

Review



Accessing Metals from Low-Grade Ores and the Environmental Impact Considerations: A Review of the Perspectives of Conventional versus Bioleaching Strategies

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Abstract: Mining has advanced primarily through the use of two strategies: pyrometallurgy and hydrometallurgy. Both have been used successfully to extract valuable metals from ore deposits. These strategies, without a doubt, harm the environment. Furthermore, due to decades of excessive mining, there has been a global decline in high-grade ores. This has resulted in a decrease in valuable metal supply, which has prompted a reconsideration of these traditional strategies, as the industry faces the current challenge of accessing the highly sought-after valuable metals from low-grade ores. This review outlines these challenges in detail, provides insights into metal recovery issues, and describes technological advances being made to address the issues associated with dealing with low-grade metals. It also discusses the pragmatic paradigm shift that necessitates the use of biotechnological solutions provided by bioleaching, particularly its environmental friendliness. However, it goes on to criticize the shortcomings of bioleaching while highlighting the potential solutions provided by a bespoke approach that integrates research applications from omics technologies and their applications in the adaptation of bioleaching microorganisms and their interaction with the harsh environments associated with metal ore degradation.

Keywords: low-grade ore; metal recovery; metallurgical methods; bioleaching; environmental pollution

1. Introduction

Initially, only precious metals were prioritized, but with the development of various industries and their demand for unique metals for production activities, the focus shifted to include the assorted metals demanded [1]. For many years, we have witnessed the development and improvement of technologies to accommodate the mining and processing of various types of metals [2,3]. Furthermore, these technological advancements have been adapted to facilitate the extraction of minerals of interest from low-grade ores, which is an expected consequence of the alarming depletion rates of high-grade ore reserves. This will increase the exploitation of previously underutilized low-grade ores [4,5]. Surprisingly, in the nineteenth and early twentieth centuries, low-grade ores were considered worthless, and mining activities were concentrated on ore bodies containing at least 5% or more of the metal of interest [6]. Such decisions were based not only on the venture's efficiency and profitability but also on the technological capability of the time [7]. However, the current state of affairs, as well as the need to meet rising demand, have altered industrial outlooks, with process considerations now including ore bodies containing less than 1% of the metal of interest.



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Low-grade ores are obtained from a variety of sources, including newly discovered reserves or mine waste containing previously overlooked metals, most likely as a result of mining activities that focused on a different target metal during the initial mining exploration. The target metal was frequently at a higher concentration, making extraction easier [8,9]. Regardless of the source, conventional mining of low-grade ores has been shown to cause more environmental issues than it is worth by generating more waste, which eventually leads to soil and water pollution [10,11], and, eventually, climate change. These environmental problems are the effects of mining activities on the biophysical environment, which consequently lead to environmental degradation. Furthermore, the presence of mine tailings is regarded as a health risk as well as an environmental challenge, and the reduction in toxic element concentrations through re-mining processes is regarded as an environmental beneficiation initiative. This initiative is a necessary strategy for mitigating the long-term environmental damage caused by transformation reactions and the production of acid mine drainage (AMD). In the presence of water-activating microbial solubilization activities, these unavoidable biochemical reactions occur [12]. However, re-extraction processes, particularly those using conventional methods, may prove unprofitable due to low yields and may release toxic gases into the environment [13].

Conversely, when addressing the challenges of mining low-grade ores, we must consider both the economic and opportunity costs. The latter is the justification that focuses on environmental sustainability in all mining strategies, which is an important consideration for future generations. As a consequence, in order to address the challenges of mining low-grade ores, current technological developments must incorporate an environmentally friendly approach as well as seek alternatives that ensure profitability. This review will look at the progress that has been made in this regard.

In addition to investigating these advancements, there has been a noticeable shift in focus in recent years toward biotechnological methods to address environmental pollution issues associated with traditional smelting methods and the use of toxic chemicals. However, in industrial-scale mining, these biotechnological alternatives have yet to attain popularity or commercial acceptance. The stalemate in the situation encountered thus far in improving the bioleaching capabilities of the microorganisms used for this operation is likely one of the major reasons that have kept the biotechnological approach a niche application since its discovery [6,14]. The allure of biomining, however, remains its undeniable environmental friendliness when compared to process operations such as smelting and electrowinning. Because of this appeal, as well as the current inclination toward environmental considerations, bioleaching will continue to be the focus of research efforts aimed at unlocking its potential for metal extraction from ores. Furthermore, bioleaching offers more appealing advantages, such as energy efficiency and broad-spectrum applicability to a diverse range of ore types and grades, as well as the processing of ores or mixtures containing very low concentrations of target metals [15,16]. However, the reliance on microorganisms as the primary role players in bioleaching degradation processes carries the burden of inefficiency caused by a plethora of multifactorial effects, with the ultimate outcome being attributable to poor process kinetics [13]. Metal toxicity, which is a direct result of their activities in the environment, is typically a major challenge for even these robust microorganisms. The increasing concentration of bioleached metals impedes further activities and hurts the entire biochemical process [17,18]. Despite this significant disadvantage and the slow kinetics, the method is regarded as a promising process for recovering valuable metals from mineral sulfides. As a result, it is still regarded as a credible alternative method to traditional leaching methods, particularly for low-grade ores [19–25]. As a result, attention is still firmly focused on developing strategies to address these slow kinetics issues. Several studies have revisited this problem in recent years using newly available techniques, some of which will be discussed in this review.

2. Current Sources of Metals

The global demand for a wide range of metals used in various industries has risen exponentially. As a result, the mining industry is addressing this challenge by devising strategies to boost output. According to Johnson [1], metal demand will more than double in the next two decades, if not sooner. This is a likely driver of the current exploitation of low-grade ores. Consequently, focused research has been conducted to develop methods for assessing the potential environmental impact of mining process variables, such as ore grade and deposit type. Such assessments can provide information on the amount of ore that can be extracted, as well as the amount of tailings that can be produced for one unit of metal, based on the deposit's grade [26]. However, the viewpoint that low-grade ore mining and processing has the potential for additional waste generation and profitability limitations is juxtaposed.

Aside from the information derived from the nature and quality of low-grade ore bodies, their constitutions present additional challenges to mining considerations due to their mineralogical complexity, which is frequently manifested in polymetallic states [6,11]. A polymetallic ore can contain several metals in its composition, such as Cu, Zn, Pb, Ag, and Au, and as a result, extracting a single metal from it may not be economically viable and may cause several environmental issues [10,11]. This implies that when dealing with low-grade ore mining, some of the challenges can be overcome by developing and implementing efficient technology to recover a group of metals rather than just one [10]. Despite these obstacles, profitable low-grade ore mines, such as the Aitik mine in Gällivare, Sweden, are currently in operation. Although there are conflicting reports on the actual copper content of the ore, with Mozaffari [27] reporting 0.4% copper content and Johnson [6] reporting 0.24% copper content, both reports show that recovery is feasible and successful for copper ore with a copper content less than 1%. Another example is Talvivaara, a lowgrade ore mine in Finland with a Ni-Zn-Cu-Co complex ore deposit that began operations in 2009. The mine's ore deposition averages 0.22 wt.% Ni, 0.50 wt.% Zn, 0.13 wt.% Cu, and 0.02 wt.% Co [28,29]. This mine's profitability was linked, among other things, to the easy access to the ore located on the land surface, which made it less expensive to mine [6].

Mining companies have also evolved a focus on waste from mineralogical and metallurgical processes as potential metal sources [30]. This waste, which was previously generated during the processing of high-grade ores, was thought to have no economic value and was discarded. However, mining companies no longer take this stance in recent times. The characterization of these mine wastes, which have revealed significant quantities of potentially valuable metals, is now the focus of research [31]. Furthermore, advances in flotation technology, an efficient technique for concentrating target minerals, have improved the re-extraction of precious metals from mine tailings, implying that their use as a raw material in mining is economically viable. The low cost of mineral beneficiation associated with mine tailings is an advantage, as the ore is already milled and available on the surface, eliminating the capital costs of excavation required to access the metals of interest [32,33]. This competitive advantage was demonstrated in the mining of cobalt in the Democratic Republic of Congo's province of Katanga, where cobalt is mined from mine tailings containing 0.2%–1% cobalt, which is characteristically in the range of low-grade ores [33,34]. According to Van Zyl et al. [35], there is currently approximately 75 major mine tailing re-mining projects underway around the world. The ERGO project, located in Johannesburg, South Africa, is one successful venture that re-mines gold from mine tailings [35]. Other examples include the Kaltails project, which was established to reprocess tailings from mine dumps in Kalgoorlie, Western Australia, in order to obtain gold [36,37]. The continued survival of these re-mining projects can only mean that the savings from avoiding excavation and milling processes allow the mining projects to be viable and profitable.

3. Technological Improvement in Metallurgical Ores Processing Methods

The pertinent enquiry of what technology to use during extraction of metals from lowgrade ores must, without prevarication, factor in the environmental impact and product recovery efficiency. Environmental impact can also be exacerbated by the type of mining process. In open-pit mining, at least 1–3 times quantities of overburden materials are produced than the ore extracted; however, in underground mining, the produced barren rock is often less than 10% of the volumes of ore produced [26,38]. This is due to the high cost of underground mining, which makes it necessary to only focus on the orebody. The overall mining process and environmental impact are shown in Figure 1. In summary, the process of ore mining and metal processing involves ore excavation, pulverization and concentration, conversion of concentrates into oxides, reduction, and refining. Although process optimization initially targeted high-grade ores, there is still room for improvement in relation to processing low-grade ore/tailings. This is because of the fast-paced depletion of high-grade ores on the one hand, and the difficulties faced by the mineral processing industries to efficiently process low-grade ores and metal-contaminated sites on the other hand. The problem has been worsened by the failure of the conventional processing methods to address environmental pollution [39], which includes contaminated sediments and soils, aquatic environment, soil nutrients, and vegetative cover, leading to severe public health concerns.



Figure 1. The process of mining from ore exploration, mining, waste generated, and environmental impact.

In this regard, significant progress has been made in terms of research. The abundance of literature demonstrates that researchers have made commendable efforts to improve the processing of various types of low-grade ores. Spooren et al. [40] observed in their review that the efficient treatment of diverse and complex low-grade ores necessitates the use of a new metallurgical systems toolbox comprising existing and innovative mineral processing, metal extraction, metal recovery, and matrix valorization processes. Tabelin et al. [41] focused on ways to address challenges in ore processing, metal extraction, and E-waste management using emerging technologies and sustainable socio-environmental strategies in another review.

Mochizuki and Tsubouchi [42] upgraded several types of low-grade iron ores from various countries to the desired high-grade ores by removing gangue components (Si, Al, and P) via hydrothermal treatment with various solvents. Mokmeli [43] investigated the economics of low-grade chalcopyrite deposits hydrometallurgical treatment as a function of ore grade and extraction recovery. Following the drawbacks of the Bayer process for leaching mineral acids, which include high energy consumption, waste generation with gaseous emission, and concentrated acid/base emission [44–46], the use of microorganisms has received considerable attention. In this regard, Shah et al. [47] investigated the recovery of aluminum from low-grade bauxite via the bio-hydrometallurgical route, discovering that

high yields of organic acid and other metabolites produced by a marine-based *Aspergillus niger* enabled the sustainable and economical production of aluminum from bioleaching low-grade bauxite ore. Brinci and Gok [48] also upgraded the low-grade boehmitic bauxite by removing silica via floatation, resulting in a high-grade bauxite concentrate with an A/S ratio of 8.54. A Bauxite A/S ratio of less than 8 results in the sintering process, which is well known for being energy intensive and emitting gases [49–51]. Screening/washing, gravity separation, and flotation are the other beneficiation methods used to reduce the reactive silica content in bauxite [52]. The siliceous gangue minerals, kaolinite and illite, are known to be closely associated with bauxite ore, and this complex association has a negative impact on mineral particle liberation, which is linked to a low A/S ratio [48].

Heap leaching is another traditional method for extracting precious metals with the help of microorganisms. It is typically used in acid leaching of copper oxide ores, oxidative acid leaching of secondary copper-sulphide ores, and cyanide leaching of gold ores [53]. However, as observed by Ghorbani et al. [54], its implementation is hampered by the difficulty of maintaining high heap permeability, particularly when leaching fine-grained ores. As a result, researchers are focusing more on bioleaching, which is thought to be more sustainable for extracting metals such as copper, gold, and zinc. Biological leaching is less expensive than the energy-intensive pyrometallurgical process or other leaching methods that use chemicals under harsh conditions. Using gold as a case study, Calvo et al. [55] investigated how the decline in gold ore grade affects energy expenditure and the associated environmental impact using current technology. The authors conclude from the case study that the lower the ore grade, the greater the energy requirements for ore processing. They also point out that technological advancements only partially reduce energy costs and that high energy demands necessitate the use of fossil fuels, which raises GHG emissions.

The feasibility of beneficiating low-grade skarn phosphate from the Mactung Tungsten deposit was investigated by Li et al. [56]. The anticipated benefit is two-fold: extracting phosphorous for the production of phosphoric acid and fertilizer, as well as cleaning the environment. Phosphate retention in mine tailings is harmful to the aquatic ecosystem because it causes eutrophication of lakes and algae blooms. Thus, both direct and reverse flotation were effective in producing acceptable concentrates from low-grade phosphate rocks containing carbonate and silicate gangue minerals. Additionally, with regard to phosphates, Arroug et al. [57] investigated acid leaching methods for the beneficiation of rejected low-grade phosphate washing sludges. Tailings from phosphate washing sludges have low P_2O_5 , high carbonates, and silica content. The authors discovered that the type and concentration of organic acid, as well as the ore's milling conditions, had a significant impact on the ore's upgrade. Above 7%, concentrations of acid were found to attack phosphate minerals, as well as dissolve carbonates.

Sudagar et al. [58] investigated the effect of low-grade metakaolin on compressive strength and heavy metal absorption, and the authors concluded that low-grade kaolins can be useful as construction materials and adsorbents. Magnetic separation techniques were used to beneficiate low-grade rare earth ore [59], and it was discovered that effective upgrading of the rare earth element ore required a combination of dry and wet high-intensity magnetic separation techniques followed by froth flotation. Because rare earth elements are mostly found in deposits that are not concentrated enough to be economically exploited, upgrading is required [60]. The importance of rare earth elements cannot be overstated because they are used to make permanent magnets used in wind turbines and traction motors for electric vehicles. As a result, froth flotation is an important step in concentrating metals and a mixture of chemicals that act as collectors or depressants and are used to improve selectivity during target metal separation from gangue material, as described by Tian et al. [61]. Concentrates from flotation can be subjected to pyrometallurgy, hydrometallurgy, and bioleaching processes for further processing of metals of interest [62], as detailed in Figure 1.

However, it is important to emphasize that the current pyrometallurgical and hydrometallurgical processes cannot efficiently recover metals from low-grade ores because they were designed for high-grade ores [63]. This is due to the difficulty of striking a balance between extracting valuable metals from very low concentrations in the matrix material while lowering the concentration of potentially hazardous metals to stringent concentration limits and achieving extraction with minimal impact on the physicochemical properties of the matrix materials because additional processing, such as valorization, would be required [40].

3.1. Pyrometallurgical Process

Pyrometallurgy is a traditional method for extracting and purifying metals through high-temperature calcination, roasting, reduction, and refining. The entire process consumes a lot of energy and adds a lot to the carbon footprint. Although pyrometallurgical energy requirements vary depending on ore grade and mineralogy, the current exploitation of low-grade ores may have a significant impact on mining process energy requirements [64–66].

It is undeniable that the mining industry is under economic pressure to reduce energy consumption and associated greenhouse gas emissions [5]. The major barrier is in the pyrometallurgy process, where the use of higher-temperature furnaces and blast furnaces is an essentially integrated aspect of metal recovery [65–67]. Previously, Agarwal et al. [68] estimated that an electric furnace requires 1200 kWh per ton of dry deep-sea manganese nodule ore. Concerns about pyrometallurgical process air pollution have prompted governments to impose stringent restrictions through emission regulations and the implementation of acceptable limits to ensure significant levels of environmental protection. Mining companies are required to comply throughout their business cycle, from the beginning to the end, where they are expected to implement land reclamation and clean-up activities. It is also necessary to obtain legal documents pertaining to pollution mitigation and prevention in accordance with each country's mining legislation. The majority of these laws uphold environmental considerations, and they even require the use of best practices and best available technology (BAT) to ensure the protection and restoration of the sites of mining operations [69]. So far, technological advancement in pyrometallurgical processes has sought to reduce energy consumption and carbon emissions while maintaining process efficiency [65]. South African company Evraz Highveld Steel and Vanadium Limited is an example of a company with a technological modification designed with such a consideration [70]. Their initial process employed submerged-arc furnaces (SAF), a technology that has since revealed a number of technical challenges. Submerged-arc furnaces were converted to open slag baths (OSB) for maximum iron and vanadium production in 2004. The OSB furnaces, on the other hand, are limited by their high energy consumption due to their open-arc configuration. This configuration and its implications for energy demand entail a relatively high electricity cost, which fails to balance profitability associated with vanadium (product) recovery. This has prompted a paradigm shift toward improved equipment and operational processes. Several important energy-saving factors have been identified. One such contentious factor identified was the effect of coal source, which was mitigated by limiting suppliers to prevent plant instability caused by the varied composition of the coal, which was linked to poor metallurgical performance, poor metallurgical control of the OSB, and inconsistencies in kilns for the solid-state reduction process. Finally, when compared to SAF, the system was able to achieve improved product recovery while using less energy per ton of metal produced. Furthermore, the use of OSB allowed for lower coal consumption and the use of less expensive coal, such as seam 2 coals with lower reactivity than seam 5 coal with higher reactivity used in SAF.

Blast furnaces are critical pieces of equipment for iron production and are commonly used in mining countries. These massive furnaces have recently been upgraded to accommodate volumes in excess of 5000 m³. In Korea, for example, a 6000 m³ blast furnace was built. Upscaling furnaces has been shown to cause non-uniformity, which increases energy consumption and, ultimately, disrupts secure, stable operation. The development of oxygen blast furnaces (OBF), which are thought to be superior to conventional furnaces,

can be viewed as a solution to energy-saving efforts. The latter has the potential for greater energy flexibility as well as other environmental advantages. The main distinction between these furnaces is that conventional blast furnaces use hot air with less than 5% oxygen and a high nitrogen (N_2) concentration, whereas the OBF uses 99.0% pure oxygen [71]. Nonetheless, when compared to conventional blast furnaces, the use of oxygen in blast furnaces was found to double productivity due to the absence of N_2 [72]. Surprisingly, Takahashi et al. [72] make the rather broad but dire assumption that energy-saving efforts related to conventional blast furnaces have likely been exhausted and that the possibility of developing additional energy-saving methods is unlikely. Further technological advancements in the OBF have resulted in the addition of a top gas remover process (TGR-OBF) with the ability to significantly reduce carbon dioxide emissions [65]. Currently, the OBF is the most productive technology, with the potential for significant reductions in energy consumption and environmental impact [8]. Although technological advances are important, particularly when they can significantly reduce negative environmental impact and energy demand, the US DOE (2007) warns that these effects are limited. Recently, Chetty et al. [64], proposed the incorporation of mineralogical monitoring as an important step toward understanding furnace energy needs assessments, based on the understanding that the composition of the ore plays an important role in furnace efficiency. Their research utilized quantitative mineralogy to differentiate ore types with similar grades and investigated their energy consumption in furnaces. Their findings suggested that this would lead to better decision making regarding the handling of ore variability, allowing furnaces to run more efficiently and thus reducing energy consumption.

The advancement of new technology has resulted in improved methods that have added environmental benefits, rendering some older technologies obsolete and unpopular. A good example is manganese extraction, which is conducted in the presence of a reductant due to its stability in acid or alkaline oxidizing conditions. Normally, coal or carbon is used as the reductant to convert manganese dioxide to manganese oxide; however, this reaction is energy intensive, with several authors, including, Abbruzzese et al. [73], Jiang et al. [74], and Welham [75], indicating that the temperature requirement is between 800 and 1100 °C. Surprisingly, the use of other reductants, such as cornstalk, bagasse, ammonium sulfate, pure sulfur, and so on, reduced the reaction temperatures to a range of 450-570 °C [76]. Despite the lower energy requirements, the process remains unpopular due to the high amount of fine dust smoke produced by the plants, as well as the high investment and operating costs [77,78]. Furthermore, applying this process to the extraction of low-grade ores will present additional technological, economic, and environmental challenges [79]. Because low-grade ores are polymetallic, the various metals in the ore must be recovered in order for the process to be profitable. This becomes an issue in the case of low copper-bearing minerals with high arsenic and other impurities. If copper concentrates contain more than 0.2 percent arsenic, they are considered "dirty" and are rejected by smelters [41,80].

3.2. Hydrometallurgical Process

Concerns about air pollution caused by pyrometallurgical processes have prompted governments to impose stringent restrictions through emission regulations and the implementation of acceptable limits to ensure significant levels of environmental protection. Compliance is required for mining companies throughout their business cycle, from the start of operations to the end, where they are expected to implement land reclamation and clean-up activities. It is also necessary to obtain legal documents pertaining to pollution mitigation and prevention in accordance with mining legislation specific to each country. Most of these laws have an environmental theme and even require the use of best practices and best available technology (BAT) [81].

In terms of energy consumption, hydrometallurgical techniques appear to be the best alternative to smelting and refining routes, and they have great potential for treating various concentrates while also demonstrating increased metal recovery and reduced air pollution [82]. Hydrometallurgy is a process that uses chemical reactions to extract metals of interest from ores, concentrates, and recycled materials. Sulfuric acid (H_2SO_4), hydrochloric acid (HCl), ferric sulfate, nitric acid (HNO₃), ferric chloride, sodium chlo-

materials [78,83-86]. Metal recovery in hydrometallurgy is frequently achieved through the use of H_2SO_4 , but this has a negative impact on the environment occurring from accidental spillages. The likelihood of ground water contamination occurring from H₂SO₄, which are transported through pipes that burst and leaks that cause seepages into surrounding land, is very high [86]. However, technological advancements have resulted in their replacement with chemicals less harmful to the environment. Furthermore, hydrometallurgical processes have evolved in recent years to meet the challenge of mining low metal concentrations in ores. Historically, the majority of zinc metal was extracted from zinc sulfide ores. However, due to the current depletion of zinc sulfide ores, the exploitation of zinc oxide ores has increased [85,87]. Following gangue material separation using conventional flotation methods, the concentrated ore was leached with a solvent to prepare the leached solution for subsequent electrowinning [77,85]. However, low-grade zinc oxide ores, particularly those containing high concentrations of iron, silicon, chloride, and calcium, are processed in a different manner. This process uses a lot of acid, and the purification process is complicated, since it is difficult to separate zinc oxide from the slurry due to the formation of silica gel, which reduces zinc recoveries [77]. Although pyrometallurgical processes can achieve high zinc recoveries from oxidized zinc ores, the associated high energy and production costs have prevented widespread industrial acceptance [77]. To overcome the hydrometallurgical process's poor zinc leaching, Santos et al. [88] and Ju et al. [89] propose that impurities be kept insoluble, which can be accomplished by using alkaline agents, such as sodium hydroxide and ammonia solutions, in a selective alkaline process. To prevent the solubility of impurities, such as Fe_2O_3 , SiO_2 , MgO, and CaO, this process requires a high pH of about 6–7. Treating ore containing high alkaline gangue, such as zinc oxide ore, with alkaline solution reduces the consumption of leaching agents when compared to sulfuric acid leaching, and it also reduces the complexity of the purification process [90]. Despite research into the use of specific alkaline processes [88,89,91–93], challenges leading to poor zinc leaching were still encountered, and Rao et al. [77] reported further enhancement of zinc extraction from low zinc oxide ore, through the addition of organic ligand–nitrilotriacetic acid, improves the stability of zinc complexes.

ride, and other inorganic chemicals are used in the leaching of metals from various raw

Another case in point is the extraction of alumina (Al_2O_3) from bauxite, a major raw material [94]. Gibbsite, boehmite, and diaspora are all types of alumina minerals, with diaspora being the most common in China. The Bayer process has always been the principal method for producing Al₂O₃ from bauxite raw material [95], although the bauxite ores must have a sufficient alumina (A) to silicon (S) ratio to use this process. However, due to the abundance of bauxite ores with high silica and low alumina (low-grade ore), this becomes a challenge [96]. Several studies have been carried out in order to improve the Al_2O_3 grade of such ores and meet the Bayer process requirements [49,95,97]. There has been significant progress, with the inclusion of an additional desalination step used to raise the Al_2O_3 grade to above 60%. Desalination, also known as ore grade improvement, is accomplished through washing or flotation. The latter is thought to be preferable and recommended. However, because carbonate minerals have similar flotation properties to alumina minerals, a single flotation process is not recommended for bauxite containing carbonate minerals. Guan et al. [95] recently reported an additional step after flotation in which hydrochloric acid leaching is used to dissolve the carbonate minerals, increasing the A/S ratio even further. Sukla et al. [97] proposed bio-beneficiation (the use of microorganisms) as a potential solution to the problems associated with conventional methods due to the limitations that may still be encountered during conventional bauxite purification.

Hydrometallurgical leaching is reliant on the use of chemical reagents, and for a time, sulfuric acid was the primary acid used in the industrial extraction of metals such as nickel

from saprolitic ores. The availability and low cost of sulfuric acid were two factors that contributed to its widespread use [98]. Because of their recyclable nature, other acids, such as hydrochloric acid and nitric acid, have recently been considered as alternatives to sulfuric acid for nickel leaching [99]. Furthermore, comparative studies were conducted to compare other acids to sulfuric acid in the leaching of metals from low-grade ores. Astuti et al. [99] investigated the efficacy of citric, sulfuric, nitric, hydrochloric, lactic, and oxalic acids in the leaching of low-grade saprolitic ores from two different mining areas in Indonesia. Surprisingly, citric acid and sulfuric acid were more effective in nickel than other acid solutions, providing motivation for the potential use of citric acid in nickel leaching. Table 1 lists various leaching acids and their functional abilities under various conditions. This table emphasizes the high recovery of target metals in short periods, ranging from a few minutes to a few hours.

Leaching Leaching Ore Type Leaching Reagent **Common Parameter** Reference Period Efficiency

Table 1. Application of hydrometallurgy for leaching valuable metals from low-grade or	e and
mine tailing.	

Low-Grade Ore								
Low-grade vanadium-bearing titanomagnetite	Low-grade dium-bearing nomagnetite Hydrochloric acid HCL) HCL) HCL) HCL) HCL) HCL) HCL) HCL) HCL		10 min	[100]				
Low-grade zinc silicate ore	H_2SO_4	94% of Zn	The effect of particle size and concentration of H_2SO_4	180 min	[85]			
Low-grade zinc oxide ore	NH ₄ Cl–NH ₃ solution	90.3% Zn	Effect of particle size and influence of NTA concentration	60 min	[77]			
Low-grade chalcopyrite concentrate	Sulfuric acid electrolyte	96% Cu	Effect of the particle size, nitrite concentration, and acid concentration	2 h	[101]			
Low-grade manganese dioxide ores	H_2SO_4	92.8% Mn and 24.6% Fe	The effect of sulfuric acid concentration and particle size	60 min	[102]			
Low-grade Ni–Mo ore	Sodium hypochlorite sodium hydroxide and sodium carbonate	96.3% Mo	Sodium hypochlorite and sodium carbonate concentrations	5 h	[103]			
Mine tailings								
Tailings of oxidized ores	Sodium hydrosulfide (NaSH) and potassium amylxanthate (KAX)	45% Cu and 83% Co	Size distribution of particles	14 min	[30]			
Copper sulfide tailings	98% H ₂ SO ₄	98.45% Cu, 21.41% Zn, 56.13% Mn, and 17.25% Fe	Sulfuric acid concentration and leaching time	2 h	[104]			
Pyrite flotation tailings	H_2SO_4 and ferric iron	79.6% Cu and 43.7% Zn	Reagent concentrations	2 h	[105]			
Molybdenite flotation tailings associated with galena	Sodium hydroxide	98% Pb and 98% Mo	Concentration of sodium hydroxide	1 h	[91]			

3.3. Environmental Impact of Waste Generated from Conventional Processing Methods

Ample evidence of mining's impact on surrounding ecosystems demonstrates the process's many devastating consequences, including changes in the interspecific interactions of resident organisms [7,106]. Even with advancements in conventional methods for mitigating these environmental impacts, waste generated during the extraction and processing of mineral ores is unavoidable. As a result, careful planning for these wastes and their management is essential. Most governments address this by imposing regulatory

measures and policies governing mine waste treatment and storage. However, oversight and adherence continue to be challenges.

Mine tailings and overburden are the two major types of solid waste associated with all mining processes (waste). Their environmental effects vary, but mine tailings are thought to be more problematic [26,38]. These mine tailings have been linked to several documented cases of water pollution, with most environmental regulatory bodies agreeing that water contamination from mining should be considered one of the world's top three ecological security threats [6,7,107–109].

The presence of amalgamated sulfide minerals, such as pyrite and pyrrhotite, in most precious metals contributes to the toxicity of mine tailings. These sulfide-containing minerals are responsible for acid production, which promotes metal solubilization in tailings [12]. Although the sulfide minerals coexist with the carbonate minerals that are responsible for neutralizing the produced acid [110], these carbonate minerals are usually insufficient to completely neutralize the concentration of acids present. The most likely reason is that they are not present in sufficient quantities to adequately counteract acidic effects. Ultimately, the failure to completely neutralize all H⁺ within mine tailings that come into contact with water results in the production of AMD. The gold and coal deposits in South Africa are an example of this, where the neutralization effect is overwhelmed by the production of this acid, as demonstrated by the visible common problem of AMD in South Africa [111]. Similarly, even when complete neutralization is achieved in the presence of sufficient carbonates, the product derived from it is referred to as contaminated neutral drainage (CND) [112]. CNDs are still regarded as a problem for the environment because toxic elements, such as Se, As, Ni, Zn, and Co, are frequently present in high concentrations in these bodies of water, posing a health risk to microorganisms, plants, and animals [12,113]. This is exacerbated by environmental phenomena, such as bioaccumulation and biomagnification [114,115].

It is critical to recognize the contribution of comminution to chemical reaction rates through the crushing and milling of rocks and ore. As a result of the increased surface area, favorable reaction kinetics in the presence of oxygen and water are facilitated. The tailings' sulfide components undergo an oxidative reaction that uses oxygen to generate sulfuric acid, which promotes metal leaching or solubilization [38,116]. This phenomenon is thought to be a natural part of the weathering process. This weathering process is accelerated, however, due to the increase in surface area (fine particles). Because of this rapid reaction, AMD is considered one of the most serious environmental issues in the mining industry [108,117,118]. The continuous production of acid from mine waste causes a drop in pH, which creates favorable conditions for autochthonous microorganisms found in these wastes. These microorganisms contribute significantly to metal solubilization processes by accelerating acid generation and, in most cases, enhancing metal mobilization to other parts of the environment [108,119,120].

Water polluted by AMD is not safe for drinking. Furthermore, the purification process is considered costly and is influenced to some extent by the type and concentration of toxic metals that must be removed from the polluted water. The rising cost of the process frequently forces the abandonment of this polluted water source, which has a heavy toll on residents in settlements near mining operations, which is exacerbated when other sources of potential potable water are not nearby [121]. Contaminated water may also pose a risk when used for irrigation because some plants have the ability to bio-accumulate metals that will eventually affect humans or animals who consume them [122].

Mine tailing stockpiling remains the preferred method of storage and/or disposal. Often, designated mine dumps are established in the vicinity of major mining cities (Figure 2). Unfortunately, this method of storage (disposal) introduces pollution to areas that are not necessarily close to mines, with winds dispersing dust particles containing toxic metals, such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, and Sn. The accumulation of mine tailings around cities and suburbs is extremely hazardous to human health, with some of them containing significant amounts of radionuclides [123]. Most pertinent to note is the cyanide pollution that is frequently associated with gold extraction processes [107].



Figure 2. Mine tailing dumps. Pictures taken in Gauteng province, South Africa.

Mine tailings have been discovered to be inadvertently storing low-grade ores. Although the grade of the mineral they contain may be too low for economical extraction at the moment, there is a possibility that these wastes will hold economic value in their mineral content in the future [124]. This understanding is most likely why some mining companies practice continued storage, even after tailings have been re-extracted and reprocessed. According to Blight [124], some South African gold mine tailings have been re-mined and reprocessed at least three times in the last century. As a result, they are stored in a location where, if the need to re-extract arises, an ease of access is guaranteed, as opposed to when the tailings are rendered inaccessible by being buried beneath old mines. However, reprocessing tailings generates waste, which poses environmental problems. Consequently, not all reprocessed tailings are permanently stored for future reprocessing. Some are investigating its use in the production of bricks as a building material in the construction industry [125]. According to Beulah et al. [126], although the bricks are more expensive than traditional bricks, this cost-effective method of producing brick is environmentally friendly. Another example is the use of tailing as a substitute for fine aggregates in the manufacture of cement [127].

For those tailings that are continuously stored, certain metals demand may still be met, to some extent by re-extracting and reprocessing tailings. The key to this re-use strategy, however, is the adoption of storage methods that reduce the environmental impact of these old and new mine tailings. Combining technological advances and waste management strategies should be integrated to ensure that both aspects work in synergy [128]. Most government policies address this issue by requiring waste management licenses for the storage of mine tailings and dump sites as part of the overall regulation of mining activities.

4. Environmental Considerations and Alternative Metal Ore Extraction Methods

So much has been done to reduce the environmental impact of traditional methods, and progress has been made over the years [70,77,85,87,99]. Despite these efforts, pollution and its negative effects on surrounding ecosystems are an unavoidable by-product of mining activities. As a result, research into mitigation and amelioration strategies is ongoing. The biomining strategy, which takes advantage of microorganisms' bioleaching activities, is one such intervention. Several authors agree that the potential benefits of bioleaching in the exploitation of low-grade ores will be significant, particularly in addressing challenges identified by the use of conventional methods [15,63,129–131]. To support this claim, studies comparing the metal extraction efficiency of chemical and bioleaching methods have been conducted. Nguyen and Lee [132] investigated the efficiency of chemicals (sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) and bioleaching (mixed cultures) processes in the removal of arsenic (As) and other heavy metals from mine tailings. In comparison to chemical leaching, bioleaching achieved higher removal efficiency for all metals (except zinc) after 300 h of incubation.

Previously, Bayat and Sari [133] compared the efficacy of the bioleaching process using *At. ferrooxidans* for the recovery of metals from sludge to the use of H_2SO_4 and ferric chloride while holding all other conditional parameters constant. Bioleaching had the highest metal removal efficiency, followed by sulfuric acid and ferric chloride, according to their findings. However, the bioleaching process, required a longer operational period. This was similar to the findings of Szolucha and Chmielewski [134] when using bioleaching to treat low-grade uranium ore, with uranium recovered at 75%–15% w/w in 55 days compared to 64%–13% w/w in 31 days when H_2SO_4 and H_2O_2 were used. A review of several studies revealed similar trends of longer operation time (Table 1). However, in terms of residence times, chemical leaching has been observed to range from a few minutes to hours, as opposed to the days required for the bioleaching process. Critically, the bioleaching process exposes mineral sulfides to acids, and the presence of these acids promotes further metal leaching.

Microbial communities rely on biological activity, which is frequently regulated by various proteins, including enzymes, which are influenced by a variety of parameters, including pH. Moreover, any significant change in environmental conditions often necessitates a period of acclimation and adaptation, which slows several biochemical functions and, as a result, has a significant impact on process outcomes. Despite the longer operating time required, bioleaching is still regarded as a viable alternative to traditional chemical reactions [132,133]. The allure of bioleaching among researchers stems from the credible consideration of its status as a characteristic of "green technology," involving microorganisms, mostly autotrophs, with the ability to fix CO₂, much like plants, whereas processes such as smelting are notoriously associated with CO_2 emissions (Johnson 2014). Bioleaching also has a lower carbon footprint than traditional methods due to its ability to occur at atmospheric pressure and lower temperatures (20–80 $^{\circ}$ C), as well as lower associated energy costs [135]. Despite the fact that the bioleaching process produces an acid by-product (which is usually recovered to prevent environmental pollution), it is still considered relatively eco-friendly due to the moderate concentration of acids produced by microorganisms. The low-cost advantage of bioleaching can be realized in both low-grade ores and mine tailing leaching, with the latter achieving a higher yield. However, it is important to note that the scaled-up bioleaching process undergoes similar pre-requisite energy-consuming blasting and grinding treatments, as shown in Tables 1 and 2. This step is critical for the efficiency of the process.

Dump leaching, heap leaching, in situ leaching, and tank leaching are commercial bioleaching processes for extracting metals, such as copper, cobalt, and uranium [136]. Nonetheless, bioleaching's demonstrated capabilities have not been sufficient to gain widespread acceptance or consideration as a replacement for conventional methods. This is because of the slow dissolution kinetics. Furthermore, the possibility of compromised metal leaching yield due to high metal concentration and its associated toxicity, inhibiting microbial activity or survival, has limited the bioleaching process's large-scale application by Pathak et al. [15] and Clark et al. [137] who reviewed heap leaching strategies and described the limitations of slow kinetics in the bioleaching process. It is estimated that 300 days are required to obtain results with laboratory-scale bioleaching experiments and that when scaled up, it will usually take at least 900 days to obtain reasonable metal extraction. Furthermore, the immediate inclusion of freshly dug-up mineral heaps, which is standard industrial practice, can be hampered by the long lag phase of bacterial growth, with incubation and biological treatment lasting up to three years [138]. This lengthy incubation period has discouraged the use of bioleaching and reduced its feasibility for commercial scale metal sulfide mining processes [13]. To alleviate the lengthy lag phase associated with the heap leaching strategy, perhaps a preparatory treatment should include the introduction and mixing of small inoculating heaps from old sites to new heaps, with an incubation period provided before their addition to older heaps. This has the potential

to improve bioleaching efficiency by shortening the lag phase and acclimation periods required by microorganisms. In particular, the constraints and limitations associated with bioleaching necessitate multi-directional solution-driven research aimed at addressing these challenges in order to improve this bioprocess.

Table 2. Application of acidophilic microorganisms in bioleaching of low-grade ores.

Ore Type	Microorganisms	Amount Leached	Common Parameters	Leaching Period	Reference				
Low-Grade Ore									
Sulfidic tailing of Golgohar Iron Mine	Mixed culture of Leptospirillum ferriphilum, Acidithiobacillus caldus, Sulfobacillus sp., and Ferroplasma sp.	53% Cu, 84% of Ni, 56% of cobalt, and 35% Fe	Bacterial inoculation, media pH, ore particle size, stirring rate, and pulp density	21 days	[4]				
Low-grade uranium ore	Aspergillus niger	71.4% U	Media pH and pulp density	7 days	[139]				
Low-grade manganese ore	Pure and mixed cultures of <i>Acinetobacter</i> sp. MSB 5 and <i>Lysinibacillus</i> sp.	70% Mn from Acinitobacter sp., 67% Mn from Lysinibacillus sp., and 74% Mn from mixed culture	Bacterial inoculation, media pH, ore particle size, stirring rate, and pulp density	21 days	[140]				
Low-grade laterite ores	Aspergillus and Penicillium strains	36% Ni, 54% Co, and 0.76% Fe	Media pH, ore particle size, stirring rate, and pulp density	60 days	[141]				
Oxide low-grade ores (mining residue)	Aspergillus niger	68% Cu, 46% Zn, and 34% Ni	Media pH, ore particle size	14 days	[142]				
Low-grade granitic chalcopyrite	Sulfolobus	85% Cu	Media pH, ore particle size, stirring rate, and pulp density	30 days	[143]				
Mine tailings									
Lead-zinc mine tailings	Acidithiobacillus ferrooxidans	0.82% Pb, 97.38% Zn, and 71.37% Fe	Media pH, ore particle size, stirring rate, and pulp density	50 days	[144]				
Concentrated arsenic mine tailings	Acidithiobacillus ferrooxidans	>85% of As	pH and pulp density	20 days	[145]				
Mine tailings	Indigenous sulfur-oxidizing bacteria	96.13% Cu, 97.59% Zn, and 40.43% Pb	Effects of initial pH	13 days	[146]				
Mine tailings	Single and mixed cultures of Acidithiobacillus thiooxidans and Acidithiobacillus ferrooxidans	34.4% 31.5%, and 47.1% of Cu for <i>A. thiooxidans,</i> <i>A. ferrooxidans,</i> and mixed cultures, respectively	Bacterial inoculation, media pH, ore particle size, and stirring rate	6 h	[132]				

Particle size below 74 µm, stirring rate above 120 rpm, pH below 2.5 and 6.5 for bacteria and fungi, respectively.

5. Technological Improvements in Bioleaching Processes

There are significant differences in developments related to traditional mining methods versus the use of bioleaching for the same endeavor. As previously discussed, the primary focus of attention for development in conventional mining is on modifications to related equipment to improve extraction efficiency, sometimes with environmental consideration. Perhaps this is not the case for bioleaching strategies, because the capability of microorganisms is limited not only by the type of ore or potential environmental damage but also by the microbial enzymatic reactions (specificity) involved and the ability of microorganisms to withstand high concentrations of metals deposited in the resident environment, as well as the pH [147,148]. Consequently, technological advances in bioleaching are the culmination of research and investigation aimed at improving the diverse capabilities of various bioleaching microorganisms. Progress in this area of research, however, is frequently hampered by the investigative tools and techniques available at the time of research. As a result, the study of bioleaching processes has evolved in tandem with advances in molecular biology and biochemistry techniques. Furthermore, as is often the case with scientific investigations, each new discovery presents new challenges in our understanding of aspects of bioleaching mechanisms. Other factors critical to bioprocess effectiveness complicate matters in some cases. This section explains some of the aspects of microbial metal dissolution mechanisms that have opened up new research avenues.

5.1. Research Progress and Biomining Process Improvement

The evolution of bioleaching can be traced back to early studies that assumed natural leaching processes were devoid of biological contributions and were instead influenced by a chemical weathering process [149]. However, convincing evidence of microorganisms' active role in the previously assumed natural chemical weathering process, demonstrated in the mid-twentieth century, triggered a cascade of subsequent research [150]. This includes everything from growing and identifying microorganisms to applying them in bioleaching [151–153] and, later, understanding the molecular factors that allow them to survive and grow in such harsh environments [154]. Nevertheless, molecular research and evolving technology have revealed that their roles are far from being fully understood.

5.1.1. Accumulation of Metals in Solution, Toxicity and Its Effects on Microbial Activity

The dependence of microbial activity on various environmental factors (such as nutrients and trace elements) and its impact on process efficiency is limiting. Adeleke [155] explains that this problem could be solved by incorporating bioaugmentation strategies that provide nutrition and energy requirements in processes that use microbial leaching to extract metals of interest. This is significant because, while metal ore or mine tailings contain a variety of compounds that contribute to nutritional growth, they are frequently deficient in some vital elements required for proliferation and biomass growth. Increased biomass is critical for peak enzyme production, which is required to catalyze chemical reactions. Meeting the nutrient growth requirement is thus essential for bioprocess efficiency. Furthermore, synergistic co-factor effects in bioleaching environments have been identified as important. Co-factors, for example, include some nutrients that, while only required in trace amounts, play a critical role in improving enzyme activities. Nickel, for example, has been shown to play an important co-factor role in a number of enzymatic reactions [156]. Thus, it is critical to maintain a balance of these trace element requirements for specific bioleaching microorganisms, particularly in conditions where tailing analysis has revealed a trace element deficiency. The progressive leaching process and the resulting low pH typically increase metal toxicity while also inhibiting enzyme (protein) activity. The nickel requirement is the challenge posed by the metal's increase. The ability of such an increase to replace metals in metalloproteins, thereby affecting their ability to bind to active sites of enzymes such as dioxygenase or sulfite oxidase, causes indirect oxidation stress in cells [18]. When Chen et al. [17] investigated the toxicity of nickel to At. thiooxidans, they discovered that a high nickel concentration of about 600 mM inhibited bacterial growth and its sulfur reduction ability. Although metal toxicity does not completely inhibit acidophilic bacteria growth, it can significantly reduce growth at high concentrations, as demonstrated by Ramos-Zúñiga et al. [157]. The effect of cadmium at concentrations ranging from 0 to 200 mM was also tested on A. ferrooxidans strains at 75, 100, and 200 mM, and a significant growth reduction was observed when compared to cells grown in the absence of cadmium. It corroborates previous research and observations by Sampson and Phillips [158], who found that increasing concentrations of nickel, cobalt, and copper inhibited the oxidation ability of mesophilic cultures of Thiobacillus ferrooxidans and Leptospirillum ferrooxidans, with copper having the greatest inhibition. Chen et al., [159] investigated other metals

and their toxicity concentrations to bioleaching microorganisms, finding that 300 and 400 mg/L Cu (II) and Cd (II) inhibited the growth of Thiobacillus thiooxidans, respectively. Pradhan et al. [160] discovered that 5 g/L and 0.03 g/L of V⁴⁺ and Mo⁶⁺inhibited the growth of *At. ferrooxidans*, respectively. As a result, the efficacy in the bioleaching process necessitates a high tolerance to metal toxicity [161].

Another study that contributed to our understanding of the bioleaching process was the role of evolutionary adaptations and their significance in microbial survival in the harsh environment of mine tailings and metal ores. It became clear that a thorough understanding of these evolutionary adaptations was required to connect the dots regarding metal toxicity and tolerance. Some studies have shown that developed mechanisms linked to the evolution of these bioleaching microbial communities can be used to combat metal toxicity. Early research isolated nickel-resistant microorganisms from harsh environments and assessed their toxicity adaptation mechanisms [162]. Extracellular detoxification, intracellular sequestration, cation transport system modification, and active transport by efflux pumps are among the mechanisms identified [17].

To summarize, increased biomass is required for the bioleaching process to be effective. When toxic element concentrations and pH levels reach extremes, bacterial growth is inhibited, and the process is compromised. Although microorganisms have evolved to meet the challenges of metal toxicity in their environments, they have not been able to overcome the extreme increases in metal concentrations that are frequently achieved during bioleaching activities. Our understanding of the tolerance levels for the various environments and metals to which these organisms are exposed will allow us to develop metal toxicity management strategies in biomining operations.

5.1.2. Improving and Adapting Bioleaching Organisms for Biomining Operations

Although bioprospecting in mines allows for the collection of microorganisms with natural bioleaching capabilities, these desired qualities are usually enhanced in laboratory environments to improve biomining process efficiency [147]. This is accomplished in microorganisms by selectively pre-adapting them through repeated culturing in media containing gradually increasing concentrations of the target metal. This procedure aims to improve the tolerance and metal dissolution ability of microorganisms prior to their use in bioleaching systems, as well as their efficiency under scaled-up process conditions [148,163].

Kim et al. [164] demonstrated the importance of pre-adaptation in their study by comparing the abilities of an adapted and un-adapted bacteria strain in leaching spent catalysts, such as Ni²⁺, V⁴⁺, and others. Adaptation was achieved in their study by serial sub-culturing with increasing concentrations of metals until maximum tolerance capacities were achieved. The comparison of the bioleaching process using these two groups of organisms revealed that adapted bacteria leached over 90% of Ni²⁺ and V⁴⁺ in 40 h, whereas un-adapted bacteria leached around 70% of Ni^{2+} and V^{4+} during the same period. In another study comparing the bioleaching abilities of adapted and un-adapted A. ferrooxidans, Xia et al. [165] discovered that adapted bacteria leached 47.5% of Cu²⁺ while un-adapted bacteria leached 39.5%. Adaptation was achieved in this study by serially increasing chalcopyrite pulp densities from 1% to 5%, and bioleaching experiments were carried out under 5% chalcopyrite conditions. Ultimately, this adaptation strategy is based on reducing the time required for the required lag phase in bacterial growth, and there is evidence that a pseudo-continuous-type approach significantly improves biomass generation and substrate utilization efficiency in most fermentation and biodegradation processes [165]. The main disadvantage of this strategy, which incorporates serial sub-culturing to improve tolerance and adaptation, is that it is time consuming and may introduce mutations that change the physiological advantages derived from previous exposure to harsh environments [147,166].

Most studies on metal toxicity, tolerance, and adaptation found that a full complement of these desirable traits is not typically found in a single native microorganism but rather in a consortium, which is typical of the operating conditions found in natural environments. Table 2 shows that the efficacy of bioleaching from a consortium is greater than that of a single species. Early studies and applications tended to concentrate on achieving bioleaching with a single population of organisms [139,141]. However, recent research suggests that this approach is unrealistic, as there is now abundant evidence of the importance of interspecific interactions and how they are critical to metabolite production and degradation processes in the natural environment [4,167,168]. These communities of microorganisms make significant contributions to the degradation of complex compounds through the use of their individual enzymes. As a result, a practical approach to bioleaching strategies must apply biomimicry of what is common in the natural environment to the degradation of these ores by allowing biodegradation to occur in a similar manner. Few studies have used techniques such as polymerase chain reaction (PCR) and quantification/real-time polymerase chain reaction (qPCR/RT-PCR) to investigate community dynamics in relation to metal toxicity/tolerance and adaptation in bioleaching processes [169-171]. The application of these techniques has enabled the real-time monitoring and quantification of the dynamics of important genes or microbial communities as metal concentrations rise. This is critical for understanding the active roles that individual organisms play within population dynamics during bioleaching activities. A few recent studies have shown that microbial genes coding for ferrous iron and sulfur oxidation play an important role in bioleaching processes [17,172,173]. This knowledge is important in the selection and application of microbial consortia, particularly in enrichment processes, to ensure the ideal balance is achieved for the organisms that are critical to bioleaching efficiency within a determined population.

5.1.3. Expanding our Understanding of Bioleaching Using (Meta) Genomics

Genomics is a branch of molecular biology that focuses on understanding the structure, function, and evolution of genomes, allowing for unprecedented insights into microorganism genetic and metabolic potential [116]. It is especially useful when studying organisms isolated from niche environments that have evolved survival traits. Genomics studies enable us to identify similarities and differences between the organism under study and other microorganisms in the same genus. Such information is useful in developing strategies for bioproducts enhancement and industrial applications. In 2001, the first draft genome of a bioleaching microorganism was published, and it came from the well-studied bioleaching bacteria At. ferrooxidans. This published draft genome has been used to construct or predict important metabolic abilities. Sulfur assimilation, quorum sensing, extracellular polysaccharides, and iron and sulfur oxidation are all examples [17,174]. The value of genetic studies lies in the indirect improvement of the bioleaching process based on a better understanding of the organisms' genetics and physiology [175]. Presently, over 55 bacterial and 36 archaeal draft genomes of bioleaching microorganisms have been published [14]. This has allowed for more accurate predictions of the genetic and metabolic potential of bioleaching microorganisms, such as models for energy metabolism in A. caldus and A. thiooxidans, and models for overall metabolism in *Sulfobacillus* spp. and *Ferrovum* spp. [176–179]. However, one limitation of these predictions is that the majority of whole-genome sequencing projects published have only been performed on bioleaching microorganisms that have been kept in the laboratory for several years, if not decades [166]. Concerns have been raised about the evolutionary ability of these isolates, as well as the possibility of genetic changes, such as mutations and gene loss, which may have resulted in genome rearrangement [166]. Furthermore, most of these organisms' robustness has not been tested in significant field application and/or commercial bioleaching work. Relevantly, both metabolic and functional predictions derived from such an old stock of microorganisms, such as metal resistance, growth parameters, and metabolite production, may influence the outcomes of bioleaching application [180]. As a result, it is critical that research be tailored to the specific environments in which the biomining process will take place and that organisms derived from within should then be used for feasibility and field studies leading to applications. Nonetheless, the importance of functional gene predictions, particularly in bioleaching genomics, is critical for progress toward process improvement [181,182].

5.2. Recent Developments in Bioleaching Bench Applications

5.2.1. Impact of Flotation Reagents on Bioleaching Microorganisms

Metals of interest are typically concentrated in mineral processing using flotation reagents, such as collectors, depressants, and frothers, to increase recovery. Flotation reagents separate metals of interest from gangue by increasing the aeration of these metals in water and facilitating the attachment of air bubbles required for the metal of interest's levitation. The presence of these flotation reagents, on the other hand, presents a set of challenges for microorganisms involved in bioleaching. Furthermore, the results differ depending on the perspectives of the various researchers. In some cases, flotation reagents absorbed into concentrates or tailings may have an impact on the activity of bioleaching microorganisms, either a positive or a negative one. So far, contradictory results from various studies indicate that residual flotation reagents in concentrates and tailings may inhibit or reduce the oxidation ability and growth of bioleaching microorganisms or may contribute to improving these microorganisms' bioleaching ability [60,183–186].

Few studies have been conducted with the goal of understanding the potential effects of interactions between bacteria and flotation reagents. Dehghan and Dianati [184] investigated the effects of lead-zinc flotation reagents on the activity of bioleaching bacteria using three organic reagents (including xanthate collectors and gasoline) and two inorganic salts. Their findings indicated that the presence of flotation reagents had a positive effect on the activity of the bacteria (individually or as consortia), with zinc bioleaching being greater in the presence of flotation reagents than in the absence of flotation reagents. This beneficial effect was attributed to the addition of salts, which served as carbon and energy sources for the bioleaching bacteria. Jafari et al. [187], Christel et al. [138], Zhang et al. [188], and Zeng et al. [189] found that flotation reagents positively influenced the activity of bioleaching bacteria. Dong and Lin [185] conducted another study in which they compared the effects of five different flotation reagents on the bioleaching of copper from chalcopyrite. Copper extraction was reduced by 45% by butylamine, 36% by ethyl xanthate, 20% by isoamyl xanthate, 13% by isopropyl xanthate, and 11% by butyl xanthate when compared to the control. Guo et al. [190], Pacholewska et al. [191], Dopson et al. [192], Okibe and Johnson, [193], and Tuovinen [194] all found similar results. However, the authors' differing results raise the question of how the effects of flotation reagents can differ and have different effects on the bioleaching process. This lack of agreement highlights the likely dissimilarities that result from environmental uniqueness, implying that organisms from different environments may be behaving differently, supporting our assertions that studies for different mine areas should be conducted, and organisms from that region may likely work best for the particular ore.

To summarize, answers may be found in understanding the mechanisms of attachment used by microorganisms to make contact with and interact directly with the metal in order for leaching activity to occur. To attach to the metal, bioleaching microorganisms must produce EPS, which is dependent on the ions on the mineral surface. *Acidithiobacillus ferrooxidans*, for example, attaches to pyrite via an electrochemical interaction with the negatively charged pyrite surface mediated by exopolymer-complexed iron (III) ions [138,195]. The residual flotation reagents on the concentrate and tailing can change the properties of the mineral surface, which can either positively or negatively influence the bioleaching process [185,187,194]. Before commercialization decisions are made, a credible strategy for gaining this understanding will involve the execution of small-scale trial runs that integrate optimization with a focus on the flotation type and specific consortia involvement to determine the effect it will have on the organisms involved [60,185,193].

5.2.2. Impact of Ferrous/Ferric Ions Concentration on Bioleaching Experiments

The oxidation of Fe^{2+} to Fe^{3+} in the presence of oxygen is the primary mechanism used by bioleaching microorganisms for metal dissolution. This Fe^{2+} is the main source of energy, whereas the biologically regenerated Fe^{3+} initiates metal dissolution [161,196]. These reactions occur in a series of steps and are mutually dependent. The rate of metal the bioleaching process is critical [197,198]. Therefore, before being used for bioleaching, the prepared inoculum must reach a significant concentration of Fe²⁺. When bioleaching experiments using an inoculum strength containing 9 g/L of Fe²⁺ solution were used, Xiang et al. [198] observed a maximum of 95% copper leached in 5 days compared to 12 days without this specific concentration of Fe²⁺. However, as a precaution, it was suggested that a suitable concentration of Fe²⁺ should be at least 6.25 g/L. Dong et al. [196] obtained similar results by increasing copper leaching from five different copper sulfide minerals early in the leaching process by adding adequate amounts of ferrous ions.

Ferric irons were found to have beneficial effects on *At. ferrooxidans* involved in bioleaching in a study conducted by Chen et al. [17]. The bacterial tolerance to nickel in the presence of ferric irons was the basis for this study. They discovered that 600 mM nickel inhibited the growth and sulfur metabolism of *At. ferrooxidans* completely. However, when traces of ferric iron (between 0.5 and 5 mM) were added, *At. ferrooxidans* were able to tolerate a high concentration of nickel, and as the ferric iron concentration was increased, metal resistance genes and sulfur metabolism were significantly enhanced. When trace amounts of ferric irons were used, this effect was found to be beneficial. However, once the concentration was increased above 5 mM, no further beneficial effects on the growth of *At. ferrooxidans* were observed.

5.2.3. Catalyst Application

The reason for adding a catalyst is to try to speed up electron transfer by lowering the activation energy and thus increasing the rate of reaction. Furthermore, metal ions, such as Ag⁺, Hg²⁺, Bi³⁺, Cu²⁺, and Co²⁺, have been used and found to have superior catalytic properties to non-metallic catalysts [15,25]. Non-metallic catalysts have a low bioleaching yield and require a lot of catalyst to achieve significant process efficiency [15,87]. Guo et al. [23] investigated the catalytic effects of silver ions (Ag^+) and copper ions (Cu^{2+}) on the dissolution of realgar (As₂S₂) and discovered that the leaching efficiency was increased to more than 86% compared to 4% for As₂S₂ leaching without Ag⁺. In this case, the catalytic effect of Cu²⁺ was not as strong as that of Ag⁺. This study also discovered that these catalysts can be used for either chemical or bioleaching methods to improve metal dissolution. Zeng et al. [22] investigated the catalytic effects of copper ions on the bioleaching of cobalt from lithium batteries by At. ferrooxidans in another study. Their findings revealed that almost all cobalt (>99%) was solubilized in the presence of 75 g/L copper ions in 6 days, while only 43.1% was solubilized in 10 days without the catalyst. Metal ion catalyst research is primarily conducted in the laboratory, with industrial applications appearing to be scarce. It is possible that elucidating the organism's up- and down-regulatory mechanisms during these reactions will provide information on how to improve and manipulate the efficient dissolution rate. Furthermore, improved bioleaching kinetics will lead to increased process efficiency and economics in commercial applications [15].

5.2.4. Metabolomics Application in Bioleaching

The petroleum-refining processes of hydroprocessing and fluid catalytic cracking (FCC) generate massive amounts of spent catalysts containing toxic and valuable metals [199]. These spent catalysts are typically disposed of in approved dumpsites that pose a risk to the environment [200]. These wastes are being investigated as a secondary source of valuable metals, such as Ni, V, Mo, Co, W, Al, and others, in order to solve environmental issues while also meeting current metal demand. Metal extraction from spent catalysts can be accomplished via a hydrometallurgical or bio-hydrometallurgical process. Mouna and Baral [201] used a bio-hydrometallurgical approach to leach lanthanum from spent fluid catalytic cracking catalyst (SFCCC) using the fungus *Aspergillus niger* in a study published in 2019. Lanthanum recovery efficiency of 63% was observed at 1% pulp density but decreased as pulp density increased due to the SFCCC's inhibition effect on *A. niger* activity.

Hydrochloric acid had a high recovery efficiency of 68% to chemical leaching. In this study, bio-hydrometallurgy (a greener process) was recommended because the leaching efficiencies of the two were comparable. However, because these metals have low recovery rates (less than 50%), efforts must be made to develop technologies that allow for high recovery rates [199,202].

5.3. Metabolomics Application in Bioleaching

At the present time, readily available high-throughput genomic technologies are hastening progress in understanding the diversity and genetic make-up of bioleaching microorganisms isolated from extreme environments, albeit they are most often decades-old laboratory isolates [178,203]. Consequently, the expansion of metabolomics applications in tandem with genomics aim to become routine preliminary inclusions in both bioprospecting and inoculum development research for bioleaching. The identification and quantification of low-molecular-weight compounds known as metabolites produced at a specific point in time from a sample understudy (metabolome) is defined as metabolomics [204–207]. Metabolomics is distinct, in that the measurement and quantification of metabolites provides information about an organism's functional status [208]. Simply put, the RNA or transcriptome contains potential genes with unknown functions. Rather than just a prediction, metabolomics provides an immediate answer as to what organisms are capable of producing under specific conditions or premised by catabolite induction. Identifying a larger array of these small molecules released during bioleaching, which may be associated with bioleaching effectiveness, according to Brisson et al. [209], could potentially contribute to a better understanding of the bioleaching process. This is supported by metabolomics studies conducted by the same researchers who investigated and tested metabolites exuded by a *Paecilomyces* fungus as potential contributors to the bioleaching of rare earth elements from monazite [209]. They chose eight of the identified organic metabolites for abiotic testing, and two of the identified metabolites, citric and citramalic acid, significantly increased leaching. Thus, metabolomics is regarded as a significant advancement that will improve the economic feasibility of industrializing bioleaching processes.

6. Research Outlooks

This review identified several gaps that researchers need to address in terms of lowgrade processing and its environmental impact. One of the gray areas identified was the critical need for technological development of pyrometallurgical processes to address the process's high energy requirements, which make product recovery from low-grade processing costly, unsustainable, and harmful to the environment.

Other gray areas were identified in biomining, which is expected to play a significant role globally in the efficient extraction and processing of low-grade ores on an industrial scale while causing minimal environmental damage. The incubation period, which is determined by the lag phase of bacteria growth, is one area in need of investigation. The lengthy period has hampered the adoption of bioleaching on an industrial scale, making commercial metal sulfide processing impossible. There is also a need for research, ranging from identifying suitable microorganisms to comprehending the molecular factors that are critical to their survival in harsh environments. The need for such research demonstrates that understanding microorganisms is a fertile field of study.

This review buttresses that little research has focused on polymerase chain reaction (PCR) and quantification/real-time polymerase chain reaction (qPCR/RT-PCR) to understand dynamics in toxicity, tolerance, and adaptation in bioleaching processes. Another unexploited area in terms of research is the complexity and unique interspecific interactions, as well as the diverse chemical profiles and ingenuity that come with the evolution of different organisms, particularly within consortia and signal transduction. It should also be noted that knowledge of an important step regulated by cell-to-cell communication mechanisms known as "quorum sensing" (QS), as seen in Gram-negative bacteria's role in bioleaching, is lacking. Though much is known about the electron transfer that contributes to the survival of *At. ferrooxidans* replication, studies that look at other bioleaching organisms besides model organisms are scarce. However, the general lack of agreement among researchers on the effects of flotation reagents on the bioleaching process may be due to dissimilarities caused by environmental uniqueness, implying that organisms from different environments behave differently. As a result, studies concerning a specific mine area must be conducted using organisms from that region. Finally, it was discovered that most studies on metal ion catalysts are conducted on a laboratory scale, with little or no application on an industrial scale.

7. Concluding Remarks

Significant progress has been made in understanding the environmental impact of mining activities. However, the importance of mining in a variety of applications, such as the generation of metal for domestic and industrial purposes or income, implies that the mining process will continue as long as there is a demand that must be met. As a result, this review highlighted the current challenges in processing low-grade ores, technological advancement, and the subsequent environmental implications. This review's major conclusions are highlighted below

- As a consequence of the global rate of decline in high-grade ores, the possibility of low-grade ore reserves serving as major future metal sources is high. Furthermore, mine tailings represent an easy-to-process source of metals with high profit potential. The nature, mining, and processing of low-grade ores present challenges that would otherwise impede profitability. The continuous technological evolution of available processes, on the other hand, has the potential to mitigate this effect.
- 2. The environmental impacts of mining processes are a major challenge for the mining industry, regardless of ore grade. This review focuses on changes implemented in terms of the types of chemicals used or energy-saving methods aimed at meeting the required environmental standards as outlined by various governments of mining countries. Some of these advancements in traditional methods have resulted in a preference for hydrometallurgy over pyrometallurgy. Although hydrometallurgy presents greater product recovery challenges than pyrometallurgy, the latter faces challenges due to the process's exhaustion of innovative possibilities and its inherent high carbon footprint. Meeting and resolving the problem of developing effective methods for processing low-quality-grade ores remains at the heart of the modern mining debate.
- 3. Although bioleaching has its own set of challenges and continues to use many of the primary energy-intensive units found in conventional mining, such as milling and crushing, it currently meets the majority of environmental safety requirements but falls short in terms of process efficiency in terms of reaction time for metal recovery. As a result, its industrial application and commercial acceptance are limited. Consequently, bioleaching modification influenced by re-defined optimization methods based on the understanding of the genetic make-up and metabolites produced by microorganisms involved may be the key to process improvement. Such knowledge may elucidate microbial functioning under specific conditions, such as high metal concentration, increasing its feasibility and acceptability for industrial application. Current advances in molecular techniques and omics technologies provide effective tools for enhancing our understanding of these extremophiles and will be critical in unravelling their mystery.

Finally, continuous technological advancement of traditional methods has the potential to improve metal recoveries from low-grade ores while reducing the environmental pollution. Nonetheless, despite the challenges that bioleaching faces, it remains an appealing technology in comparison to conventional methods in this current era. It may also be beneficial to intensify molecular studies on bioleaching microorganisms in order to accelerate the potential for bioleaching process improvement. Author Contributions: Conceptualization, G.N.I. and R.N.; writing—original draft preparation, R.N., G.N.I. and N.C.; methodology, G.N.I.; software, R.N.; validation, G.N.I., N.C. and T.S.M.; formal analysis, R.N., G.N.I. and N.C.; investigation, R.N. and G.N.I.; resources, N.C. and T.S.M.; data curation, R.N.; visualization, R.N., G.N.I., N.C. and T.S.M.; supervision, G.N.I. and T.S.M.; project administration, T.S.M.; funding acquisition, N.C. and T.S.M. All authors have read and agreed to the published version of the manuscript.

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