfates of the form $\text{MFe}_3(\text{SO}_4)_2(\text{OH})_6$, where M can be K^+ , Na^+ , NH_4^+ , Ag^+ , or H_3O^+ ^{5,6} (Changqiu et al., 2006). These hydroxysulfate precipitates are known as jarosites, and their precipitation reaction is represented as follows (Equation 6):



In media with a high concentration of NH_4^+ , jarosites specifically form as ammoniojarosites, with the formula $\text{NH}_4\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6$ (Changqiu et al., 2006).

Jarosite formation can also be analyzed using Eh-pH diagrams. As shown in Figure (3), the stability region for $\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$ is defined by phase equilibrium lines at Eh values above 360 mV and pH levels greater than 2.8. Specifically, the stable region of $\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$ exists within an Eh range of 360–800 mV and a pH range of 2.8–5 (Kaile et al., 2017).

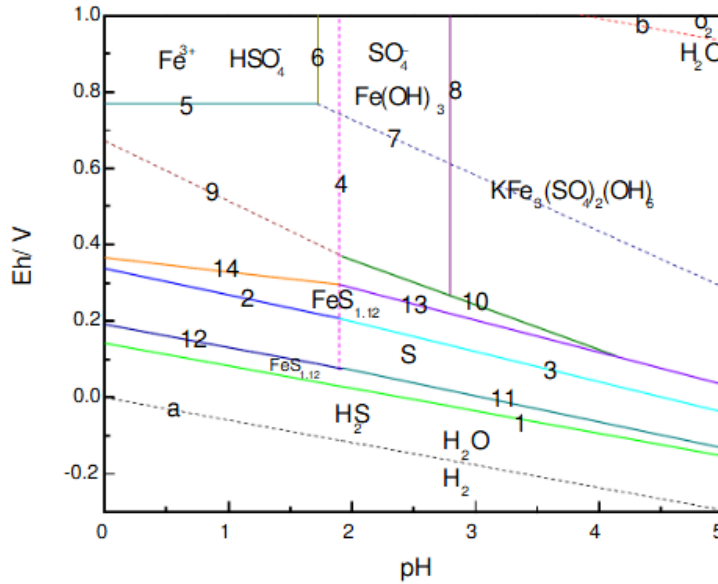


Figure 3: Eh-pH diagram for FeS_{1.12}-H₂O system, at temperature=298K and Pressure=1.013*10⁵ Pa (Kaile et al., 2017)

Chapter 3: Experimental Procedure

3.1. Materials and Methodology

3.1.1 Sample characterization and preparation

A proper baseline characterization of mine tailings encompassing their physical and chemical characteristics, is a fundamental prerequisite for evaluating potential strategies for reuse, recycling, and further processing. Examining factors such as particle size distribution, density distribution, and elemental composition is key to assessing the viability of recovering any remaining valuable minerals or metals within the tailings. Characterization also plays a crucial role in identifying potentially hazardous contaminants that could be mobilized during direct use or reprocessing, as well as minerals that may affect geotechnical stability if the tailings are repurposed (Mulenshi et al., 2021).

A mine tailing sample was obtained from an abandoned mine located in the vicinity of Leoben, Austria. Sample characterization and preparation were carried out by another research team of the project and this section is based on the project report Montanuniversität Leoben (Mine tailing, 2024) under the research leader Dr. L. Bokányi.

3.1.1.1 Wet Sieve Analysis

The feasibility of primary gangue removal was evaluated via a wet sieve analysis. Following the mass ratio of certain size fractions were merged purposely. The results of sieve analysis and size fractions of merged data are shown in Table (4).

Table 4: Results of sieve analysis and merged size fractions

X_i (mm)	X_{i-1} (mm)	m (g)	$m/\sum m$ (%)	$F(x)$ (%)	
63	45	646.32	9.39	100.00	29,.19
45	31.5	188.2	2.73	90.61	
31.5	22.4	428.34	6.22	87.88	
22.4	16	435.51	6.33	81.65	
16	11.2	310.57	4.51	75.33	
11.2	8	348.04	5.06	70.81	29.69
8	4	667.42	9.70	65.76	
4	2	481.78	7.00	56.06	
2	1	546.21	7.94	49.06	
1	0.5	461.94	6.71	41.13	
0.5	0.25	416.22	6.05	34.41	23.55
0.25	0.125	361.92	5.26	28.37	
0.125	0.063	380.69	5.53	23.11	
0.063	0	1209.94	17.58	17.58	
		Σ 6883.1			

Based on the sieve analysis data from the project report, the empirical particle size distribution function by mass (Figure 4) was plotted.

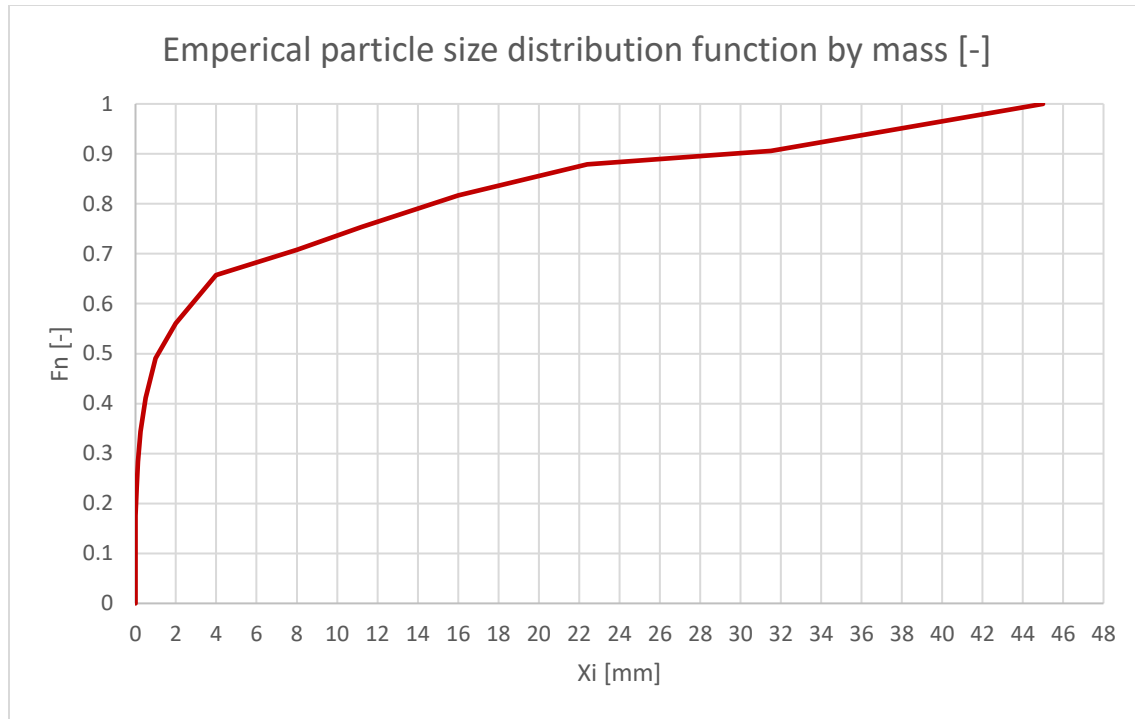


Figure 4: Particle size distribution of the sample based on sieve analysis

3.1.1.2 Density Fractionation

Each merged size fraction was subjected to density fractionation at 2.7 kg/dm^3 in Na-polytungstate solution to identify the presence of dense particles in each size fraction. The results of density separation indicate that particles larger than 11.2 mm contain no heavy particles (Table 5). Consequently, screening at 11.2 mm is validated as an initial unit operation to reduce material flow by 30% for subsequent bioleaching processes.

Table 5: Density separation in Na-polytungstate at 2.7 kg/dm^3 of joined size fractions

Size fraction	ΔM (%)	$\rho > 2.7 \text{ (kg/dm}^3\text{)}$	$\rho < 2.7 \text{ (kg/dm}^3\text{)}$
0-0.063	17.58	28.12	71.88
0.063-1	23.55	23.78	76.22
1-11.2	29.68	9.51	90.49
11.2-63	29.91	0	100

3.1.1.3 Sample preparation

The fraction less than 11.2 mm was subsequently dried and ground using a planetary ball mill to achieve a particle size of less than 0.5 mm.

3.1.1.4 Phase Analysis

Phase composition was determined by a project team for the particles < 0.5 mm by X-ray powder diffraction analysis on a Bruker D8 Discover instrument, with Cu-K α radiation (40 kV, 40 mA generator settings). The measurement was done in Bragg-Brentano geometry, with symmetric 2.5° axial Soller slits. Recording was done with LynxEye XE-T position sensitive detector (PSD), 2° window opening. The scan was recorded in the 2-110 °(2 θ) range, with 0.007 °(2 θ)/24 sec counting time. The powder specimen was arranged in top top-loaded shallow background Si single crystal sample holder. Identification of crystalline components was done by Search/Match, on ICDD PDF2 (2005) database in the Bruker DiffraC*Plus* EVA software. Quantitative composition was calculated with Rietveld refinement in Bruker TOPAS4, instrumental profile determined on SRM640a Si standard. Crystallographic information for the identified phases was taken from ICSD and AMCSD databases. Amorphous content was determined by the “Amorphous Hump” method. Results are shown in Table (6), which also contains the RDX results of the mine tailings.

Table 6: Phase analysis of the sample

Phase Name	Tailings < 0.5mm
Quartz	43.2
Chlorite IIb	5.6
Muscovite 2M1	8.8
Calcite	4.1
Albite	11.0
Hematite	0.8
Hornblende (Fe,Mg)	1.7
Smectite 15A	1.2
Illite 2M1	11.0
Magnesioferrite	-
Gahnite	-
Fassaite	-
Zircon	-
Amorphous	12.6
Sum	100.0

3.1.1.5 Chemical Composition Analysis of Feed

The sample was characterized by its chemical composition before subjecting to bioleaching to identify which elements were present and their quantity. An accredited laboratory, Kisanalitika Ltd., performed the chemical composition analysis of the tailing sample using a Spectrogreen FMX46 inductively coupled plasma optical emission spectrometer (ICP-OES). This analytical technique was chosen for its precision in detecting and quantifying trace metals in complex matrices, ensuring reliable measurements.

The chemical analysis of the tailing sample reveals that copper (Cu) is present at a concentration of 8680 mg/kg, the highest among the analyzed metals, suggesting that the sample is relatively enriched in copper. zinc (Zn) was detected at a concentration of 102 mg/kg, representing a comparatively significant presence, though much lower than that of copper.

Arsenic (As) is the second most abundant element in the sample, with a concentration of 3300 mg/kg, indicating a notable presence of this metalloid. lead (Pb) and gold (Au) were found at similar concentrations, measuring 26.6 mg/kg and 28.4 mg/kg, respectively, which are moderate in comparison to the more abundant elements.

Silver (Ag) was recorded at 4.37 mg/kg, making it the least concentrated metal in the sample. This distribution of metals highlights copper as the predominant element, with arsenic also present in substantial amounts, while silver is notably sparse in comparison to the other metals (Table 8).

Table 7: Chemical analysis of sieved feed

Metal	Cu	Zn	Pb	As	Au	Ag
Concentration (mg/kg)	8680	102	26.6	3300	28.4	4.37

3.1.2 Cultivation of Bacteria

Silverman-Lundgren's 9K nutrient solution was prepared to support the growth and activity of the bacterial culture. The composition of this nutrient medium per liter is as follows:

- Solution A: 3 g of ammonium sulfate - $(\text{NH}_4)_2\text{SO}_4$, 0.1 g of potassium chloride - KCl, 0.5 g of dipotassium hydrogen phosphate - K_2HPO_4 , 0.5 g of magnesium sulfate heptahydrate - $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0144 g of calcium nitrate tetrahydrate – $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, and 700 ml of deionized water.
- Solution B: 44.24 g of ferrous sulfate heptahydrate - $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 10 ml of 1N sulfuric acid - H_2SO_4 , and 300 ml of deionized water.

After combining Solutions A and B, which provide basal salts and an energy source for the bacteria, the pH of the final solution was adjusted to 2.1-2.2 with further H₂SO₄ (Silverman and Lundgren, 1959).

The bacterium *Act. ferroxidans* (Pech), isolated from acid mine drainage in Slovakia, was selected as the bioleaching agent. This particular species was chosen due to its demonstrated resilience in toxic heavy metal environments, as observed in the previous study with other *Acidithiobacillus* species. For inoculation, 10% of an actively cultured *Act. ferroxidans* in Silverman-Lundgren's 9K medium was used (Figure 5). To ensure a robust and viable culture, a double inoculation was performed at five-day intervals before the experiment, which allowed for bacterial regeneration and an optimal environment for leaching activity.



Figure 5: The culture of *Act. Ferroxidans* (Pech) in 9K which used as the inoculum

3.1.2 Bioleaching experiments

The bioleaching experiments were conducted in 250 ml Erlenmeyer flasks over 10 days using a Wise Cube Shaker set at 150 rpm rotation speed with the freshened culture of *Act. ferroxidans*. Based on the previous bioleaching studies in the literature, it can be observed that pulp density has a significant impact on mineral yield. Therefore, to assess the limitations of pulp density, three different solid concentrations; 1%, 5%, and 10% (m/v) were selected. Sterile, freshly prepared 9K solutions without *Act. ferroxidans* served as control setups, maintained at the same initial pH and in equivalent pulp densities. The room temperature ranged from 32°C to 36°C throughout the experiment.

Samples of 20 ml were extracted from the clear zone of the leachate at intervals on the 3rd, 6th, 8th, and 10th days. Samples including the control (9K) were given sample IDs based on the sampling date, and pulp density (Table 7). Each sample was subjected to pH and ORP

measurements since they can reflect the bioleaching efficiency. pH and ORP were measured by Hach Multimeter (with intellical PHC845 electrode).

The samples were acidified with 1-2 drops of nitric acid for conservation. The acidified samples were stored at 4°C before being transported for chemical analysis. After each sampling, 20 ml of sterile 9K solution was added back to the bioleaching flasks to maintain consistency in volume.

Table 8: Samples IDs of the experiment

Sampling date	Pulp density	Control (9K)	9K+Pech
3	1%	S1	P1
3	5%	S2	P2
3	10%	S3	P3
6	1%	S4	P4
6	5%	S5	P5
6	10%	S6	P6
8	1%	S7	P7
8	5%	S8	P8
8	10%	S9	P9
10	1%	S10	P10
10	5%	S11	P11
10	10%	S12	P12

At the end of the 10-day experiment, the leachate suspensions were filtered using Whatman filter papers and laboratory funnels. Solid residues retained on the filter paper were washed with deionized water and air-dried for 12 hours. The dry weight of these residues was recorded for future subsequent analysis.

3.1.3 Chemical assaying and mineral recovery calculation

Metal concentrations in each liquid leachate sample were analyzed by Kisanalitika Ltd. using a Spectrogreen FMX46 ICP-OES device.

Following the chemical assay results, mineral recovery rates were subsequently calculated for every mineral considering different pulp densities basically as follows (Equation 7,8 and 9):

$$Recovery (\%) = \frac{Mineral\ content\ in\ solution\ (mg)}{Mineral\ content\ in\ feed\ (mg)} * 100 \quad (7)$$

$$\begin{aligned} Mineral\ content\ in\ solution\ (mg)) \\ = Mineral\ concentration\ in\ liquid\ leachate\ (mg/l) \\ * volume\ of\ liquid\ leachate\ (l) \end{aligned} \quad (8)$$

$$\begin{aligned}
 & \text{Mineral content in feed (mg)} \\
 &= \text{Mineral concentration in feed (mg/kg)} \\
 & * \text{applied solid content based on pulp density (kg)}
 \end{aligned}
 \tag{9}$$

The flowsheet of applied methodology is as follows (Figure 6):

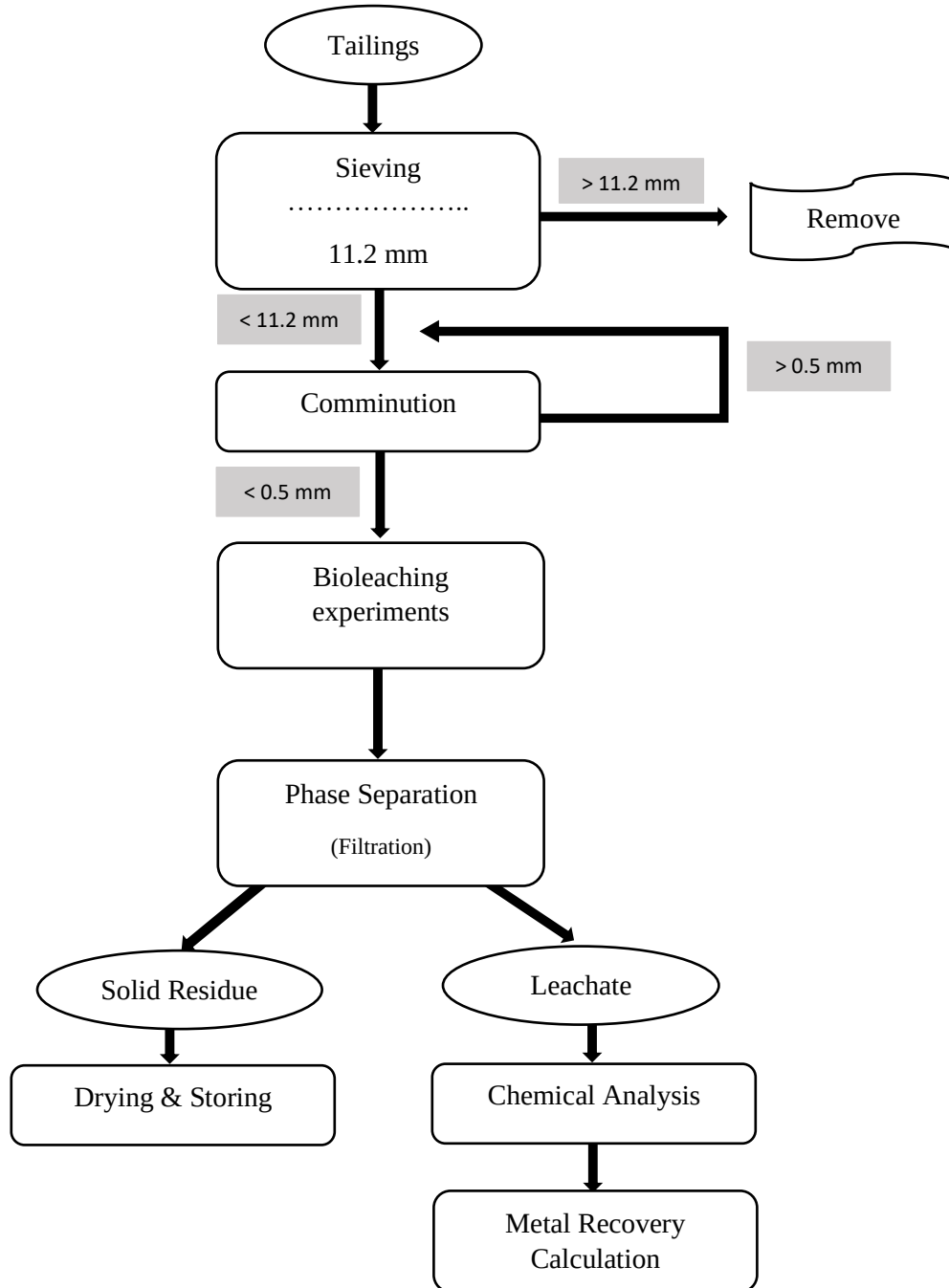


Figure 6: Flowsheet of the applied experimental methodology

Chapter 4: Results and Discussion

4.1 Mineral Recovery

After the 10-day residence time of the bioleaching and control experiments (Figure 7), metal recovery values were calculated based on the results of chemical analysis. The calculated recovery percentages after the 10-day experiment, presented in Table (9), were used to generate graphs depicting the recovery of each metal at different pulp densities in both the bioleaching experiments and the control setup.

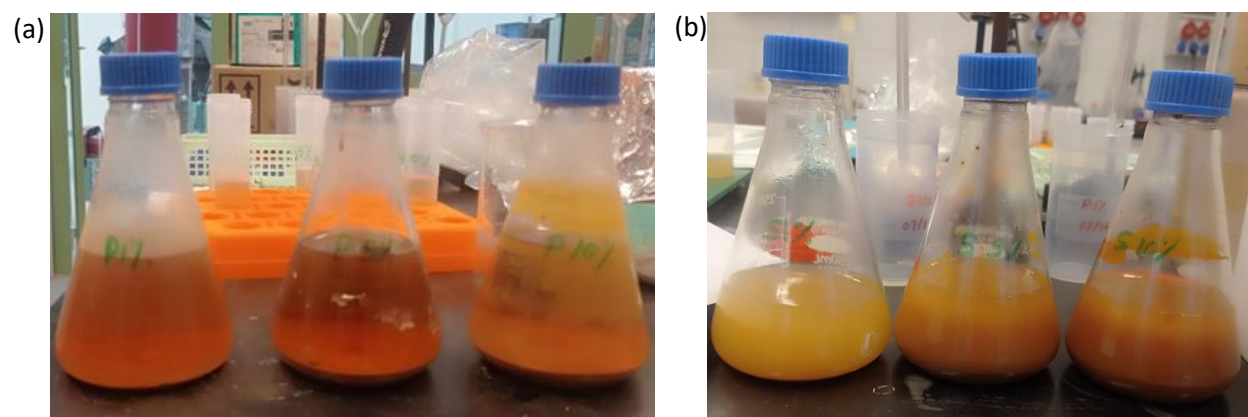


Figure 7: (a) Bioleaching samples (b) Control-sterile 9K samples at 1%, 5% and 10% pulp densities (standing from left to right) after 10 days of residence time

In examining the data, several outliers were identified, where metal recovery values exceeded 100%. Notably, in the bioleaching sample (9K + Pech), Pb at a 1% pulp density appeared as an outlier. Similarly, in the control setup, Pb and silver Ag at 1% pulp density were identified as outliers. These anomalous values are likely due to analytical inconsistencies during chemical assays or sampling errors. Therefore, the exceeding values were corrected to 100% when generating graphs. Increasing the number of replicates could help mitigate such variability and improve data reliability.

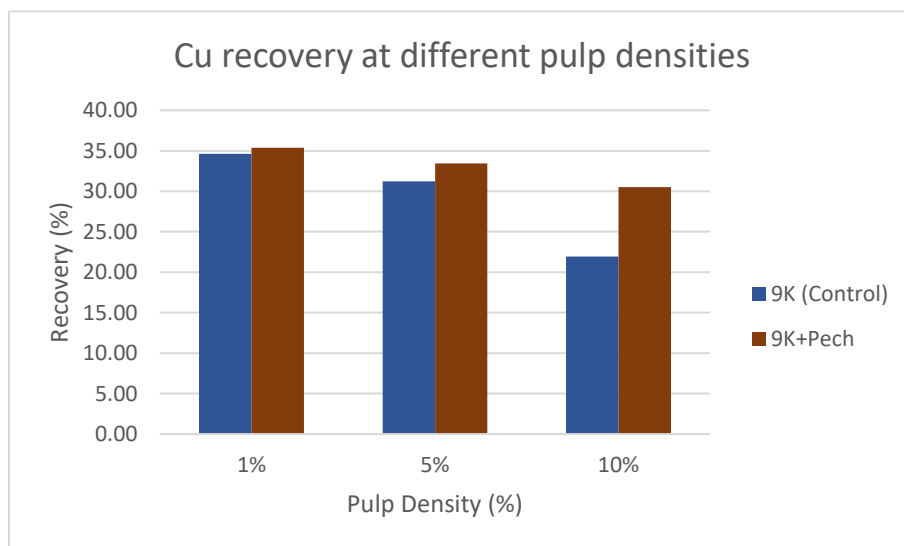
In certain instances, specific metals showed higher recovery rates in the sterile 9K solution (control) compared to the bioleaching samples. For example, Zn at a 1% pulp density, Pb across all three pulp densities, Au at a 1% pulp density, and Ag at a 1% pulp density demonstrated comparatively elevated recovery percentages in the sterile 9K solution. This discrepancy could be primarily attributed to the formation of jarosite, and co-precipitation of leached metal ions with jarosite. Additionally, the possibility of human error during the experiment may have contributed to these observed differences.

Table 9: Concentrations (mg/l) of metals in the leachate after the 10-day experiment and their recovery values.

ID	Cu		Zn		Pb		As		Au		Ag	
	*C (mg/l)	*R (%)	C (mg/l)	R (%)	C (mg/l)	R (%)	C (mg/l)	R (%)	C (mg/l)	R (%)	C (mg/l)	R (%)
P10	24.5	35.37	0.578	71.13	0.31	147.46	4.73	17.69	0.115	51.54	0.011	32.31
P11	114	33.44	0.925	22.88	0.275	26.61	12.5	8.72	0.355	31.96	0.014	8.09
P12	217	30.51	1.32	16.11	0.265	12.79	2.61	0.87	0.66	29.54	0.019	5.54
S10	23.7	34.64	0.668	81.42	0.53	249.02	1.51	5.56	0.132	58.42	0.338	785.72
S11	109	31.22	0.89	22.06	0.388	37.30	4.35	2.84	0.358	32.09	0.023	12.58
S12	166	21.92	1.2	14.53	0.34	16.45	0.563	0.19	0.64	28.37	0.02	5.86

*C indicates the concentration of particular metal in leachate and *R indicates the recovery % of respective metal

The recovery data indicate that most metals achieved higher recovery rates at a 1% pulp density, with a consistent decline in recovery as pulp density increased to 5% and 10%. This trend is particularly evident in the cases of copper (Cu) and gold (Au), both of which demonstrate notable recoveries across all pulp densities. Specifically, Cu recovery was 35.37% at 1% pulp density, slightly decreasing to 33.44% at 5% and 30.51% at 10%. Cu exhibited better solubility in bioleaching than chemical leaching (Figure 8).

**Figure 8: Recovery of copper at different pulp densities**

Similarly, Au showed a high recovery of 51.54% at 1%, which decreased to 31.96% at 5% and 29.54% at 10% pulp density. The elevated recovery of Au at 1% pulp density is especially noteworthy due to the high value of gold as a precious metal. Additionally, gold exhibited slightly lower solubility in bioleaching compared to chemical leaching at a 1% pulp density, suggesting the potential issue of co-precipitation with jarosite (Figure 9).

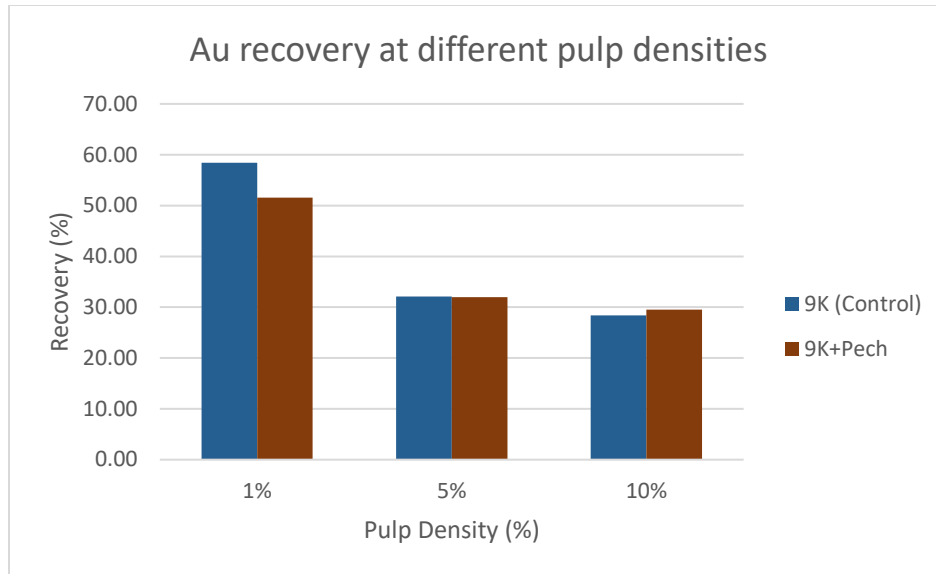


Figure 9: Recovery of gold at different pulp densities

Zinc (Zn) demonstrated a higher recovery rate at a 1% pulp density, reaching 71.13%, with recovery rates decreasing notably as pulp density increased. Additionally, Zn showed slightly higher leaching efficiency in the 9K medium compared to bioleaching conditions (Figure 10).

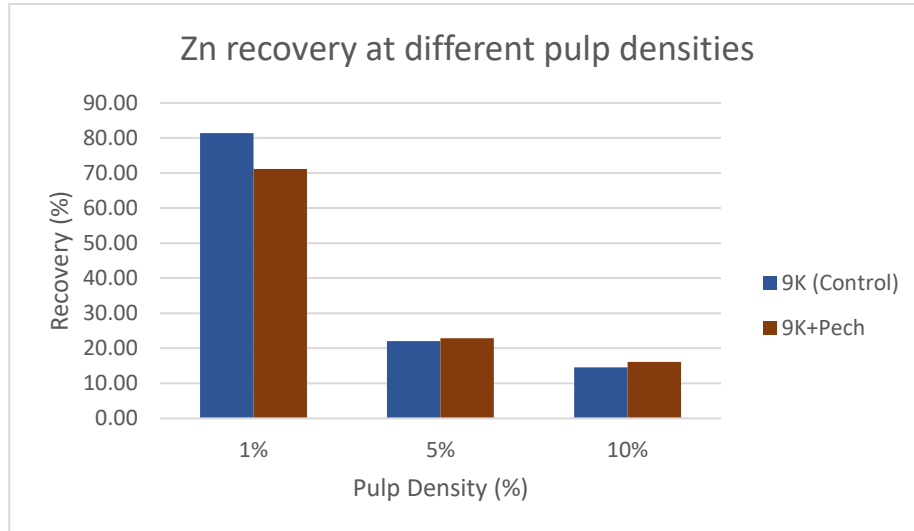
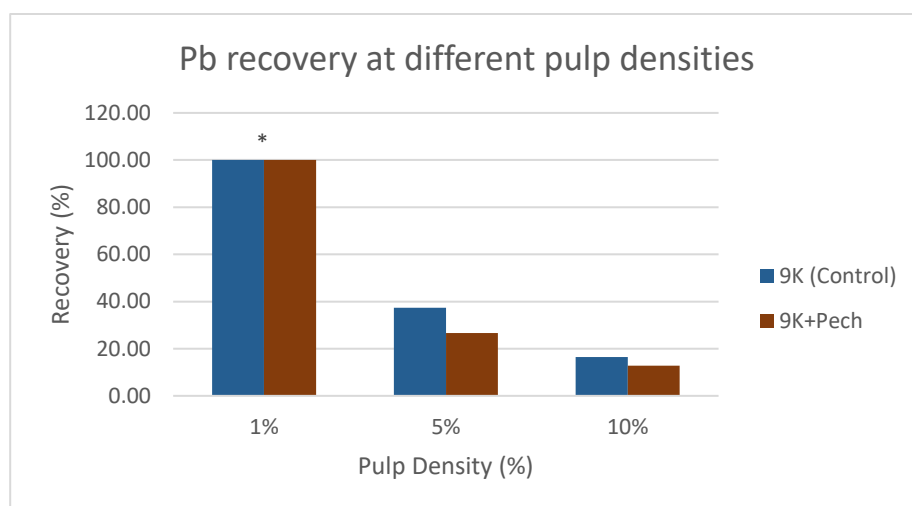


Figure 10: Recovery of zinc at different pulp densities

Lead (Pb) exhibited maximum possible recovery at 1% pulp density while resulting in moderate recoveries at 5% and 10% pulp densities (Figure 11).



*Recovery rate has been adjusted to 100%

Figure 11: Recovery of lead at different pulp densities

Arsenic (As) generally exhibited lower recovery rates across all pulp densities, with moderate solubility observed at a 1% pulp density. Notably, arsenic showed its lowest recorded recovery at a 10% pulp density, indicating limited extraction efficiency under higher pulp density conditions (Figure 12).

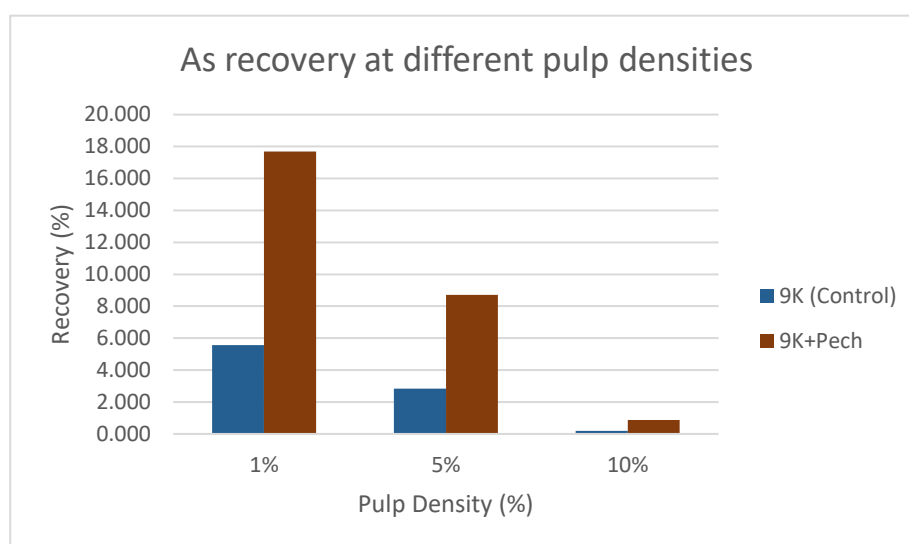
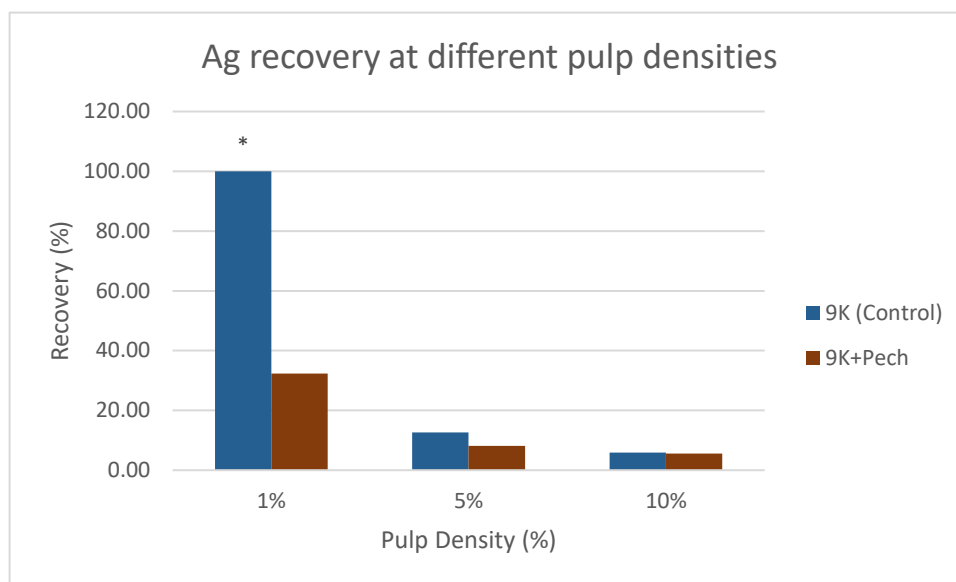


Figure 12: Recovery of arsenic at different pulp densities

Silver (Ag) demonstrated significant recovery at 1% pulp density, with recovery decreasing as pulp density increased. Additionally, Ag exhibited greater solubility in the 9K medium than in bioleaching, achieving full recovery under chemical leaching conditions with 9K (Figure 13).



*Recovery rate has been adjusted to 100%

Figure 13: Recovery of silver at different pulp densities

These findings highlight the significant impact of pulp density on metal recovery efficiency. The overall trend suggests that lower pulp densities may enhance metal recovery, particularly for economically valuable metals such as Cu, Au, and Zn, which benefit from higher recoveries under these conditions. Conversely, higher pulp densities appear to hinder recovery, potentially due to increased solid-to-liquid ratios that limit metal dissolution or hinder microbial activity during bioleaching.

4.2 pH and ORP Analysis

4.2.1 pH

The bioleaching samples exhibited an initially elevated pH level, which subsequently decreased and stabilized, averaging around 1.9 on the first day of sampling and later settling within a steady range of 1.7–1.77 (Figure 14). The initial rise in pH is likely due to the consumption of hydrogen ions during the oxidation of ferrous to ferric ions since *Act. Ferrooxidans* (Pech) utilize ferrous ions as their energy source. Following this, ferric ions undergo hydrolysis, releasing hydrogen ions back into the solution, thus would be the reason for lowering the pH. 9K medium can undergo chemical leaching which is known as ferric leaching by using ferric ions as the main oxidant. Therefore, the samples containing only 9K medium showed a similar trend, though with a distinctly higher pH range than the bioleaching samples, which suggests the presence of *Act. Ferrooxidans* which is the iron-oxidizing bacteria, significantly accelerated the bioleaching process. This indicates the effective role of these bacteria in facilitating the biochemical reactions

necessary for metal recovery. As found in the literature, jarosite is likely to form at a pH above 2.8, indicating that the pH of the experiments has not contributed to the jarosite formation.

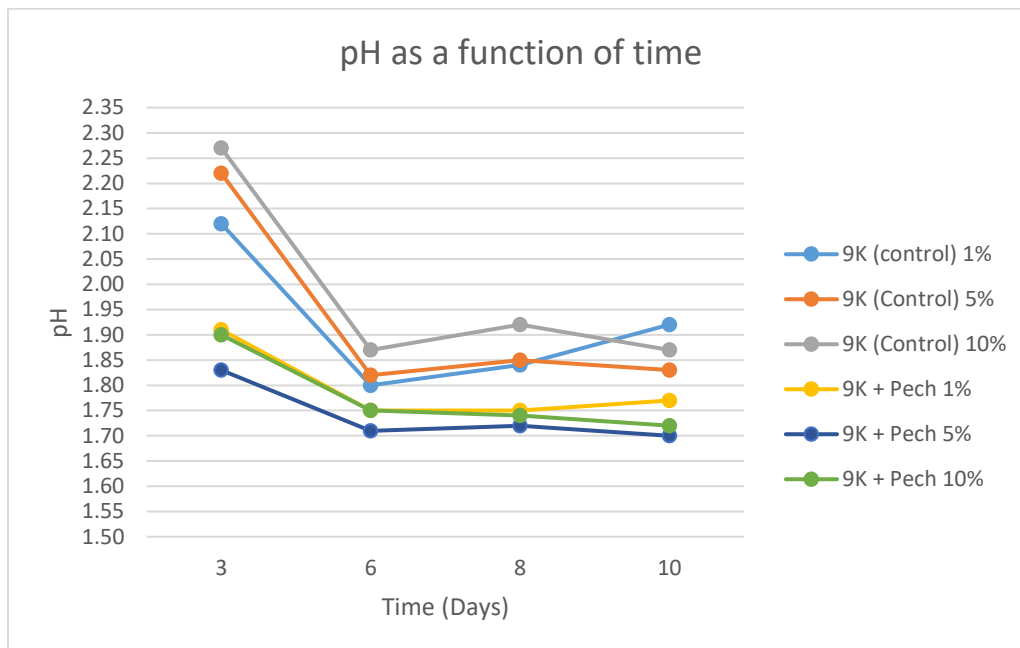


Figure 14: pH of samples both control and bioleaching as a function of time

4.2.2 ORP

The ORP measurements (Figure 15) reveal distinct ranges for the bioleaching samples compared to the control setup. Specifically, bioleaching samples displayed ORP values between 401.4 and 585.6 mV, whereas the control setup containing only the 9K medium showed a range of 419.2 to 331.8 mV. Literature indicates that an ORP range of 400-500 mV is optimal for copper (Cu) recovery. The bioleaching sample with 1% pulp density displayed an ORP above the optimal range, indicating the need for a control mechanism to maintain the ORP within the ideal range. According to the literature, jarosite formation typically occurs within an ORP range of 360-800 mV. Given that most ORP values measured in this study fall within this range, it is likely that jarosite has formed, with ORP levels playing a significant role in facilitating this process.

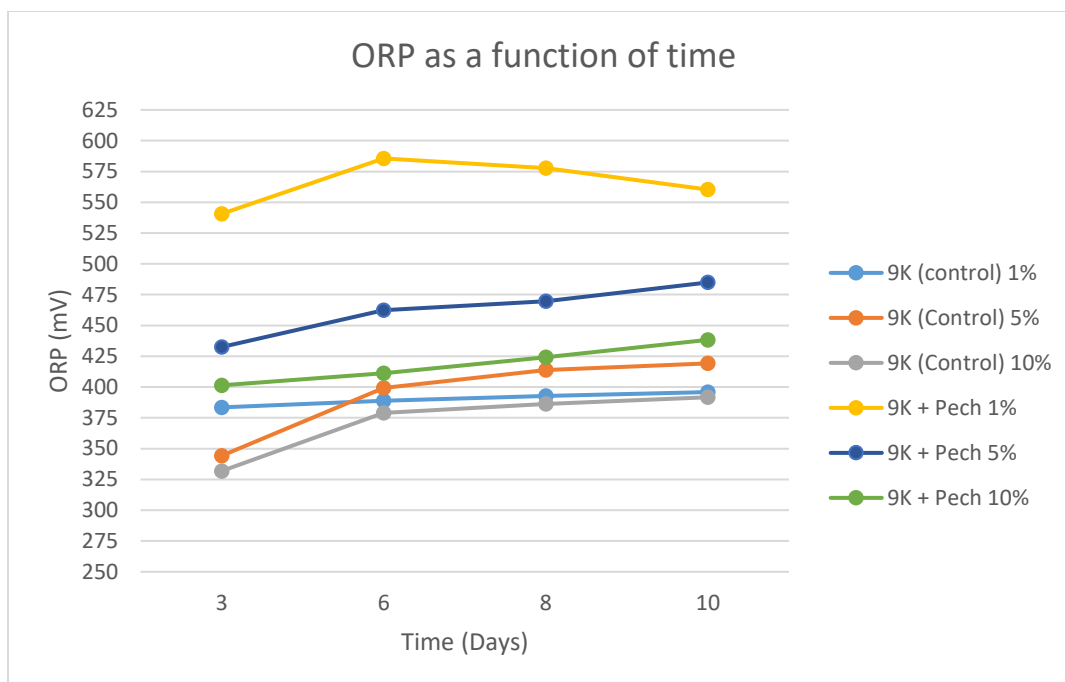


Figure 15: ORP of samples both control and bioleaching as a function of time

4.3 Solid residues

The solid residues remaining after the 10-day bioleaching setup, following filtration, washing, and drying, show a distinct colour difference between the bioleaching and the control samples. Specifically, the residues from the bioleaching experiments display a darker colour compared to those in the control setup (Figure 16).

This colour variation likely reflects differences in the composition of the residues, as the residue typically contains a combination of un-leached feed material and precipitates formed during the leaching process. The darker colour in the bioleached residues suggests a greater extent of jarosite precipitation in the bioleaching samples than in the control setup, which was treated solely with 9K medium.

Additionally, there is a possibility that the leached metals themselves could have precipitated along with the jarosite or become associated with un-leached feed particles, rather than remaining dissolved in the leachate. This would mean that some metals, despite being mobilized during bioleaching, may have re-precipitated, potentially reducing their concentration in the solution phase and impacting overall recovery.

The formation of jarosite and potential metal re-precipitation could be indicative of environmental conditions within the bioleaching setup that favour the stabilization of metals as solid-phase precipitates. This process may be interfered with the dissolution of metals, possibly hindering the bioleaching efficiency. The observed colour difference, therefore, not only highlights the chemical

changes in the residue composition but also suggests that jarosite formation and metal precipitation play roles in the bioleaching dynamics, affecting the recovery potential of the system.

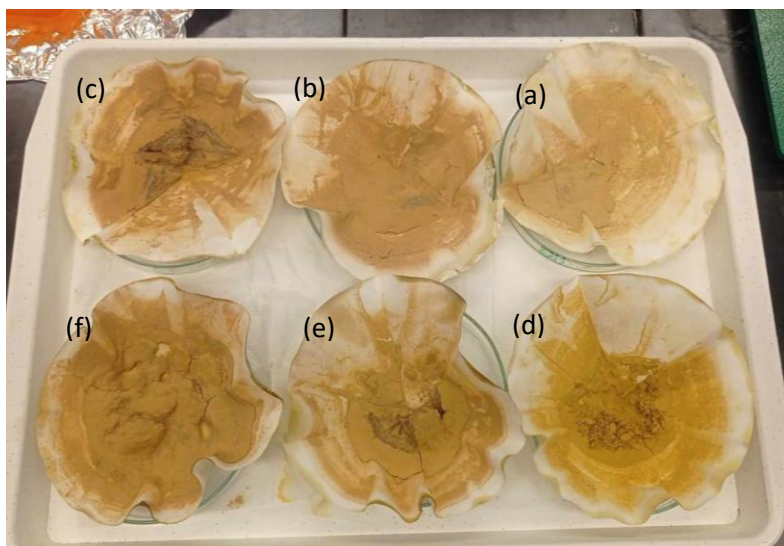


Figure 16: Open air-dried filtrate after the 10-day experiment (a) 9K-Control at 1% (b) 9K-Control at 5% (c) 9K-Control at 10% (d) 9K+Pech at 1% (e) 9K+Pech at 5% (f) 9K+Pech at 10% pulp density

Chapter 5: Conclusions

The study aimed to *identify* the feasibility of bioleaching for extracting valuable minerals from tailings at the historic Leoben mine site. Initial characterization of the samples via XRD, optical microscopy, and chemical analyses revealed the presence of valuable metals, including copper, zinc, gold, and silver. Following this, batch kinetic bioleaching tests with *Acidithiobacillus ferrooxidans* (Pech) were conducted alongside sterile 9K medium (control) tests at varying pulp densities.

Results indicated that the solubility of all the minerals was decreased when increasing the pulp density. Bioleaching proved more effective than chemical leaching, particularly for copper at increased pulp densities, while arsenic was significantly better leached in bacterial conditions compared to the sterile 9K solution. In contrast, zinc, silver, and gold recovery was higher in the sterile 9K experiment. Pb exhibited the same solubility in both experiments.

The bioleaching experiments achieved approximately 35% recovery of copper, 70-80% of zinc, 50-60% of gold, and 30% of silver. Arsenic recovery reached 18%, and its role in potentially inhibiting bacterial action remains inconclusive. Notably, the high recovery rates for copper, zinc, and especially gold underscore the potential of bioleaching for these metals, with gold highlighted as a promising target for future studies due to its high economic value.

The pH remained optimal throughout the experiment; however, some samples exhibited ORP values within a range conducive to jarosite formation, and outside the optimal range for copper

leachability. This highlights the need for a mechanism to control the ORP. Overall, the findings establish a prospective basis for advancing bioleaching as a sustainable approach for metal recovery from Leoben mine waste.

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