

Exploring the potential of acidithiobacillus in bioleaching: keys to sustainable metal extraction

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ABSTRACT

The Acidithiobacillus genus is a set of acidophilic bacteria that has a crucial participation in the bioleaching process, because they can oxidize iron and sulfur and contribute to the sulfide mineral dissolution, releasing metals such as: copper, gold, and other valuable items. This systematic review compiled the environmental factors and optimal conditions for the use of the Acidithiobacillus proteobacteria in the bioleaching process in the Scopus database since January 2014 until December 2024. PRISMA's guidelines were used for the identification, selection, and analysis of relevant primary studies. The results and discussions of each article were examined, synthesizing the answers to the questions posed and highlighting the most relevant findings along with their implications for the field of biomining. In conclusion, it is evident that biomining with Acidithiobacillus emerges as an innovative and sustainable alternative for the metal extraction, offering environmental, economic and technical advantages compared to traditional methods. Despite it, the associated technical challenges are recognized, such as the control of leaching conditions, where factors such as: pH, temperature, redox potential, oxygen concentration, and the presence of metal ions can influence the growth and the activity of Acidithiobacillus, as well as the kinetics and leaching mechanisms. Research opportunities were identified for improving this innovative technology.

Keywords: Sustainable biomining; Bioleaching; Acidithiobacillus; Heavy metals; Systematic review.

1. Introduction

The Acidithiobacillus genus belongs to the phylum Proteobacteria. These bacteria can oxidize sulfide compounds and play an important role in the bioleaching of minerals [1, 2]. Acidithiobacillus spp. are particularly relevant in the bioleaching of heavy metals such as cadmium, copper, cobalt, and nickel. Therefore, the application of this technique is essential for both metal recovery and environmental bioremediation [3, 4]. In addition, Acidithiobacillus species contribute to bioleaching and biological desulfurization processes through their ability to oxidize sulfur compounds. This metabolic activity promotes the solubilization of metals such as copper during bioleaching [5, 6]. Moreover, these bacteria can convert sulfur dioxide into elemental sulfur during the desulfurization, providing a sustainable approach to reduce air pollution [7, 8].

The Acidithiobacillus genus plays important ecological roles as sulfur-oxidizing bacteria. They are key in processes such as bioleaching and biological desulfurization [9]. Their physiological diversity allows them to adapt and to diverse environmental conditions, including different temperatures and pH, which gives them metabolic versatility for the oxidation of sulfur compounds and the solubilization of metals in industrial processes [10, 11]. Furthermore, the formation of biofilms contributes to their survival in extreme environments. These characteristics make the Acidithiobacillus genus a fundamental player

in industrial applications such as the valuable metals recovery [12, 8].

Biomining is a technology that uses biological systems to recover and extract metals from waste minerals generated by mining activities [13]. The term "biomining" is often used synonymously with bioleaching. However, bioleaching specifically refers to the process in which metals are solubilized during bioprocessing or biological processing [14].

Bioleaching is a widely used technology that allows the efficient extraction of low-quality and low-concentration copper ores. This technique offers both economic and environmental advantages [15, 16]. However, the limited efficiency of bioleaching restricts its industrial application [17].

The acidophilic proteobacteria of the genus Acidithiobacillus spp. from the Proteobacteria family, play a critical role in bioleaching processes [7]. These bacteria are extremely resistant to acidic environments and can survive and thrive at very low pH conditions (< 2), which is common in mineral leaching [19]. Acidithiobacillus ferrooxidans is one of the best-known representatives of this genus. It has been extensively studied because it can oxidize iron and sulfur, contributing to the dissolution of sulfide minerals and releasing metals such as copper, gold, and other valuable elements [14, 20, 21]. Furthermore, Acidithiobacillus ferrooxidans can extract these metals from metal sulfide minerals at temperatures around 30 °C [14, 19, 22-

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25]. *Acidithiobacillus ferrooxidans* oxidize ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). This process not only improves the solubility of metals but also facilitates the dissolution of sulfide minerals such as bornite and chalcopyrite [26]. This oxidation is essential because it converts copper minerals and other metals into soluble forms that can be more easily recovered [27]. On the other hand, *Acidithiobacillus ferrivorans* also contributes to bioleaching, although has been less extensively studied. This species is effective in iron oxidation and can be used in the recovery of metals such as gold and uranium. Its activity expands the application potential of *Acidithiobacillus* species in modern mining [28, 29]. The ability of these bacteria for oxidizing sulfur compounds, such as hydrogen sulfide (H_2S) and sulfite (SO_3^{2-}) is also crucial. This process generates sulfuric acid, which in turn contributes to metal solubilization [30].

The metabolic diversity of these bacteria, together with their ability to adapt to different environmental conditions, highlights the importance of selecting strains appropriately to optimize metal recovery processes [30, 31]. Continued research on *Acidithiobacillus* species and their mechanisms of action is essential for improving bioleaching efficiency and promoting more sustainable mining practices.

The study of *Acidithiobacillus* species in bioleaching and biomining is important for understanding their physiological diversity and specific ecological roles in these industrial processes [32]. Additionally, it provides crucial information about the effectiveness of different *Acidithiobacillus* species in sulfur oxidation, metal solubilization, and their adaptability to extreme conditions. This knowledge can also lead to improvements in metal extraction efficiency and the sustainability of these processes. Considering the aforementioned information, the main purpose of this systematic review is to explore and evaluate the use of the genus *Acidithiobacillus* spp. in bioleaching. This biomining technique is innovative, focusing on its impact on metal recovery and its potential for environmental bioremediation [33, 34]. To address this topic thoroughly, a detailed analysis of the scientific literature from the last 10 years, available on the Scopus platform was conducted. The study aimed to identify the environmental factors and optimal conditions that play a fundamental role in the bioleaching process mediated by the *Acidithiobacillus* proteobacteria. This approach allows a deeper understanding of the relevance and potential of this technique for sustainable metal recovery and the mitigation of adverse environmental impacts [4, 35].

2. Methodology

The PICOS (Population, Intervention, Comparison, Outcome, and Study design) guidelines allow the formulation of the research questions in a precise and structured manner [36]. This approach allowed to clearly define the inclusion and the exclusion criteria of the relevant studies for this review [37], as well as a transparent and objective evaluation of the quality of the collected evidence. The PICOS guidelines are part of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology, which allows the selection and analysis of the most appropriate articles to the study topic. PRISMA provides a rigorous and transparent framework for the identification, selection, evaluation, and synthesis of relevant scientific literature, ensuring the quality and integrity of the systematic review process [38].

2.1. Formulation of questions with the PICOS guidelines

The application of the PICOS guidelines is in Table 1. Based on PICOS's guidelines, the questions were formulated as follows:

- Question 1: How many species of the *Acidithiobacillus* genus with biomining potential have been identified and what detailed information is available on their taxonomy and genome?
- Question 2: What are the specific environmental conditions necessary for the occurrence of bioleaching optimally using *Acidithiobacillus* bacteria?
- Question 3: What are the limitations and technical challenges associated with the use of *Acidithiobacillus* in bioleaching?

- Question 4: What are the environmental effects of bioleaching and the use of *Acidithiobacillus* compared to conventional metal extraction methods?
- Question 5: What is the currently known genetic mechanism of bioleaching of the *Acidithiobacillus* genus and what are the key aspects that remain to be understood in order to achieve a complete understanding?
- Question 6: What are the efficiencies achieved in biomining using *Acidithiobacillus* and how do they compare with those obtained using other chemical methods?

2.2. Initial search

The Scopus database was used after the definition of the inclusion criteria, keywords, types of documents, and the 11-years period (January 2014 - December 2024) for the selection of the articles. Biomining was considered as a general topic of study and keyword, obtaining 489 articles as the first result. The inclusion criterion was then used and the bioleaching technique was defined. The selection of the articles continued, specifying the metal (copper) and the bacteria used in this process (*Acidithiobacillus*). Finally, as part of the initial search, a range was delimited for the year of publication of the articles between January 2014 and December 2024 (11 years). This study only worked with Scopus. Duplication of articles were not found.

2.3. Systematic Literature Search based on PRISMA declaration

The keywords combination was: TITLE-ABS-KEY ((biomining OR bioleaching) AND *acidithiobacillus* AND (metal OR taxonomy OR mechanism)) AND PUBYEAR > 2013 AND PUBYEAR < 2025 AND (LIMIT-TO (LANGUAGE, "English")). The 625 documents were obtained in Scopus, which is a database that is especially useful for conducting research, evaluating the scientific production of institutions and authors, and for making informed decisions in the academic field; furthermore, it covers a wide range of disciplines and offers a multidisciplinary view of research [39, 40].

2.4. Inclusion criteria

It was considered that the keywords were found within the title or were included within the abstract of the article. Likewise, a range of publication years was delimited from 2014 to 2024. Only scientific articles in English were chosen. Only articles that provided relevant information for answering the research questions of the study were included.

2.5. Exclusion criteria

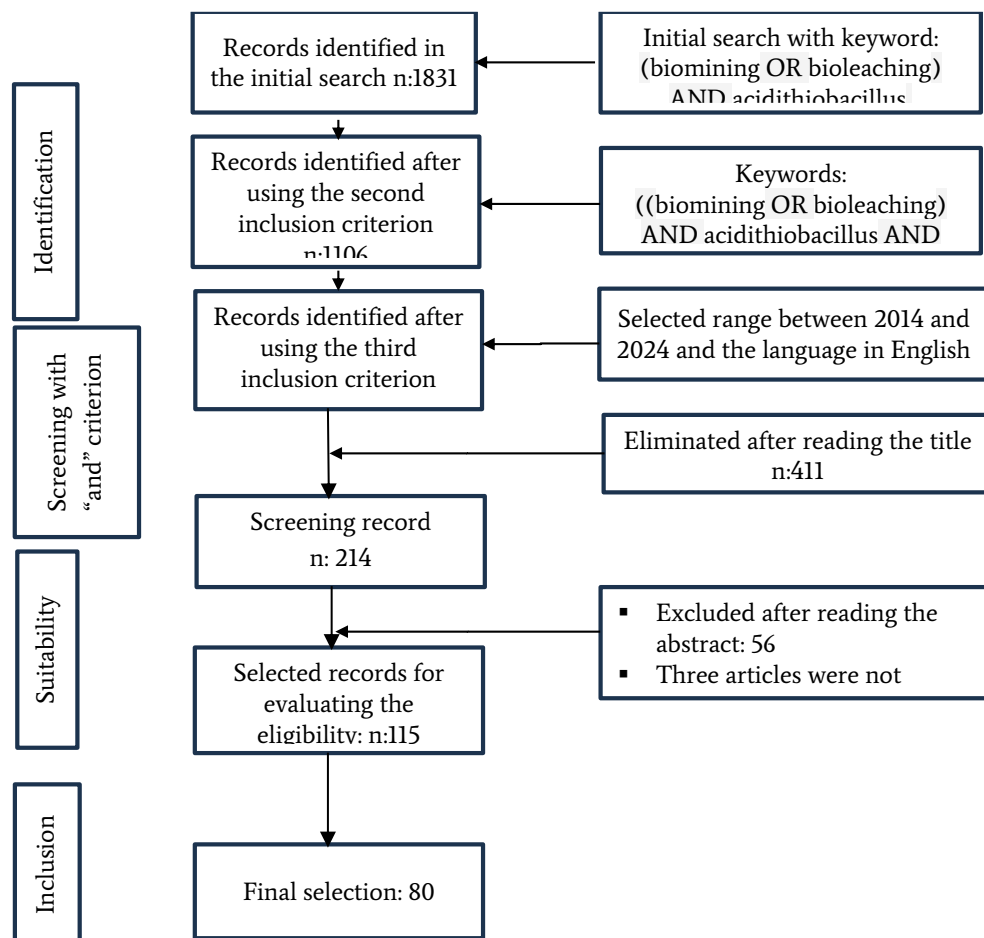
PRISMA was applied to select relevant articles on the use of *Acidithiobacillus* in sustainable and innovative biomining. Studies related to bacteria different to *Acidithiobacillus* used in bioleaching and papers that did not answer the research questions were not included. Researches that did not mention biomining, bioleaching, *Acidithiobacillus*, and metals in the title or abstract were discarded. Following these steps, the selection of relevant articles was guaranteed and the exclusion of those that do not contribute to the purpose of the study about the potential of *Acidithiobacillus* in sustainable and innovative biomining. With these criteria, a total of 58 articles were obtained that were useful in answering the research questions (Figure 1).

2.6. Article selection

The details of the article selection process are described in Figure 1. The document has 128 references, of which 80 were used for answering the six research questions. The other 48 articles were used for the following sections: (a) introduction; (b) methodology; (c) challenges, perspectives and opportunities section; (d) conclusions; and (d) recommendations for future research.

Table 1. Description of the components of the PICOS guidelines.

Guideline	Interpretation
Population	Bioleaching in the mining industry understands and evaluates the use of <i>Acidithiobacillus</i> in metal extraction, its environmental impact, and the growing need for sustainable methods in mining.
Intervention	The use of the Proteobacteria <i>Acidithiobacillus</i> is a preventive and environmental protection initiative for the production processes, goods, or services for controlling environmental pollution.
Comparison	The use of traditional mining extraction methods allows the evaluation of the effectiveness and viability of the use of the <i>Acidithiobacillus</i> genus of proteobacteria in bioleaching, highlighting its advantages and limitations in relation to existing alternatives.
Outcome	Effectiveness of the <i>Acidithiobacillus</i> proteobacteria used in bioleaching and efficiency of the process and its environmental impact, evaluating its potential in the mining industry and its contribution to environmental sustainability.
Study type	The systematic literature review examines and synthesizes previous research on the use of Proteobacteria <i>Acidithiobacillus</i> in mining bioleaching, providing a comprehensive and objective assessment of the available evidence for informing its efficacy and applications in industry.

**Figure 1.** Delimitation of the selected articles.

3. Result and Discussion

The obtained information was selected and organized in an MS Excel table that included: article number, author, article title, publication year, place of study, and abstract. Then, the articles were analyzed for answering each question.

Question 1: How many species of the *Acidithiobacillus* genus with biomining potential have been identified and what detailed information is available on their taxonomy and genome?

The main species of the *Acidithiobacillus* genus that have a notable potential in biomining applications are in Table 2. These species are crucial in bioleaching processes, where they are used for the extraction

of metals from ores through the oxidation of iron and sulfur compounds [7]. *Acidithiobacillus* species exhibit a remarkable metabolic diversity, allowing them for adapting to different environments and extreme conditions. Thus, *Acidithiobacillus caldus* is a thermophilic species that thrives at elevated temperatures. In contrast, *Acidithiobacillus thiooxidans* is specialized in oxidizing elemental sulfur. This metabolic diversity is fundamental for bioleaching applications, since different minerals and environmental conditions require specific metabolic capacities [41, 42].

Acidithiobacillus ferrooxidans is particularly relevant in this context. Its ability to oxidize iron and sulfur compounds allows the release of metals from ores [31]. This process not only improves extraction

efficiency but also reduces the need for aggressive chemical treatments, making mining more sustainable. *Acidithiobacillus ferridurans* and *Acidithiobacillus ferrivorans* are examples of less studied species that show significant potential in the bioleaching of metals such as gold and uranium [43, 44].

Like other species of the genus, *Acidithiobacillus albertensis* thrives in extremely acidic conditions. This makes it ideal for bioleaching in mines where mineral deposits occur in environments unsuitable for less adapted microorganisms [45]. The potential use of *Acidithiobacillus ferriphilus* in the bioleaching of metals such as gold and copper is remarkable. This species is especially relevant in modern mining, where the search for more sustainable and efficient methods is crucial [46].

Strain CJ-2^T (*Acidithiobacillus sulfuriphilus*) is a novel species of the *Acidithiobacillus* genus, which is a strict aerobe that oxidizes zero-valent sulfur and reduced inorganic sulfur compounds but does not use ferrous iron or hydrogen as electron donors. Strain S30A2T (isolated from the acid drainage sediment of Mengzi copper mine, Yunnan) represents a novel species of the sulfur-oxidizing *Acidithiobacillus* genus. The latter two represent novel sulfur-oxidizing species of the *Acidithiobacillus* genus, but their potential in bioleaching remains to be studied. The isolation of species of the *Acidithiobacillus* genus is essential for

advancing in biomining, providing more sustainable and efficient methods for metal extraction [47]. Members of the genus *Acidithiobacillus* (currently placed in the class *Acidithiobacillia*) are key bacteria for studying chemolithotrophic energy conversion in extreme environments. However, knowledge about the genomic and taxonomic diversity of *Acidithiobacillia* remains limited [48].

The “Type species or type strain” column in Table 2 is fundamental in microbial taxonomy because it identifies the reference strain of each genus. This classification serves as a basis for identifying and comparing other strains within the same group. Such comparison is particularly useful in the context of biomining. The inclusion of new species reflects the ongoing evolution of microbiological knowledge and highlights the importance of characterizing microorganisms with biotechnological potential. In addition, the “NCBI RefSeq/GenBank accession” column provides access numbers to genomic sequences in databases such as GenBank and RefSeq. These records allow researchers to obtain genomic data, validate findings, and perform comparative analyses more efficiently. This accessibility promotes study reproducibility, supports scientific validation, and facilitates collaboration between researchers, thereby advancing biomining and fostering new biotechnological applications.

Table 2. Species of the genus *Acidithiobacillus* that have biomining capacity: Type species or type strain and NCBI RefSeq/GenBank accession.

Taxonomic assignment	Characteristics	Biomining Capacity	Type Species or Type	NCBI RefSeq/GenBank accession
<i>Acidithiobacillus ferrooxidans</i>	Gram-negative, chemoautotrophic, iron and sulfur oxidizing bacterium.	Used in copper bioleaching [31]	ATCC 23270 ^T	CP001219 [48, 49]
<i>Acidithiobacillus thiooxidans</i>	Oxidizes elemental sulfur compounds, chemoautotrophic	Metal recovery [42]	ATCC 19377 ^T	AFOH01 [48, 50]
<i>Acidithiobacillus caldus</i> / <i>Fervidacidithiobacillus caldus</i>	Thermophilic, iron and sulfur oxidizing at high temperatures.	Represents a new species of the sulfur-oxidizing genus <i>Acidithiobacillus</i> . in	ATCC 51756 ^T	CP005986-9 [48]
<i>Acidithiobacillus ferridurans</i>	Oxidizes iron and sulfur compounds, present in acidic environments	Low-grade uranium bioleaching [43]	ATCC 33020 ^T	JABBHQ00 [48, 51]
<i>Acidithiobacillus albertensis</i>	Oxidizes iron and sulfur compounds, present in acidic environments.	Extraction of copper and other precious metals [45]	DSM 14366 ^T	MOAD01 [48, 52]
<i>Acidithiobacillus ferrivorans</i>	Oxidizes iron and sulfur compounds, present in acidic environments.	Potential in bioleaching of Copper, Zinc and Gold [44]	DSM 22755 ^T	JAAOMR00 [28, 48]
<i>Acidithiobacillus ferriphilus</i>	Catalyzes the dissimilatory oxidation of ferrous iron and elemental sulfur, as well as	Potential in bioleaching of Copper and Gold [46]	DSM 100412 ^T	JAAZTY00 [46]
<i>Acidithiobacillus sulfuriphilus</i> sp. nov./ <i>Ambacidithiobacillus</i>	Oxidizes zero-valent sulfur and inorganic sulfur compounds	Isolated from a sample of lead mine drainage [11]	CJ-2 ^T	RIZI01 [11, 48]
<i>Acidithiobacillus acidisediminis</i> sp. nov.	Sulfur oxidizer isolated from acid mine drainage sediment	Isolated from mine drainage sediment copper [53]	S30A2 ^T	JALQCS000000000 [53]

Question 2: what are the specific environmental conditions necessary for the occurrence of bioleaching optimally using acidithiobacillus bacteria?

Table 3 shows that the specific environmental conditions required for optimal bioleaching using *Acidithiobacillus* bacteria. An acidic pH, generally between 1.5 and 3.5, is crucial for the metabolic activity of these microorganisms. In addition, the ideal temperature is usually between 20 °C and 35 °C, although some species can tolerate higher temperatures. However, the presence of oxygen is also essential. These bacteria are aerobic and require oxygen both for their metabolism and for oxidizing metal sulfides [54, 55]. Likewise, the concentration of heavy metals in the medium must be controlled, because excessive levels can be toxic and negatively affect bacterial activity [24, 56]. Likewise, the availability of nutrients (such as nitrogen and phosphorus) is essential for the growth and reproduction of *Acidithiobacillus*. This nutrient availability directly influences the efficiency of the bioleaching process [57].

The knowledge of how acidophiles survive in acidic and metal-rich places is important for a better approach to metal biomining. For improving these procedures, it still will be necessary to better understand the geochemical characteristics of the sediment, the partitioning of contaminating metals, and the biological mechanisms involved [58]. Likewise, several aspects must be considered. These

include the selection of suitable microorganisms, the optimization of growth conditions, the choice of energy sources and mineral types, and the understanding of sediment geochemistry. It is also necessary to study the partition of contaminating metals and the biological mechanisms involved [58, 59]. The conditions of the applications of the *Acidithiobacillus* genus of bacteria for the bioleaching processes are in the Table 3.

A study evaluated the efficiency of metal solubilization in bioleaching processes using pure and mixed cultures of *Acidithiobacillus ferrooxidans* and *A. thiooxidans*. Both species were applied to the same mine tailings under identical conditions. The results revealed the importance of selecting appropriate bacterial strains and providing optimal growth conditions to achieve satisfactory metal solubilization and recovery [61]. On the other hand, it is important to guarantee the supply of oxygen and carbon dioxide, which are essential for the growth and oxidation of iron and sulfur by biomining microorganisms [63].

Research Question 1 focuses on the specific environmental conditions necessary for bioleaching with *Acidithiobacillus* bacteria optimally. Some of the environmental factors that influence bioleaching are temperature, pH, oxygen concentration, nutrient concentration, and the presence of toxic metals. It has been shown that the *Acidithiobacillus* bacteria is capable of growing and oxidizing iron and sulfur in a pH range between 1.5 and 4.5, and at temperatures between 20 °C and 45 °C.

Table 3. Evaluation of the selected Scopus articles for the first research question.

Conditions	Description	Importance	Author
It is one of the most studied species, due to its ability for surviving in spaces with a high concentration of metals. It lives in environments with pH levels 1.5–2.5 and has an autotrophic chemolithotrophic metabolism that uses electrons from different inorganic compounds.	This bacterium generates the formation of biofilms that adhere to the surfaces of metals, creating a link between the surface of the minerals and the rest of the microorganisms present in these acidic environments.	Allows survival in acidic environments	[25]
Acidophilic bacteria such as <i>Acidithiobacillus</i> thrive in environments with low pH [1.8, 2.5], because the bacteria are acidophilic and cannot grow below 1.2 or above 4.0. Additionally, the optimal temperature for growth in controlled environments is 30°C, but they can survive to 40°C.	<i>Acidithiobacillus ferrooxidans</i> and <i>Leptospirillum ferrooxidans</i> oxidize iron in an acidic environment and are thermophiles.	Facilitates iron oxidation activity and bacterial growth. Ensures favourable conditions for metabolic activity and efficient bioleaching.	[59]
The study describes that acidophilic bacteria (more specifically <i>Acidithiobacillus thiooxidans</i>) has optimal growth in the temperature range of 25–35°C and an optimal pH between 1 and 4.	Compared with <i>At.ferrooxidans</i> , <i>L. ferriphilum</i> has a great ability to withstand extreme conditions, such as low pH, high oxidation-redox potential and high temperature.	Greater adaptability	[60]
Within the parameters are described the pH values from 0.5 to 5.5, optimal pH from 2 to 3.5, and that an optimal temperature of 30°C can decrease the pH of the leaching medium from 1.5 to 1.0 and even lower.	<i>Acidithiobacillus thiooxidans</i> uses reduced forms of sulfur as an energy source. <i>Acidithiobacillus ferrooxidans</i> differs by using Fe ²⁺ as an electron donor and Fe ³⁺ as an alternative acceptor.	<i>Acidithiobacillus thiooxidans</i> and <i>A. ferrooxidans</i> are acidophilic and can lower the pH of the medium, which favors the solubilization of metals.	[61]
In the case of limonite biooxidation by a consortium of mesophilic acidophilic bacteria, the pH was maintained at 1.8 and the temperature at 35°C. In another experiment, the pH was maintained in a range of 1.75–1.85 and the gas supply was alternated for minimizing acid consumption, while maintaining effective solubilization of the metals present in the limonite. Furthermore, in the experiments with <i>A. thiooxidans</i> , the pH was adjusted to values of 1.0, 1.5, and 2.0 and the temperature was maintained at 30°C.	Bacteria of the genus <i>Acidithiobacillus</i> , such as <i>A. ferrooxidans</i> , <i>A. thiooxidans</i> , and <i>A. caldus</i> , function aerobically to generate sufficient acidity and dissolve oxidized minerals, thus promoting the extraction of base metals such as cobalt and nickel from limonitic deposits.	Prevents the development of low redox potentials that accelerate the reductive process. The observations found highlight the metabolic versatility and adaptation capacity of <i>A. thiooxidans</i> to different environmental conditions.	[63]
Some strains of <i>A. ferrooxidans</i> use elemental sulfur as energy and require slightly acidic conditions (pH < 2) at a temperature < 30–45°C, for nickel solubilization.	<i>Acidithiobacillus ferrooxidans</i> uses elemental sulfur as an energy source for mediating the dissolution of goethite at acidic pH, facilitating nickel extraction.	Maintaining a low pH and a temperature of 30°C favors the activity of <i>Acidithiobacillus ferrooxidans</i> in the reductive solution.	[19]

Furthermore, oxygen concentration has been found to be a critical factor for the growth and metabolic activity of *Acidithiobacillus* bacteria. Regarding the concentration of nutrients, it has been shown that the presence of nitrogen and phosphorus is essential for the growth of the *Acidithiobacillus* bacteria. Finally, the presence of toxic metals such as arsenic can inhibit the activity of *Acidithiobacillus* bacteria and reduce the efficiency of bioleaching.

Figure 2 shows the optimal pH and temperature conditions for different *Acidithiobacillus* strains, as well as their tolerance to heavy metal. Strains such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus caldus*, and *Acidithiobacillus thiooxidans* stand out for their ability to grow in highly acidic environments, with optimal pH values between 1 and 2. They can also tolerate temperatures ranging from 25°C to 45°C [62, 64, 65], which demonstrates their capacity to thrive in extreme conditions. Furthermore, these bacteria have a remarkable resistance to high concentrations of heavy metals [19, 25, 60, 61].

The size of the circles represents the concentration of metals associated with each strain. Some strains, such as *A. ferrooxidans* and *A. thiooxidans* can tolerate high concentrations of metals such as Cu, Zn, Fe, and Mn. These bacteria are able to grow under extreme conditions and tolerate high concentrations of metals. Therefore, they could be used in bioremediation processes, and in the bioextraction of valuable metals. They may also contribute to the recovery of resources from mining or industrial waste [31, 66]. Bioleaching and biomining are successfully applied in the extraction of metals such as copper, gold, and uranium, using methods such as heap leaching and agitation tank leaching [58]. One of the main challenges in heap leaching is the low

oxygen concentration in deep environments, which affects the efficiency of the process. To address this limitation, several strategies have been proposed, including the implementation of forced aeration. This technique increases the mineral leaching rate by improving the oxygen supply. Oxygen concentrations between 1.5 and 4.1 mg/L have been found to be optimal for microbial activity [55]. Oxygen limitation results in severe suppression of the growth of acidophilic and aerobic autotrophic microorganisms, directly affecting the copper bioleaching process.

Recoveries of 80% of zinc and 85% of nickel have been reported in the heap bioleaching operation of the Talvivaara Sotkamo mine, located in northeastern Finland. This process remains effective even at ambient temperatures below –30°C, which is one of its most remarkable characteristics [33]. Bioleaching of low-grade metal sulfides is carried out in heaps of crushed minerals. In these environments, biomining microorganisms face extreme conditions such as high osmotic potential caused by accumulated ions, toxic concentrations of metals like iron and copper, and strong temperature variations depending on the climate [67]. At the Zijinshan plant, copper recoveries of up to 80% were achieved even with low bacterial activity. This was possible due to high ferric ion concentrations, low pH (around 2), and high temperatures (above 30°C). However, variations in crush size and stacking method negatively affected metal recovery [13].

Heap bioleaching operates under acidic conditions, typically with pH values between 1.5 and 2.5. In some cases, the pH can drop below 1.2, which favors the growth of *Acidithiobacillus*. However, pH levels that are too low can inhibit its development and affect leaching rates [13].

Question 3: what are the limitations and technical challenges associated with the use of acidithiobacillus in bioleaching?

Table 5 shows the limitations presented in the analyzed case studies. The *Acidithiobacillus* genus is fundamental in biooxidation and bioleaching processes, but faces several limitations that can affect its efficiency. Thus, the activity of *Acidithiobacillus* can be affected by changes in temperature and the concentration of toxic metals. Acid production during mineral oxidation can inhibit its growth, creating a hostile environment. In addition, it competes with other microorganisms that are more efficient in mineral oxidation, which limits its effectiveness. This limitation highlights the need to maintain optimal conditions for the operation of these microorganisms. Any deviation from these conditions can significantly reduce the efficiency of bioleaching and, consequently, affect metal recovery [69]. Although some strains of *A. thiooxidans* can reduce ferric iron under aerobic conditions, their capacity is lower than that of *A. ferrooxidans*. It suggests limitations in the metabolic efficiency of *A. thiooxidans*, affecting its performance compared to other species.

The use of acidophilic bacteria for catalyzing the reduction of oxidized minerals. This process facilitates the recovery of valuable metals that are often considered extraction waste [62]. The variability in metabolic efficiency between different strains highlights the need for properly selecting microorganisms for optimizing metal recovery processes [62].

The main limitations of *Acidithiobacillus* in bioleaching include the formation of passivation layers on minerals, dependence on oxygen supply, and the requirement of specific pH and temperature conditions for optimal performance. Additionally, its sensitivity to environmental changes and competition with other microbial species emphasize the importance of understanding microbial dynamics to optimize bioleaching processes [27].

Although *Acidithiobacillus* can tolerate certain levels of heavy metals such as iron, copper, and zinc, excessive concentrations become toxic and reduce bioleaching efficiency. This limitation poses a significant challenge in contaminated environments, where heavy metals are commonly present. Their toxicity affects the viability of *Acidithiobacillus* and limits its use in metal recovery under harsh environmental conditions [60].

The dissolution of minerals such as chalcopyrite is hampered by the formation of passivation layers, which limit the release of copper. The optimization of leaching conditions (such as pH and temperature) is essential for enhancing *Acidithiobacillus* activity. The presence of these passive layers highlights the need to develop strategies that mitigate their formation. Such strategies are essential to improve metal recovery in industrial bioleaching processes [16].

Optimizing reactor design to improve mass transfer, pH control, and aeration involves high costs and technical complexity. This requirement can limit the commercial viability of processes that use *Acidithiobacillus*. Therefore, reactor design becomes a critical engineering factor for developing efficient and cost-effective bioleaching processes [61].

Future challenges include the improving the understanding of these processes at the molecular level. This knowledge will enhance their effectiveness in metal recovery and help mitigate environmental impact [13]. Another key challenge for the future is the better understanding of the interaction between *Acidithiobacillus ferrooxidans* and other microorganisms that are present in bioleaching environments [25].

It is necessary to investigate the metal resistance mechanisms of *Acidithiobacillus ferrooxidans* in greater depth. Although some strategies, such as efflux pumps and copper chaperones, have been identified, it remains unclear how these systems are regulated. It is also possible that other resistance mechanisms still remain unknown. A complete understanding of the metal resistance of this microorganism could have applications in bioremediation. This knowledge would allow the use of *Acidithiobacillus ferrooxidans* for cleaning contaminated soils or waters. As a result, the process could become more efficient and

effective. Bioleaching technology still has limitations, such as microbial leaching efficiency and reaction cycle [75]. One of the main challenges is the improvement of existing bioleaching microorganisms, so that they remain highly active under more complex reaction conditions [76]. Another challenge is the complexity of the leaching mechanism between microorganisms and minerals [77]. For overcoming these challenges, researchers have expanded their knowledge of metal microbes and their role in metal extraction and recovery thanks to advances in molecular biology techniques [78].

Regarding the limitations and technical challenges associated with the use of *Acidithiobacillus* in bioleaching, several obstacles have been identified. One of the main challenges is the control of metal toxicity, since some metals can inhibit bacterial activity and reduce bioleaching efficiency.

Another factor is the need for maintaining specific environmental conditions for bacteria of the *Acidithiobacillus* genus for growing and reproducing optimally. Additionally, it has been found that bioleaching with *Acidithiobacillus* can be slower than conventional metal extraction methods, which can affect the profitability and efficiency of the process.

Despite these challenges, the biomining with the use of *Acidithiobacillus* is positioned as an innovative and sustainable alternative for metal extraction, offering environmental, economic and technical advantages compared to conventional methods. Research opportunities were identified that could improve the efficiency and profitability of *Acidithiobacillus* bioleaching in the future.

Question 4: what are the environmental effects of bioleaching and the use of acidithiobacillus compared to conventional metal extraction methods?

The environmental effects of using *Acidithiobacillus* in bioleaching are shown in Table 6. Bioleaching is an effective alternative to traditional metal extraction methods, highlighting its lower environmental impact and greater efficiency in the use of resources. This technique represents advances towards more sustainable mining practices by reducing emissions of polluting gases and mitigating the generation of toxic waste. Bioleaching not only contributes to the environment by reducing the consumption of energy, water, and chemical reagents. It also allows the extraction of metals from minerals with low concentrations. This capability helps optimize resource management and promotes more sustainable production processes.

When considering an option for metal extraction from a material, chemical extraction is usually selected due to its fast reaction rate and convenient handling [80]. However, bioextraction has many advantages, including low energy requirements, low chemical consumption, and low operating costs, in terms of environmental friendliness [81, 82].

Greenhouse gas emissions from bioleaching can be as low as 37.50% of those from traditional processes [83]. Terrafame uses a commercial bioheap leaching process for treating ores and for producing nickel, cobalt, zinc, and copper in Finland. According to an environmental analysis, the carbon footprint associated with the production of one kilogram of nickel sulphate at Terrafame is 1.75 kg CO₂-eq, significantly lower than the industry average of 5.4 kg CO₂-eq [84].

It implies that the biopile leaching-based processing route generates approximately 60% less carbon emissions compared to alternative technologies. Furthermore, this method consumes about 90% less electricity and thermal energy than traditional smelting and high-pressure acid leaching routes. These results demonstrate its high energy efficiency and sustainability [84, 85].

Bioleaching can reduce energy consumption in the range of 30% to 50% compared to traditional methods because microorganisms perform much of the extraction work at lower temperatures and pressures [15, 85]. A decrease in the gold grade increases energy requirements and greenhouse gas emissions due to the greater use of fossil fuels. Therefore, the lower the ore grade, the higher the energy requirements for ore processing [86].

Table 5. Evaluation of the selected Scopus articles for the second research question.

Limitations	Description	Importance	Author
<i>Acidithiobacillus</i> activity can be affected by extreme environmental conditions, such as changes in temperature and concentration of toxic metals. Acid production during mineral oxidation can inhibit its growth, creating a hostile environment. Furthermore, it competes with other microorganisms that are more efficient in mineral oxidation. Finally, its dissolution rate of metallic minerals can be limited by its growth and metabolic activity, which negatively impacts metal recovery in industrial processes.	<i>Acidithiobacillus</i> is a genus of acidophilic microorganisms that thrive in extremely low pH environments, such as acidic lakes, hydrothermal systems, and mining-impacted environments. These prokaryotes are known for their ability to oxidize reduced iron and/or sulfur, allowing them to catalyze the oxidative dissolution of metal sulfide minerals, such as pyrite (FeS). This process severely acidifies the environment, often to a pH <3.	The activity of <i>Acidithiobacillus</i> is beneficial in biotechnology, especially in a process known as "biomining", where they are used for facilitating the extraction and the recovery of base metals (such as copper, cobalt, nickel, and zinc), precious metals (mainly gold), and uranium.	[69]
Although some strains of <i>A. thiooxidans</i> demonstrated the ability for reducing ferric iron under aerobic conditions, this ability was limited compared to other species, such as <i>A. ferrooxidans</i> . Furthermore, it was observed that <i>A. thiooxidans</i> did not show a clear dependence on the electron donor used, suggesting certain limitations in its metabolic efficiency compared to other <i>Acidithiobacillus</i> species.	<i>A. thiooxidans</i> did not show a clear dependence on the electron donor used, suggesting certain limitations in its metabolic efficiency compared to other <i>Acidithiobacillus</i> species. The generation of sufficient acidity is crucial for counteracting acid consumption in the dissolution of oxidized minerals, but can limit efficiency by not allowing low redox potentials.	It is important to balance the necessary acidity and the acceleration of the reductive process. These limitations could influence its applicability in biooxidation and bioleaching processes in specific environments.	[63]
Resistance to heavy metals is one of the limitations of <i>Acidithiobacillus</i> in bioleaching. These bacteria can tolerate certain levels of heavy metals, such as: iron, copper, zinc, and arsenic, but if these exceed certain limits, then they can be toxic or inhibitory for the bacteria, which decreases their efficiency in bioleaching.	<i>Acidithiobacillus thiooxidans</i> can grow with sulfur, Na ₂ S ₂ O ₃ , or K ₂ O ₆ S ₄ as substrates, but its oxidation capacity is weak with minerals such as pyrite, sphalerite, and galena.	It affects the efficiency of biooxidation by limiting the ability of <i>Acidithiobacillus thiooxidans</i> for oxidizing metal sulfide minerals.	[60]
Some of the limitations identified include the formation of passivation layers on the surface of minerals, the dependence of bioleaching reactions on oxygen supply, and the need for accelerating the aerobic oxidation of elemental sulfur for improving the efficiency of the extraction of copper.	Limitations include sensitivity to changes in the environment, competition with other microbial species, and need for specific pH and temperature conditions for optimal performance.	It is important to understand these limitations for designing strategies that maximize bioleaching efficiency and minimize unwanted microbial competition.	[27]
The low leaching efficiency is caused by the high lattice energy of chalcopyrite and the formation of a "passivation" layer composed of jarosite and elemental sulfur, because the dissolution of copper is very slow and limited. The control of leaching conditions where factors such as pH, temperature, redox potential, oxygen concentration, and the presence of metal ions can affect the growth and activity of <i>Acidithiobacillus</i> , as well as the kinetics and leaching mechanisms of chalcopyrite.	The growth of <i>Acidithiobacillus ferrooxidans</i> is inhibited by certain compounds, such as polyvinyl pyrrolidone (PVP), which can reduce biological activity in the bioleaching process.	It affects the ability of the microorganism for catalyzing the oxidation of minerals, decreasing the efficiency of the process.	[16]
The design of bioleaching reactors must optimize mass transfer, pH control, aeration, agitation, and distribution of microorganisms, which implies high cost and technical complexity.	<i>Acidithiobacillus</i> spp. are acidophilic microorganisms that require extreme pH conditions for their growth and metabolic activity, which limits their application in environments with neutral or alkaline pH. Additionally, there is the possibility of competition with other microbial species present in the bioleaching environment, which may affect the efficiency of <i>Acidithiobacillus</i> spp. in the solubilization of heavy metals.	It is crucial to understand the adaptability of <i>Acidithiobacillus</i> spp. for guaranteeing its effectiveness in the bioleaching of heavy metals in different environmental conditions.	[61]

Bioleaching consumes less water than conventional heap leaching (0.3 tons of water per 1 ton of ore) [87]. It is estimated that bioleaching processes may require up to 50% less water compared to conventional mining, although specific data may vary depending on the type of ore and the used process [85]. The bioleaching process consumes much less water and energy, representing only 38.35% and 12.85% of what conventional mining consumes [83].

More than 12 billion tons of solid waste generated annually worldwide. In this context, bioleaching emerges as a sustainable alternative for recovering metals from solid matrices while avoiding the use of harmful chemical processes [88]. The recovery of metals from low-quality sources, such as low-grade ores, can include several metals simultaneously. This is especially true in polymetallic deposits, where combinations of metals such as zinc, copper, lead, silver, gold, cobalt, and nickel are commonly found [89].

The exact amount of recoverable metals is associated to the specific mineralogical composition of the deposit and the technologies used for extraction. With the advancement of techniques such as bioleaching, the efficiency in the recovery of multiple metals has significantly improved. This improvement increases the economic viability of exploiting these resources. It also allows the growing demand for metals in various industries to be met in a more sustainable way and with a lower environmental impact [90].

It is possible to recover some metals from low-grade sources through biomining processes, including copper, nickel, zinc, cobalt, as well as precious metals such as silver and gold [91]. Furthermore, metals such as cadmium and uranium are also adequate to extraction under suitable conditions [92]. Other metals that can be recovered from low-grade sources through techniques such as bioleaching include arsenic, indium, and manganese. However, recovery efficiency can vary; for example,

copper may require longer bioleaching times for achieving optimal recovery levels [93]. The use of *Acidithiobacillus* bacteria presents several challenges related to acid generation and the risk of corrosion in equipment. In addition, the release of heavy metals raises questions about its long-term safety. Bioleaching using *Acidithiobacillus* is positioned as an attractive alternative due to its environmental benefits

and ability for recovering metals from low-grade sources. However, it also faces challenges, such as managing the potential toxicity of released metals. In contrast, traditional methods such as pyrometallurgy and hydrometallurgy are often faster and more efficient under certain conditions but carry greater environmental risk and are resource intensive.

Table 6. Evaluation of the selected articles from Scopus for the third research question.

Comparison with traditional methods	Description	Importance	Author
Bioleaching produces fewer polluting gases than conventional metal extraction methods such as sulfur dioxide, nitrogen oxide, and carbon monoxide, which contribute to the greenhouse effect and acid rain. Likewise, bioleaching makes it possible to take advantage of low-grade minerals and mining waste, which reduces the amount of solid waste that is produced and deposited in the environment.	<i>Acidithiobacillus thiooxidans</i> is an acidophilic, chemoautotrophic, gram-negative, and rod-shaped microorganism.	It can eliminate the sulfur passivation layer.	[60]
Bioleaching reduces the consumption of energy, water, and chemical reagents, and avoids the generation of toxic waste such as cyanide and arsenic, compared to conventional methods. In addition, <i>Acidithiobacillus</i> can degrade organic and inorganic contaminating compounds present in minerals or the environment.	These microorganisms operate at ambient temperatures, facilitating the leaching of minerals through their metabolism.	It allows the extraction of metals at lower temperatures, resulting in lower energy consumption.	[79]
Bioleaching is a cleaner, more efficient and more economical process than conventional methods, because it uses microorganisms such as <i>Acidithiobacillus</i> for dissolving metals of interest from minerals selectively, reducing the consumption of energy, water, and chemical reagents, as well as the generation of solid waste and gas emissions.	These bacteria can operate in extreme conditions and recover metals from low-grade ores that would be discarded by traditional methods.	It allows the exploitation of resources that would otherwise be unprofitable, minimizing waste.	[26]
It allows metals to be extracted from minerals with low concentration or from mining waste, reducing the consumption of energy, water, and chemical reagents, avoiding the generation of toxic waste.	<i>Acidithiobacillus ferrooxidans</i> is an acidophilic bacterium capable of the formation of biofilms on elemental sulfur in environments with high copper concentration.	This ability for forming biofilms in the presence of high concentrations of copper is relevant for biomining and metal extraction in environments with the presence of this metal.	[25]
Bioleaching reduces greenhouse gas emissions, the generation of large tailings and acid mine drainage, and is also very successful in the extraction of gold, copper, or uranium. The use of <i>Acidithiobacillus</i> has challenges such as the generation of acid that contributes to the corrosion of equipment. Heavy metals and other toxic elements can also be released into the environment, which can affect human health and biodiversity.	Bioleaching uses microorganisms such as <i>Acidithiobacillus</i> for solubilizing metals, rather than energy-intensive processes such as smelting and roasting that generate toxic emissions.	It is crucial to reduce the environmental footprint of mining and minimize damage to human health and ecosystems.	[58]

The selection of extraction method depends on factors such as the type of mineral, economic efficiency, and project-specific environmental considerations. The Table 7 illustrates the complexity involved in selecting methods for metal extraction and treatment within wastewater management. Each method presents a unique set of disadvantages and advantages, which must be carefully evaluated according to the specific operational and environmental needs. The trend toward more sustainable methods (such as bioleaching) must be balanced against the need for efficiency and speed in metal recovery. This analysis is essential for guiding informed decisions in the wastewater treatment and mining industry.

Question 5: what is the currently known genetic mechanism of bioleaching of the acidithiobacillus genus and what are the key aspects that remain to be understood in order to achieve a complete understanding?

The genetic mechanism of bioleaching in the *Acidithiobacillus* genus is based on the oxidation of iron and sulfur, processes mediated by a series of specific enzymes [31]. This process involves reduced inorganic sulfur compound (RISC) oxidation systems, such as the Sox system, which catalyzes the oxidation of sulfur, elemental sulfur, thiosulfate, and sulfite to sulfate. Essential proteins such as SoxYZ, SoxB, SoxCD, and SoxXA facilitate electron transfer through cytochromes. Although genes that contribute to sulfur oxidation have been identified in these microorganisms, their activity may be low in some cases [91]. *Acidithiobacillus thiooxidans* presents at least ten proteins or protein complexes associated to sulfur metabolism. These components allow the

bacterium to perform disproportionation reactions and sulfur oxidation. Key genes such as *doxD*, *tetH*, *sqr*, *sor* and *hdr* have been identified as essential for sulfur metabolism [102]. Additionally, different *Acidithiobacillus* species possess genes that encode sulfur oxidation pathways, such as the Sox pathway. For example, *Acidithiobacillus thiooxidans* has two copies of this pathway (Sox I and Sox II), whereas *Acidithiobacillus ferrivorans* only contains Sox II [7, 29]).

Despite these advances, significant knowledge gaps remain, such as the lack of information on the enzyme of sulfite oxidation in *A. ferrooxidans* and *A. ferrivorans*, leaving a key aspect of the sulfur oxidation process unresolved [13, 103]. Furthermore, differences in the genetics associated to sulfur metabolism have been observed among *Acidithiobacillus* species [103].

Regarding iron oxidation, other members of the *Acidithiobacillus* genus show significant differences in their complexes compared to *A. ferrooxidans*. For instance, *A. ferrivorans* possesses two distinct iron oxidation pathways: (a) the protein rusticyanin (*rus*), an iron-oxidizing cytochrome c, and (b) a gene encoding iron oxidase (High Potential Iron Protein [HIPIP]) known as *iro* [103]. Among the genus, only *A. ferrivorans* and *A. ferriphilus* have been identified as *iro*-possessing species. In these bacteria, the *iro*-encoded HIPIP may represent the first step in the iron oxidation pathway. However, comprehensive models describing this process are still lacking. Bioleaching concept is simple; however, the underlying mechanisms are complex because sulfur metabolisms and microbial iron rely on a complex array of enzymes for facilitating the process [103].

Although significant progress has been made in understanding the

genetic mechanisms of *Acidithiobacillus* species, several critical gaps remain. One of the main challenges is to fully understand the genetic and metabolic mechanisms that allow these bacteria to survive in extreme environments. Some genes related to iron and sulfur oxidation have already been identified, but a more complete mapping of the genome is still needed. This would help identify all genes that contribute to resistance to toxic metals and adaptation to acidic conditions [13]. The sequencing of new genomes, together with genetic editing techniques such as CRISPR-Cas9, can facilitate this process [13, 104].

Understanding specific metabolic pathways and enzymatic mechanisms that catalyze the reactions of intermediate sulfur compounds is critical for unraveling the bioleaching bioprocess [102]. Although several enzymes involved in sulfur and iron oxidation have been identified, significant knowledge gaps remain. For example, the enzyme responsible for sulfite oxidation in *A. ferrooxidans* and *A. ferrivorans* has not yet been identified, leaving this critical step in the sulfur oxidation pathway unresolved [103]. Additionally, more information is required regarding the transport of elemental sulfur (S^0) within cells and its intracellular metabolism. There are also gaps in knowledge about which proteins catalyze specific sulfur pathways and about the characterization of sulfur compounds in higher oxidation states, such as polythionates [102].

A detailed understanding of the molecular interactions between cells and minerals, as well as between cells themselves, remains a missing aspect in the bioleaching bioprocess. This knowledge requires exploring genetic networks, evolutionary traits, and advanced proteomic analyses, in addition to developing recombinant strains. With such information, it would be possible to design new bioleaching applications using acidophilic microorganisms as part of green biotechnology [91].

Integrative approaches such as transcriptomics and proteomics are required for analyzing real-time changes under leaching conditions, as well as gene editing (CRISPR-Cas9) for validating the function of specific genes. In addition, biological interaction models could simulate the relationships between different species and their environment, helping to the optimization of bioleaching conditions.

Comprehensive knowledge of these aspects will not only improve the efficiency of bioleaching but will also promote more sustainable mining and metal recovery practices. Research in these areas could open new opportunities for developing innovative biomining technologies, facilitating the transition toward greener and more efficient resource extraction methods.

Question 6: what are the efficiencies achieved in biomining using acidithiobacillus and how do they compare with those obtained using other chemical methods?

Chemical leaching (traditionally used) has environmental and economic limitations. Bioleaching using microorganisms such as *Acidithiobacillus* is a sustainable alternative. Bioleaching is an environmentally friendly and cost-effective approach for metal recovery from waste. Several experiments have been conducted using this method, achieving high metal recovery rates [4, 64]. Data about the recovery of diverse metals from different sources are presented in Table 8, comparing the efficiency of chemical leaching and bioleaching. Copper recovery from various sources shows significant variability in

efficiency between chemical and bioleaching methods. At the Hitura tailings, sulfuric acid leaching shows low recovery, ranging from 0.00 to 34.7%. In contrast, bioleaching with *Acidithiobacillus ferrooxidans* achieves 27.70%, which highlights the limited effectiveness of the chemical method in this context. However, on printed circuit boards (PCBs), chemical leaching achieves up to 96% recovery with HCl + FeCl₃, while bioleaching improves it to 85%. On PCB waste, bioleaching excels even more, achieving 99% recovery, compared to 100% obtained by chemical leaching with sulfuric acid. These results suggest that chemical leaching can be effective in certain contexts. However, bioleaching offers a competitive and sustainable alternative, particularly for electronic waste, where achieving full copper recovery is critical. The selection of the most suitable method will depend on several factors, such as material type, process economics, and environmental considerations. This reaffirms the need to optimize both methods to maximize copper recovery efficiency. Nickel recovery from various sources shows significant variations in efficiency between chemical leaching and bioleaching. In Ni-Cd battery waste, chemical leaching with H₂SO₄ achieves 97% recovery, while bioleaching with *Acidithiobacillus ferrooxidans* reaches 92%. These results indicate a high effectiveness of both methods. However, in laterites, chemical leaching with sulfuric acid shows a recovery of 68%, compared to 64% achieved by bioleaching with *Acidithiobacillus thiooxidans*, suggesting that bioleaching is competitive in this context. On the other hand, in nickel ores, recovery by hydrochloric acid leaching is 78%, but bioleaching only reaches 54%, indicating that this method may not be as effective in this specific source. Finally, in PCB waste, chemical leaching with H₂SO₄ achieves 85% recovery, while bioleaching presents 88%. This once again highlights the effectiveness of the biological method. It suggests that bioleaching may be a viable and sustainable alternative to chemical leaching, especially in contexts where environmental impact is sought to be minimized.

Zinc recovery from various sources shows a wide range of efficiencies between chemical leaching and bioleaching methods with *Acidithiobacillus ferrooxidans*. At the Hitura tailings, chemical leaching with sulfuric acid showed a recovery ranging from 0.08% to 33.0%. In contrast, bioleaching achieved only 10.50%, which indicates significant inefficiency in this context.

However, chemical leaching achieves 96% recovery in sphalerite concentrates, while bioleaching reaches only 30%. This suggests that the chemical process is better suited for this specific material. In PCB sludges, bioleaching with H₂SO₄ shows an outstanding recovery of 98.40%. This performance notably outperforms the 61% recovery obtained through chemical leaching. In zinc ores, chemical leaching achieves 85% recovery, while in mine tailings it reaches 67%. In both cases, bioleaching also shows competitive performance. In summary, chemical leaching may be more effective in certain contexts. However, bioleaching with *Acidithiobacillus ferrooxidans* proves to be a viable and efficient alternative, especially in PCB sludges. This highlights its potential for the sustainable recovery of zinc from diverse sources. Manganese recovery efficiency varies depending on the source. In Pyhäsalmi tailings, chemical leaching reaches 57.30%, while bioleaching achieves only 24.60%. This result indicates the superiority of the chemical method in this case. In laterites, chemical leaching achieves 58% recovery, while bioleaching with *Acidithiobacillus thiooxidans* reaches 86%.

Table 7. Comparison of metal extraction and treatment methods.

Method	Advantages	Disadvantage	Reference
Bioleaching (<i>Acidithiobacillus</i>)	Eco-friendly and sustainable	Slow compared to chemical methods.	[33, 95]
Chemical extraction (acids)	Fast and efficient	Use of toxic chemicals	[96, 97]
Flotation	Effective for concentrating minerals	Requires chemical reagents.	[98]
Cyanidation	High efficiency in gold and silver extraction	Use of highly toxic cyanide	[99]
Electrowinning	High purity of recovered metal	Expensive in terms of infrastructure	[100, 101]

Table 8. Comparison of metal recovery by chemical leaching and bioleaching.

Recovered	Metal Source	Recovery	Chemical Leaching	Recovery	Used <i>Acidithiobacillus</i> Species	Reference
Copper (Cu)	Tailings of Hitura	0.00 - 34.7%	Sulfuric acid	27.70%	<i>Acidithiobacillus ferrooxidans</i>	[105]
Nickel (Ni)	Tailings of Hitura	3.07 - 27.2%	Sulfuric acid	12.40%	<i>Acidithiobacillus thiooxidans</i>	[105]
Zinc (Zn)	Tailings of Hitura	0.08 - 33.0%	Sulfuric acid	10.50%	<i>Acidithiobacillus ferrooxidans</i>	[105]
Manganese	Tailings of Pyhäsalmi B	57.30%	Sulfuric acid	24.60%	<i>Acidithiobacillus ferrooxidans</i>	[105]
Copper (Cu)	Printed Circuit Boards (PCBs)	75%	Sulfuric acid (1%)	85%	<i>Acidithiobacillus ferrooxidans</i>	[92]
Nickel (Ni)	Printed Circuit Boards (PCBs)	90%	Sulfuric acid (1%)	90%	<i>Acidithiobacillus thiooxidans</i>	[92]
Zinc (Zn)	Sphalerite concentrate	96%	Lixiviación con	30%	<i>Acidithiobacillus ferrooxidans</i>	[106]
Copper (Cu)	Printed circuit boards (PCBs)	96%	HCl + FeCl ₃	94.08%	<i>Acidithiobacillus ferrooxidans</i>	[107]
Nickel (Ni)	Ni-Cd battery waste	97%	H ₂ SO ₄	92%	<i>Acidithiobacillus ferrooxidans</i>	[107]
Zinc (Zn)	PCB sludge	98.40%	H ₂ SO ₄	90%	<i>Acidithiobacillus ferrooxidans</i>	[107]
Indian (In)	LCD panels	86.30%	H ₂ SO ₄	100%	<i>Acidithiobacillus thiooxidans</i>	[107]
Tin (Sn)	LCD panels	97.07%	H ₂ SO ₄	90.20%	<i>Acidithiobacillus thiooxidans</i>	[107]
Nickel (Ni)	Laterites	68%	1M sulfuric acid	64%	<i>Acidithiobacillus thiooxidans</i>	[108]
Cobalt (Co)	Laterites and manganese phases	69%	1M sulfuric acid	83%	<i>Acidithiobacillus thiooxidans</i>	[108]
Manganese	Laterites	58%	1M sulfuric acid	86%	<i>Acidithiobacillus thiooxidans</i>	[108]
Copper (Cu)	Copper ore (converter slag)	93%	Acid ammonia	66%	<i>Acidithiobacillus ferrooxidans</i> ,	[109]
Zinc (Zn)	Zinc ores	85%	Sulfuric acid	61%	<i>Acidithiobacillus ferrooxidans 2</i>	[109]
Nickel (Ni)	Nickel ores	78%	Hydrochloric acid	54%	<i>Acidithiobacillus ferrooxidans 3</i>	[109]
Cobalt (Co)	Cobalt ores	80%	Sulfuric acid	58%	<i>Acidithiobacillus ferrooxidans 4</i>	[109]
Copper (Cu)	PCB waste	100%	Sulfuric acid	99%	<i>Acidithiobacillus ferrooxidans</i>	[110]
Nickel (Ni)	PCB waste	85%	Sulfuric acid	88%	<i>Acidithiobacillus ferrooxidans</i>	[110]
Iron (Fe)	PCB waste	85%	Sulfuric acid	78%	<i>Acidithiobacillus ferrooxidans</i>	[110]
Zinc (Zn)	PCB waste	85%	Sulfuric acid	70%	<i>Acidithiobacillus ferrooxidans</i>	[110]
Aluminum (Al)	PCB waste	85%	Sulfuric acid	77%	<i>Acidithiobacillus ferrooxidans</i>	[110]
Manganese	PCB waste	85%	Sulfuric acid	57%	<i>Acidithiobacillus ferrooxidans</i>	[110]
Manganese	PCB waste	92%	H ₂ SO ₄ y H ₂ O ₂	95%	<i>Acidithiobacillus ferrooxidans</i>	[111]
Copper (Cu)	Mine tailings	38%	H ₂ SO ₄ y H ₂ O ₂	38%	<i>Acidithiobacillus ferrooxidans</i> ,	[111]
Zinc (Zn)	Mine tailings	77%	H ₂ SO ₄ (2%)	67%	<i>Acidithiobacillus ferrooxidans</i>	[111]
Arsenic (As)	Mine tailings	37%	H ₂ SO ₄ y H ₂ O ₂	41%	<i>Acidithiobacillus ferrooxidans</i> ,	[111]
Iron (Fe)	Mine tailings	42%	H ₂ SO ₄ (2%)	47%	<i>Acidithiobacillus ferrooxidans</i>	[111]
Copper (Cu)	Printed Circuit Boards (PCBs)	No data	No data	80%	<i>Acidithiobacillus ferrooxidans</i>	[112]
Indium (In)	OLED touch screens of mobile	No data	No data	100%	<i>Acidithiobacillus ferrooxidans</i>	[113]
Cadmium (Cd)	Nickel-cadmium batteries	No data	No data	100%	<i>Acidithiobacillus ferrooxidans</i>	[114]
Copper (Cu)	Mobile phone PCB	No data	No data	99%	<i>Acidithiobacillus ferrooxidans</i>	[115]
Cobalt (Co)	Nickel ion batteries	No data	No data	65%	<i>Acidithiobacillus ferrooxidans</i>	[116]
Copper (Cu)	Spent mobile phone PCB	No data	No data	98%	<i>Acidithiobacillus thiooxidans</i>	[117]
Copper (Cu)	Computer PCBs	No data	No data	75%	<i>Acidithiobacillus thiooxidans</i>	[118]
Cadmium (Cd)	Contaminated synthetic sediments	No data	No data	80%	<i>Acidithiobacillus ferrooxidans</i>	[119]
Copper (Cu)	Industrial waste	No data	No data	94.7% In 78 days	<i>Acidithiobacillus ferrooxidans</i>	[64]
Copper (Cu)	Industrial waste	No data	No data	99.6% in 12 days	<i>Leptospirillum</i> and	[64]
Nickel (Ni)	Nickel laterite ore	No data	No data	70%	<i>Acidithiobacillus ferrooxidans</i>	[20]
Nickel (Ni)	Deposits of Ni and Co	No data	No data	100%	<i>Acidithiobacillus thiooxidans</i>	[120]
Copper (Cu)	Lateritic deposits	No data	No data	78%	<i>Acidithiobacillus ferrooxidans</i>	[121]

This suggests that bioleaching is more efficient in this material. In PCB waste, chemical recovery is high at 85%, but bioleaching only reaches 57%, indicating potential for improvement. In mine tailings, chemical leaching shows 92% recovery, while bioleaching reaches 95%. These results evidence the effectiveness of both methods in this context. The comparison of efficiencies between chemical leaching and bioleaching using *Acidithiobacillus* reveals a diverse picture for recovering metals such as: copper, zinc, manganese, and nickel. Chemical leaching can be more effective in certain sources, such as copper and nickel concentrates.

However, bioleaching proves to be a competitive and sustainable alternative, especially in electronic waste and in contexts where minimizing environmental impact is a priority [122]. In particular, bioleaching highlights its effectiveness in recovering copper from PCB waste, reaching up to 99%. The selection of the most suitable method

depends on material-specific factors, as well as economic and environmental considerations. The underlines the importance of optimizing both approaches to maximize metal recovery efficiency [123].

Figure 3 highlights that while chemical leaching typically achieves slightly higher recoveries for some metals, *Acidithiobacillus* bioleaching presents a competitive and more sustainable performance. In most cases, the differences in recovery percentages are marginal, while the biological process offers clear environmental advantages, such as lower reagent consumption and fewer toxic byproducts. Notably, bioleaching demonstrates particularly high efficiency for metals such as nickel and cobalt, reaching yields above 80%, confirming its industrial viability. These results support the transition to bihydrometallurgical processes as a sustainable alternative to conventional chemical leaching, especially for low-grade ores and e-waste.

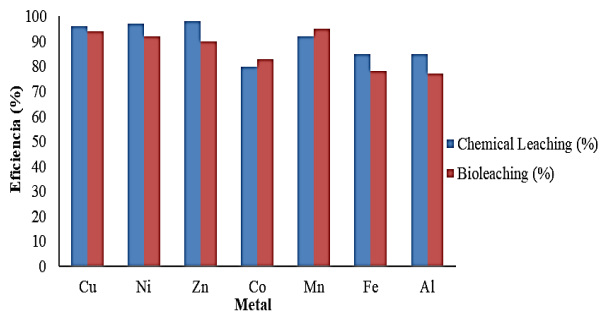


Figure 3. Efficiency comparison: Bioleaching vs. Chemical leaching.

3.1. Challenges, perspectives, opportunities and limitations

The *Acidithiobacillus* genus is crucial in bioleaching, a process that enables the extraction of metals in a more sustainable way than conventional methods. However, it faces several challenges that limit its overall effectiveness [76, 88]. One of the main challenges is the extreme environmental conditions required by these microorganisms. They thrive in acidic environments and at specific temperatures, conditions that may not be present at all mining sites [13, 67]. Variability in geological and climatic conditions can affect the effectiveness of *Acidithiobacillus*. Therefore, a detailed study of each environment necessary to optimize its use [124, 125]. Furthermore, microbial interactions with other organisms can limit the activity of *Acidithiobacillus*. This interaction creates competition that may decrease its performance in bioleaching [56, 126].

It is important to note that there are operational and environmental risks associated with the generation of sulfuric acid during bioleaching with *Acidithiobacillus*. The generation of sulfuric acid (H_2SO_4) as a metabolic byproduct during the biooxidation of sulfides by microbial consortia, predominantly of the *Acidithiobacillus* genus, can reach significant concentrations. In industrial heap leachates, values ranging from 5 to 50 g/L have been reported, depending on the mineralogy and operating conditions [58, 127]. This high acidity (pH<2.5) is the main driver of leaching, but also constitutes its main operational and environmental risk. The metabolic activity of these acidophilic microorganisms not only generates the acidic medium, but also dramatically accelerates corrosion mechanisms by forming biofilms on metal surfaces. Electrochemical investigations have confirmed that carbon steel exposed to bioleaching media active with *Acidithiobacillus* ferrooxidans can undergo uniform corrosion rates exceeding 1 mm/year, an order of magnitude higher than under sterile conditions of similar pH [128]. This accelerated degradation is attributed to the biofilm's ability to maintain hyperacidic conditions at the metal-solution interface, facilitate cathodic oxygen reduction reactions, and alter anodic dissolution kinetics. Beyond equipment corrosion, the most critical environmental risk is the accidental leaching of these hyperacidic effluents, which can generate or exacerbate acid mine drainage (AMD). These leachates are characterized by high acidity and heavy metal concentrations that exceed regulatory limits by several orders of magnitude. For example, copper (Cu) concentrations of up to 200 mg/L and zinc (Zn) of 150 mg/L have been reported in abandoned or poorly managed stockpiles [129]. Therefore, although bioleaching offers substantial advantages in reducing greenhouse gas emissions and water consumption compared to pyrometallurgy [130], its long-term technical and economic viability critically depends on the implementation of robust risk management and mitigation systems. This includes the design of infrastructure with high-alloy materials (e.g. duplex stainless steel), specialized coatings, waterproofing systems, and continuous water quality monitoring, transforming potential risks into manageable aspects of the operation [8, 131]. Another major challenge is the lack of standardized protocols for optimizing cultivation and bioleaching conditions. This absence of standardization makes large-scale implementation difficult. Despite these challenges, the prospects for the use of *Acidithiobacillus* in biomining are encouraging. Research

and development in biotechnology may lead to new techniques that increase the efficiency of this microorganism. These advances could broaden its applicability in the extraction of different metals. The integration of bioleaching with other extraction methods could improve metal recovery and optimize the use of resources [84].

Sustainability is increasingly becoming a global priority. In this context, *Acidithiobacillus* can play a key role in the transition towards greener and more responsible mining practices. Furthermore, multidisciplinary research combines microbiology, engineering, and environmental sciences may offer new opportunities for optimizing biomining processes.

These advances would allow the mining industry to adapt to contemporary demands for sustainability and efficiency [66, 132]. Finally, using industrial waste and wastewater in bioleaching processes could improve sustainability and reduce operating costs. This approach makes biomining a more attractive option from an economic perspective [66, 88, 133]. Although the *Acidithiobacillus* genus faces significant challenges in its application for bioleaching, the prospects are promising. Its continued development can substantially contribute to sustainability in mining. Moreover, it promotes more responsible and efficient practices in the extraction of mineral resources.

To identify potential regional gaps in scientific production, the geographical distribution of the reviewed studies was analyzed. The results show a marked concentration of research in Asia, particularly in China (195 publications), followed by Iran, India, Chile, the United Kingdom and Germany, each contributing 49, 39, 37 and 35 studies respectively (Figure 4).

This uneven distribution highlights significant asymmetries in research infrastructure, funding availability, and environmental data accessibility among regions, which constrain the global representativeness and applicability of advances in bioleaching. Therefore, it is recommended to promote international collaboration and encourage research in underrepresented regions, particularly in Africa, Central America, and Eastern Europe, where the biotechnological potential of the *Acidithiobacillus* genus remains largely unexplored. Expanding the geographical diversity of studies will strengthen the understanding of microbial interactions under different geoclimatic conditions and support the development of more sustainable and adaptive metal extraction strategies.

In addition to the technical and biological challenges associated with optimizing *Acidithiobacillus*-based bioleaching systems, certain methodological constraints also influence the scope of this review.

Although the literature search was conducted exclusively in the Scopus database, this choice is based on its broad coverage of scientific literature in environmental engineering, hydrology, and mathematical modelling, as well as the availability of filtering tools that facilitate the application of the PRISMA protocol. However, it is recognized that this decision may limit the inclusion of certain studies indexed in complementary databases such as Web of Science, PubMed, or regional repositories (e.g. SciELO and RedAlCy). Future research could broaden the scope of the review by integrating these sources to strengthen the geographical and thematic representativeness of the results.

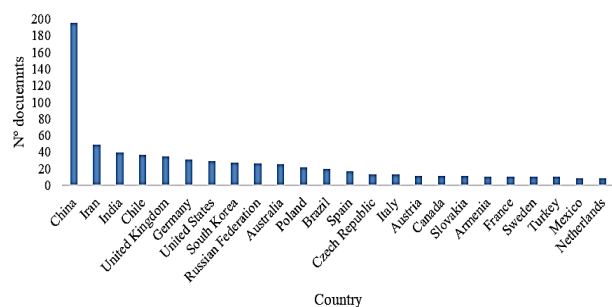


Figure 4. Geographic distribution of bioleaching studies.

3.2. Comparative perspective with recent systematic reviews

Compared with other recent systematic reviews on biomining, this study provides a more specific and in-depth synthesis focused on the *Acidithiobacillus* genus, integrating physiological, genetic, and operational aspects. While broad reviews such as Dong et al. [34] offer extensive coverage of various bacterial and fungal agents for bioleaching of metal tailings, the present manuscript distinguishes itself by analyzing the particular metabolic and genetic mechanisms that make *Acidithiobacillus* the cornerstone of industrial bioleaching. Likewise, in contrast with Naseri et al. [71], who emphasized statistical and optimization approaches (e.g. response surface methodology and Box–Behnken designs) to enhance metal recovery efficiency, our study highlights the environmental and biochemical constraints that still limit large-scale applications of *Acidithiobacillus*-based biomining.

Additionally, recent advances in genetic engineering of acidophilic microorganisms, as discussed by Chen et al. [32], point toward the potential use of CRISPR-Cas systems, recombinant strain development, and transcriptomic integration to improve bioleaching performance. The present review aligns with these trends by identifying knowledge gaps in sulfur and iron oxidation pathways and proposing future directions for multi-omics integration. Therefore, this manuscript complements previous reviews by providing a focused, technical, and critical synthesis that bridges microbial physiology, environmental conditions, and industrial applicability of *Acidithiobacillus*.

Overall, the comparative analysis highlights that while broader reviews have mapped the diversity of biomining agents and process optimizations, a genus-focused review such as the present one contributes to deepening molecular and engineering understanding, which is essential for advancing sustainable and scalable biomining technologies. Proposal of standardized experimental conditions for cross-study comparison Table 9 proposes a set of standardized experimental conditions. Standardizing experimental parameters in bioleaching studies is crucial for improving reproducibility and enabling meaningful comparisons across different investigations. The choice of microorganism and the specification of the strain are essential, as different species exhibit variations in metabolism and metal tolerance, which directly impact the efficiency of Fe^{2+} and S^0 oxidation (Gao et al. [76]; Vera et al. [91]).

The culture medium must be defined, such as medium 9K or an equivalent with a detailed composition—to ensure controlled nutrient availability and to correctly evaluate the influence of the energy source (Nguyen et al. [80]). The energy source, whether Fe^{2+} or elemental sulfur, determines the predominant metabolic pathway and the oxidation rate, highlighting the importance of clearly specifying it in experiments (Opara et al. [93]).

Physicochemical parameters such as initial pH and temperature directly affect microbial growth and enzymatic activity. Optimal pH values between 1.5 and 2.5 ensure *Acidithiobacillus* activity, whereas significant deviations inhibit growth (Vera et al. [91]). Similarly, maintaining a constant temperature within a tolerance of ± 0.5 °C is critical for mesophiles and thermophiles, as microbial metabolism is highly sensitive to thermal fluctuations (Gao et al. [76]). Aeration and dissolved oxygen levels are decisive factors for oxidation efficiency, given that suboptimal concentrations significantly reduce bioleaching activity (Vera et al. [91]).

The duration of the experiment and the recording of periodic samples allow for the capture of both the active growth phase and the complete kinetics of metal solubilization, providing critical data for process modeling (Gao et al. [76]).

Including a minimum set of variables to be reported such as pH, ORP, $\text{Fe}^{2+}/\text{Fe}^{3+}$, cell density, and dissolved oxygen facilitates performance comparisons across different studies and enhances the potential for modeling and meta-analysis (Nguyen et al. [80]). Likewise, the use of experimental controls (without inoculum or with inactivated inoculum) makes it possible to distinguish between biological and chemical leaching, thereby increasing the reliability of the results (Vera et al. [91]).

Finally, the selection of a standardized analytical method such as ICP-OES or ICP-MS for metals and the ferrocyanide method for $\text{Fe}^{2+}/\text{Fe}^{3+}$ along with a minimum number of replicates, ensures the comparability of results and reduces statistical variability (Gao et al. [76]; Opara et al. [93]). Overall, adopting these recommended experimental conditions contributes to generating more consistent and reliable data, facilitating the development of robust knowledge bases in bioleaching and metal recovery processes.

Table 9. Recommended standardized experimental parameters for bio-oxidation studies with *Acidithiobacillus* spp.

Experimental parameter	Recommended standardized condition	Scientific justification / technical observation	Suggested reference
Microorganism	Specify species and strain (e.g., <i>A. ferrooxidans</i> ATCC 23270T or <i>A.</i>	Minimum of three replicates per experimental condition	Gao et al. [76]; Vera et al. [91]
Culture medium	Half-strength 9K or equivalent defined medium (specify composition in $\text{mg}\cdot\text{L}^{-1}$)	It allows for comparing nutrient availability and evaluating the effect of the energy source.	Nguyen et al. [80]
Energy source	Fe^{2+} (10–20 mM) or elemental sulfur (5–10 $\text{g}\cdot\text{L}^{-1}$); specify which is used.	The choice of electron donor influences the oxidation rate and the predominant metabolic pathway.	Opara et al. [93]
Initial pH	1.5 – 2.5	It maintains optimal <i>Acidithiobacillus</i> activity; a pH that is too low inhibits its growth.	Vera et al. [91]
Temperature	30 ± 2 °C for mesophiles; 45–50 °C for thermophiles	Metabolism is highly temperature-dependent; maintaining a constant temperature within ± 0.5 °C is recommended.	Gao et al. [76]
Aeration / Dissolved O_2	1.5 – 4.0 $\text{mg}\cdot\text{L}^{-1}$ (record supply method and flow)	Optimal oxygen concentrations are critical for the oxidation of Fe^{2+} and S^0 ; lower O_2 levels reduce efficiency.	Vera et al. [91]
Duration of the experiment	28 days (with sampling at 0, 7, 14, 21, and 28 days)	It allows for the capture of the active growth phase and the complete kinetics of metal solubilization.	Gao et al. [76]
Minimum variables to be reported	pH, ORP, $[\text{Fe}^{2+}]/[\text{Fe}^{3+}]$, [dissolved metal], cell density, temperature, dissolved O_2	It facilitates the comparison of performance and modeling across different studies.	Nguyen et al. [80]
Experimental controls	(i) without inoculum; (ii) with inactivated inoculum	It makes it possible to distinguish between biological and chemical leaching.	Vera et al. [91]
Analytical method	ICP-OES or ICP-MS for metals; ferrocyanide method for $\text{Fe}^{2+}/\text{Fe}^{3+}$	It avoids variability between methods and improves the comparability of results.	Gao et al. [76]
Number of replicates	Minimum of three replicates per experimental condition	Reduces statistical errors and increases the reliability of the results.	Opara et al. [93]

Standardizing variables such as pH, temperature, pulp density, and aeration along with adopting common reporting protocols (e.g., using identified strains, 9K medium, and ICP-OES analytical methods) will improve the reproducibility of bioleaching assays and enable the creation of databases that allow for inter-laboratory comparisons.

Furthermore, it is recommended to include biological and chemical controls in all experiments, report complete solubilization kinetics, and use a minimum of three replicates per condition. These practices will facilitate the development of predictive models and international standardization in *Acidithiobacillus* biomining research.

4. Conclusions

The systematic review presented in this study demonstrates that the genus *Acidithiobacillus* constitutes a cornerstone in the development of environmentally sustainable bioleaching technologies. Its metabolic versatility particularly its ability to oxidize iron and sulfur under extreme conditions positions it as a biological model capable of transforming conventional mineral extraction paradigms. A comparative analysis between bioleaching and traditional chemical methods reveals that the biological pathway not only reduces environmental impact but also improves the recovery of valuable metals from low-grade ores and industrial waste.

This dual environmental and economic advantage supports the inclusion of *Acidithiobacillus*-based technologies within modern circular economy frameworks. Future advances in biomining depend on a deeper understanding of the genetic and enzymatic mechanisms governing metal oxidation and microbial adaptation to toxic environments. While recent genomic studies have identified key genes involved in sulfur and iron metabolism, significant gaps remain particularly regarding the regulation of heavy metal resistance and the characterization of unknown enzymatic pathways, such as sulfite oxidation. Integrating multi-omics tools (such as transcriptomics and proteomics) with gene-editing technologies like CRISPR-Cas9 is essential to deciphering these mechanisms and enabling the design of genetically optimized strains for industrial applications.

However, the findings also highlight that bioleaching with *Acidithiobacillus* faces technical challenges, including limited leaching rates, complex reactor design, and sensitivity to physicochemical fluctuations. Overcoming these barriers requires interdisciplinary collaboration among microbiology, biochemical engineering, and computational modeling. Developing predictive models that integrate environmental variables with microbial kinetics will enable better control and scalability of bioleaching systems. Crucially, this research underscores the importance of shifting from merely descriptive studies to mechanistic and applied research that links microbial function with industrial performance and environmental metrics. *Acidithiobacillus* should be viewed not only as a tool for metal extraction but also as a model organism for exploring resilience, adaptation, and biotechnological innovation in extreme ecosystems.

5. Recommendations for future research

The importance of *Acidithiobacillus* in bioleaching and sustainable biomining has been widely recognized due to its ability for oxidizing metal compounds, as well as its resistance to extreme environments. However, there are significant knowledge gaps that require additional research for advancing in these areas. One of the key challenges for the future is the better understanding of the resistance and the efficiency of these microorganisms in challenging environments [76].

Some future challenges include the study of the following: (a) strategies for increasing tolerance to extreme acidic conditions, (b) resistance to toxic metals and inhibitory compounds present in minerals, (c) exploration of ways for improving tolerance to osmotic stress situations, and (d) how to increase the thermotolerance of microorganisms for operating under high temperature conditions. These areas represent key opportunities for future research in the field of biomining and sustainable bioleaching. Genetic engineering

strategies and resilience pathways need to be further explored for developing more robust and effective microorganisms in metal extraction from low-quality minerals [134].

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