



Overview of Bioleaching

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Authors' contributions

This work was carried out in collaboration among all authors. Each author contributed to the completion of this paper. The final version was read by each author before it was approved.

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ABSTRACT

Aims: This study aims to present a comprehensive analysis of bioleaching, the fundamental idea behind it, the emergence of microbes and the bioleaching approaches.

Study Design: To do this, this research has been developed on a foundation of significant topics. The researcher used a quantitative approach in this particular investigation. The quantitative study is presented in tables that list the bioleaching processes and efficient microorganisms.

Place and Duration of Study: This study was carried out in Chemical Engineering department, Delta State University of Science and Technology, Ozoro, Nigeria.

Methodology: The researcher decided on a combination methodology because of the context of the present investigation. The approach of data collection and analysis employing qualitative as well as quantitative methodologies is known as combined research design.

Results: The lengthy training period of microorganisms at the laboratory scale, which is significantly impacted by other experimental variables, is one of the key difficulties faced by the bioleaching process. Therefore, the key to increasing the simplicity of bioleaching technologies in large-scale industrial production is to enhance the bioleaching microorganism's currently in use so that they can continue to be highly active under more complicated reaction conditions. Regarding the microbial

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problem, biological leaching piles of just a few genes in the offspring of acidophilic microorganisms have been documented. Although some bacterial genomes from acid mine drainage and acidic environments have been used to create replacements, these models cannot fully depict the potential for leaching; in addition, it can be difficult for researchers to obtain samples of microbes from actual production, making further research challenging.

Conclusion: In the years to come, microbial use for waste treatment and mineral processing will continue to gain importance on a global scale. The need to process ores with trace amounts of copper and gold, the potential for recycling waste spoils and tailings, financial limitations, and potential legislative changes on the environmental impact of more conventional approaches like hydrometallurgy will all contribute to this. The employment of chemolithotrophic and heterotrophic bacteria will be a significant addition, boosting the leaching rates and metal recoveries and enabling the treatment of resistant ores like chalcopyrite.

Keywords: *Bioleaching; acidithiobacillus ferrooxidans; thiobacillus ferrooxidans; heterotrophic microorganisms; metal sulphide.*

1. INTRODUCTION

Metal values for economic uses symbolize the state's global mineral resource expansion. The desire for metals has risen substantially and is predicted to continue to do so in the years to come due to the boost in industrialization brought upon with increase in population. As a result, solid wastes containing metals are concurrently synthesized together with effluents, which had detrimental consequences on the high-grade ores' depleting reserves. Thus, it is significant to attack the issue of cost-effective contamination abatement and metal recovery.

Bioleaching acknowledges that metals are driven in a way that is comparable to biogeochemical cycles, which decreases the demand for minerals including ores [1]. The extraction of mineral ores by microorganisms is an effective, profitable, and environmentally safe alternative to conventional ore processing [2]. Progressively, microbial leaching is being implemented to extract metal from sub standard ores that can't be processed efficiently and affordably by traditional approaches [3]. Many studies have been published regarding the role of microbes in the sorption of metals [4], metal dissolution [5-7], and mineral crystallization [8]. The rapid rise in minerals usage is creating a surprising deterioration in the world's high-grade mineral reserves.

Furthermore, These low-grade ores make it challenging to extract minerals, and employing conventional methods as a backup is costly because of the substantial energy and capital expenditures entailed. Due to the significant pollution such systems produce, environmental

costs pose a revelatory additional complication. Owing to the ongoing upgrading of environmental regulations, particularly those dealing to toxic wastes, cost of sustaining environmental protection will increase. Contrasting biotechnology to pyrometallurgy or chemical metallurgy, it is thought to be among the most innovative and promising approaches to these challenges. It has the potential to significantly reduce capital expenditure. Additionally, it provides a chance to lessen air pollution. Biological leaching, also known as bioleaching, presents a viable methodology to recover metal from metal ores. Bioleaching is an approach of extirpating metals and minerals from their parent aggregates by use of essentially developing biological processes. Bacteriological leaching is increasingly used to recover metal from low-grade ores that cannot be generated efficiently using traditional techniques [9]. As a recycling technology, biologically supported waste deterioration has a great potential, according to Valix [10], due to its inexpensive operating expenses, little negative environmental effects, and low energy consumption. Clearly, microbial extraction techniques seem to be more environmentally benign than conventional physicochemical ones, according to Rawlings [11]. The processing of elements like copper, uranium, gold, nickel, cobalt, and others from their varied ores is adequately assured by biological mineral refining.

1.1 Brief History

When Dave et al. [12] initially singled out *Thiobacillus ferrooxidans* (later reclassified as *Acidithiobacillus*) after being isolated, It was regarded as the earliest piece of scientific proof

of the importance of microbes in the solubilization of metals. From mine water, a viable culture was isolated using *Thiobacillus ferrooxidans*. At very low pH levels, negative chemolithotrophs could convert the sulfide component of minerals into sulphuric acid and the ferrous ion into ferric acid. Since then, industrialized bioleaching processes to extract metal from ores have begun to emerge as scientific understanding of how to utilize these bacteria has advanced [13]. The commercial use of biohydrometallurgy for copper pile leaching, in particular, was inaugurated in 1980, and several copper heap bioleach facilities have been set up since then [14]. But in recent years, the extraction of metals by bioleaching has emerged as among the most promising methods for recovering metals [15]. Accordingly, a significant number of researchers are still striving to enhance and optimize the approach nowadays [16-18].

Leaching technologies characterized as hoard and dump were utilized in the early 19th century. And this technique has thrived for the last twenty years, boosting the annualized global copper assembly from 0.2% to over 8–10%. More than two centuries ago, copper bioleaching in dumps was first observed at Rio Tinto mine. In 1980 in Lo Aguirre in Chile, the first modernistic industrial-scale biological copper leaching dumps apparently happened, generating 14,000 tpa. The first standalone mine to employ copper bioleaching, extraction with solvent, and electro-extraction was Australia's Girilambone Copper Operation, which Straits Resources oversaw and obtained a license for in 1993.

1.1.1 Ore bioleaching

A form of rock known as an ore is one that has sufficient amounts of essential minerals and metals that may be commercially mined from it [19]. Mining is used to remove the ores. The ores are typically rich in iron oxides and come in a variety of hues, including dark grey, bright yellow, deep purple, and rusty red. The use of microorganisms to enhance the extraction of metals from ores or concentrates that contain sulfide or iron is referred to as "biomining" in general [20]. Bemoaning includes the two closely related microbial processes known as bio-oxidation and microbiological leaching, both of which are helpful in extractive metallurgy. Metals like zinc, copper, nickel, and cobalt have sulfides and oxides that

are practically insoluble in water, while their sulfates are easily soluble. When the metal sulfide is converted to its sulfate, the metal is leached into solution and can then be recovered.

Microorganisms are used in bioleaching to produce the oxidation of iron sulfides into ferric sulfate and sulfuric acid. The metal contained in the oxidized metal sulfur minerals, which are outlined previously, is subsequently leached by the sulfuric acid that is produced as a result of ferric sulfate, a potent oxidizing agent. The method of solubilization is predominantly a chemical one, according to current knowledge, albeit microorganisms attached to the mineral can facilitate dissolving [21]. Due to the solubilization of the metal during this method of metal recovery, it is known as bioleaching.

2. TYPES OF MICROORGANISMS

Microorganisms that seem to be fundamental to biohydrometallurgy are categorized into two major groups. First there are chemolithotrophic microbes like *Thiobacillus ferrooxidans* (*T. ferrooxidans*) and tightly correlated species; the second are heterotrophic microbes, which include fungus, bacteria, and yeast like *Bacillus mucilaginosus*, *Aspergillus niger*, and related species.

2.1 Chemolithotrophic Bacteria

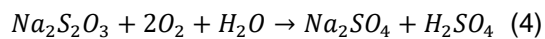
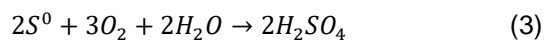
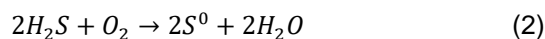
The chemolithotrophic bacteria utilized in biohydrometallurgy nowadays are acidophiles, which can thrive in a pH range of 1.5 to 4, and autotrophs, which get their energy from the oxidative assault of metal sulfides they solubilize. The chemolithotrophic bacteria obtain their nitrogen from silicate minerals in order to take in CO₂. The carbon and energy sources used mostly by heterotrophic bacteria are organic molecules. Additionally, they necessitate ideal growing characteristics, comprising pH and a nitrogen source. The main groups of heterotrophs bacterium appear to be three. The very first are mesophiles, which survive at temperatures around 30°C, such as the genera *Thiobacillus* and *Leptospirillum*; the second are temperate thermophiles, which survive at high temperatures between 40°C and 60°C; but the last are the furthest thermophiles, which thrive at temperatures around 60°C and 90°C, such as genera *Sulfolobus*, *Acidanus*, *Metallosphaera*, and *Sulfurococcus* [22].

2.1.1 Mesophiles

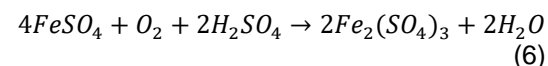
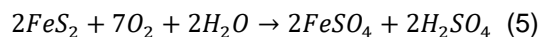
2.1.1.1 Thiobacillus

Thiobacillus is typically characterized for its capacity to oxidize sulfur compounds and elemental sulfur, however the necessary conditions such as temperature and pH may vary based on the physiology of each species. *Thiobacillus* bacteria are aerobes that might be mixotrophs, chemolithotrophs, or chemolithotrophs on an obligate or facultative basis. They occur in conditions with pH ranges of 0.5 to 10. Some are acidophiles, whereas others may grow at neutral pH levels. *Thiobacillus* are mesophiles, but whose ideal growth temperature is at 30°C. They may grow and oxidize inorganic substrates at temperatures ranging from 2 and 37°C [22]. Some *Thiobacillus* species, such as the sulfur-oxidizing *Thiobacillus caldus*, are moderately thermophilic microbes. These bacteria are also used to oxidize sulphur at 40°C and bioleach gold from pyrite and arsenopyrite [23]. Chemolithotrophic bacteria of the genus *Thiobacillus* are able of reducing a variety of sulphur compounds (i.e. S^{2-} , S^0 , S_2O_4 , $S_2O_3^{2-}$, SO_4^{2-}). Several of the oxidation processes are listed below.

Equations [1-4]



Some *Thiobacillus* species are however susceptible of decomposing complex compounds to generate energy as depicted in Equations [5-6].



Thiobacillus comprises 5 major species: *Thiobacillus intermedius*, *Thiobacillus thiooxidans*, *Thiobacillus thioparus*, and *Thiobacillus ferrooxidans*. On the basic principle of the optimal pH ranges for growth, genus *Thiobacillus* categorized into two separate groups [24]. The species that can develop extraordinarily well at neutral pH values are the

oldest of them. *T. thioparus* and *T. denitrificans* are the two species that fall into this category. *T. thioparus*, according to Wentzel [24] is liable for the oxidation of sulphur ($N-C-S^- + 2O_2 + 2H_2O \rightarrow SO_4^{2-} + NH_4^+ + CO_2 + 220 \text{ kcal/mole } O_2$) and comparable to *T. thioparus*, *T. denitrificans* utilizes the same reaction, but NO_3^- is used as the terminal electron acceptor in place of O_2 ($5S + 6KNO_3 + 2CaCO_3 \rightarrow 2CaSO_4 + 3K_2SO_4 + 2CO_2 + N_2$).

The second sort *thiobacillus* includes species like *T. thiooxidans*, *T. intermedius*, and *T. ferrooxidans* that may flourish at reduced pH level. *T. thiooxidans* is a sulfur-only oxidizer that thrives in acidic pH environments and can be required to extract sulfate deposits from minerals during indirect leaching or direct leaching of minerals in the absence of iron e.g. $ZnS + 2O_2 \rightarrow Zn^{2+} + SO_4^{2-}$ (18). *T. intermedius* is a tropical chemolithotroph with a pH values between 3 to 7 and its excretion is driven by $S_2O_3^{2-}$ as an electron donor [19]. The far more ubiquitous organism regarding biohydrometallurgy is *T. Ferrooxidans*, which is a bacterial leaching organ that has received substantial research and is susceptible with using sulfur hydrometallurgy as an energy source [23].

2.1.1.2 Leptospirillum

Leptospirillum ferrooxidans is a lesser effective iron oxidant [25]. *L. ferrooxidans*, which always oxidizes ferrous ions, might very well grow at higher temperatures than the genus *Thiobacillus*, which has an equilibrium state of almost 30°C, similarly at higher acidity concentrations relative to *T. ferrooxidans*, *L. ferrooxidans* exhibits a greater affinity for Fe^{2+} (apparent K_m 0.25 mM Fe^{2+} versus 1.34 mM for *T. ferrooxidans*, where K_m is the Michaelis constant for reactant Fe^{2+}) a decreased propensity for the inhibitory competitor Fe^{3+} . Owing to these distinctive qualities, *L. ferrooxidans* is suitable for mineral leaching in environments with a high Fe^{3+}/Fe^{2+} ratio, high temperature, and low pH. But when redox potential is low, *T. ferrooxidans* grows more quickly than *L. ferrooxidans* does in early phases of a mixed batch culture [26], and consequently, It is envisaged that it will predominate among microorganisms which metabolize iron in such a system [26]. *L. ferrooxidans* is more delicate with copper and incapable of oxidizing sulfur or sulfur compounds on its own. It also allows larger amounts of molybdenum, uranium, and silver than *T. ferrooxidans*. With sulfur-oxidizing acidophiles,

this can be accomplished without difficulty (e.g., *T. caldus*, *T. ferrooxidans*, or *T. thiooxidans*).

2.1.1.3 Thermophilic bacteria

Moderate and aggressive thermophiles are two broad categories for thermophilic microorganisms which decompose iron. For extreme thermophiles and roughly 40 to 60°C for moderate thermophiles, the ideal temperature for metal leaching and growth is observed between 65 and 85°C. The diversification of thermophilic microbes is enhanced and isolated from bioleaching conditions [27]. The majority of the bacteria are alternative autotrophs, meaning they can thrive without yeast extract, glutathione, or cysteine. *Sulfobacillus thermosulfidooxidans*, a gram-positive, spore-forming, and non-spore-forming rod-shaped mycobacterium, is a lower thermophilic bacterium. It may thrive in temperatures ranging from 28 to 60 °C, with 50 °C being the ideal temperature. The bacteria are naturally occurring autotrophs given that a very small amount of yeast extract (0.01-0.05 w/v) increases microbial activity while 0.1% (w/v) inhibits it [28]. The microorganism can utilize metal sulfide, S₀, or Fe(II) as a source of energy to grow autotrophically. With all, *S. thermosulfidooxidans* has the following key features: (1) the ability to absorb CO₂ whenever yeast extract is present; (2) the potential to grow on FeSO₄⁺ yeast extract without even any CO₂ being inexpugnable; (3) the necessity for yeast extract for the best growth; and (4) being able to oxidize CuS, FeSO₄, FeS₂, CuFeS₂, S₄O₆⁻² or NiS [29].

Sulfolobus acidocaldarius and *Acidianus brierleyi* are two well-known examples of the extreme thermophiles found in acidophilic iron-oxidizing bacteria [28,30]. The genus *Archaeobacteria* comprises both. These four genera are *sulfurococcus*, *acidanus*, *metallosphaera*, and *sulfurolobus* [22]. All of them are aerobic, very thermophilic, acidophilic bacteria that oxidize ferrous ions, sulfides, and sulfide minerals. Their optimal growing temperature is between 70 and 75°C, with a temperature range of 55 to 90°C. Between pH 1 and 5, they can grow, with pH 3.0 being just the optimum range. The species all demonstrate facultative chemolithotrophic metabolism and can be autotrophic, mixotrophic, or heterotrophic conditions. With yeast extract at large concentrations, the organisms grow heterotrophically and more rapidly with concentration levels of 0.01–0.02% (w/v) [28].

2.2 Heterotrophic Microorganisms

Numerous heterotrophic species are also present in bacterial leaching environments (yeast, fungi, and bacteria). Because *Thiobacillus sp* has an insufficient ability to extract metal from oxide, silicate, and carbonate ores, its employment is restricted in these cases. Currently, the use of heterotrophic bacteria and fungus with particular ores is being assessed. The two most important genera of fungus are *Aspergillus* and *Penicillium*, while *Bacillus*, a category of heterotrophic bacteria, is incredibly beneficial at dissolving metals. Using *Bacillus mucilaginosus* and *Bacillus polymyxa* to extract silica from bauxite and *Aspergillus niger* to dissolve aluminum from alumino-silicates are two examples of where and how heterotrophic microorganisms are employed in microbial leaching [31]. For instance, in contrast to certain other components, a *Bacillus sp.* culture medium also includes CaCO₃, essential salts, yeast extract as a nitrogen source, and 0.5% (w/v) sucrose [31]. The bacteria also need to maintain a pH that is closer to neutral and a temperature that is mesophilic.

Enzymatic abatement is necessary when heterotrophic bacteria extract a mineral from an ore. By removing organic acids from *Pseudomonas putida*, like citrate and gluconate, several heterotrophs can aid in the extraction of metals citrate from *Bacillus megaterium*, and citrate from *Aspergillus niger*, in addition to oxalate, malate, tartrate, and succinate from *Aspergillus niger*. Thermophiles or mesophiles, mixed or pure strains, described or uncharacterized strains, have all been as a bioleaching agent investigations. Among the most commonly mesophilic bacteria employed in the bioleaching process is *T. ferrooxidans*. Many researchers have taken into account either the isolated strain from a mine or the pure strain obtained from a collecting firm. It was established that a number of parameters, comprising pulp density, the characteristics of the mineral ores, temperature, particle size, and many others, had an effect on the decomposition of sulfide ores by *T. ferrooxidans*. For instance, *T. ferrooxidans* was found using ferrous ions (9 g/l) as a source of dissolving copper from covellite [32]. The optimum parameters for *T. ferrooxidans*' oxidation of chalcopyrite were low particle sizes (+45, -53 m) and the presence of 50 mg/l silver ions [33]. According to Battaglia et al. [34] the combined culture of *L. ferrooxidans* and *T. ferrooxidans* had a higher cobalt dissolution rate from cobaltiferous pyrite than a pure culture. The

mixture of *T. thiooxidans*, *T. ferrooxidans*, and *L. ferrooxidans* generated the highest leaching rates.

Several strains of the same microbe have varied bioleaching characteristics, according to some interpretations [35]. *T. ferrooxidans* (ATCC 23270) were able to dissolve following 350 hours of leaching with 40%, 42%, and 61% of the iron from pyrite concentrate, chalcocopyrite concentrate, and arsenopyrite concentrate, respectively, while *T. ferrooxidans* (DSM 583) was only able to dissolve 24%, 38%, and 40% of the iron from pyrite concentrate (pyrite + quartz), chalcocopyrite.

Clark and Norris [36] discovered, for example, that moderate thermophile bacteria could oxidize pyrite/arsenopyrite concentrations faster than mesophiles. They also find that the amount of copper that dissolves from intricate sulfide when using a *Sulfolobus*-like strain at 80°C is almost 100%. However, according to Yahya and Johnson, using a starting pH of 2.5 and temperature of 35°C, *T. ferrooxidans* outperformed *Sulfobacillus sp.* in respect of the

percentage of pyrite concentrate decomposition [37]. Mostly in occurrence of chalcocopyrite, extreme thermophiles have the potential to considerably increase the percentage of bioleaching, according to some studies. While using a batch bioreactor, Konishi et al. [38] calculated kinetics of chalcocopyrite leaching by *Acidianus brierleyi* at 65°C, pH 1.2, +38–53 particle size, and 0.5% (w/v) pulp density. Compared to *T. ferrooxidans*, which needed roughly 10 days to complete 20 dissolutions with the identical sample, approximately 100 copper dissolutions were achieved in ten days. An exceptionally thermophilic bacterium acquired from a hot, sulfur-rich coal dump was used by Gericke et al. [39] to investigate the microbial leaching of copper concentration, sulfur-rich coal dump, and Konishi et al. (2001) found good agreement with their findings. 98% copper (18.8 g/l Cu) and 96% iron (10g/l Fe) were finally recovered [39]. Although copper dissolution increases wonderfully in microbes, there's also a rich synchronous iron disintegration which lessens the copper concentration in the leachate. The usage of thermophiles is thought to have this one notable drawback.

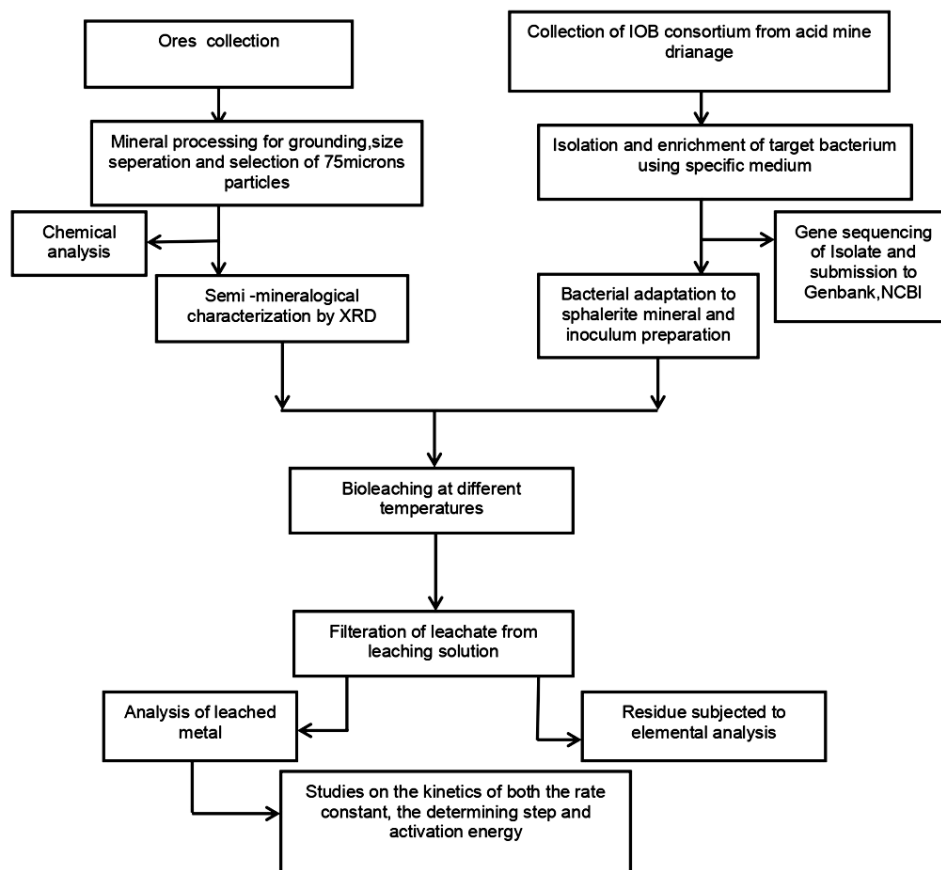


Fig. 1. Sundramurthy's [40], a diagram illustrating the bioleaching process

Table 1. Bioleaching operations and efficient microbes

Bioleaching operations	Effective microorganisms
Silica leaching	<i>Acetobacter, Bacillus, Streptococcus</i>
Manganese leaching	<i>Alcaligenes, Clostridium, Aeromonas, Pseudomonas</i>
Leaching of metal ores, including iron	<i>Pseudomonas, Escherichia, Candida, Enterobacter, Rhodopseudomonas, Micrococcus, Aspergillus, Penicillium,</i>
Phosphorous leaching	<i>Hyphomicrobium, Aspergillus,</i>

Table 2. Large-scale microorganisms used in the technique of bioleaching [37,12,41-42]

Types of organism		Optimum pH	Temperature (°C)	Energy source	Chemical substance used as a lixiviant	Metal being obtained
	<i>Acidithiobacillus Ferrooxidans</i>	2.0	28-35			
Iron Oxidizers	<i>Leptospirillum ferrooxidans</i>	1.8	30	Ferrous sulphate	Ferric sulphate	Zn, Cu, Ni, Cd, Pb.
	<i>Leptospirillum ferriphilum1</i>	1.6	40			
	<i>Acidithiobacillus Thiooxidans</i>	2.0-3.5	10-37			
Sulphur oxidizers	<i>Sulfobacillus hermosulfidooxidans</i>	1.7-2.4	40-55	Sulphur and reduced sulphur compounds	Sulphuric acid or oxidized form of sulphur compound	Cu, Zn, Ni, Al.
	<i>Sulfolobus spp. Chromo-bacterium violaceum</i>	2.0-3.0 7	55-85 28			
Cyanogenic Organism	<i>Pseudomonas aeruginosa</i>	7	37	Glycine	HCN	Ag, Pt, Pd, Au,
	<i>Pseudomonas fluorescence</i>	7	30			

Types of organism		Optimum pH	Temperature (°C)	Energy source	Chemical substance used as a lixiviant	Metal being obtained
Organic acids producers	<i>Aspergillus niger</i>	4.5	30	Carbohydrate (Glucose or sucrose)	Citric, oxalic, gluconic and malic acid	Cu, Zn, Ni, Pb, V, Al, Mo, Co, Li.
	<i>Penicillium simplicissimum</i>	5.5	22-30			

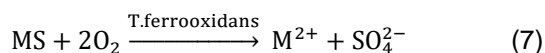
3. BIOLEACHING MECHANISMS

From the literature, a hypothesis with two distinct pathways for metal bioleaching has generally been proposed. The first type is direct leaching, which uses enzymatic mechanisms to interact directly between the sulfur dioxide surface and the bacterial membrane. Consequently, direct leaching is able to be observed when the microbes are inclined towards to the metal substrate. Within a few minutes or hours, cells can cling to dissolved mineral particles, preferentially taking advantage of surface irregularities.

Second, ferrous ions (Fe^{2+}) molecules contained in the mineral are oxidized by microbes to form ferric ions (Fe^{3+}), which are then used to indirectly oxidize damaged metal. An oxidant called ferric ion can oxidize metal sulfides and can also be converted to ferric ions by microorganisms. Iron has responsibilities as an electrons transporter in light of this supposition. It has been proposed that iron can oxidize without direct physical contact. The both direct and indirect routes for the combustion of metal sulfides are described by these equations. Tuovinen [43], Haddadin et al. [44] and Nemati et al. [45] among many others gave concise explanations of the two mechanisms [45].

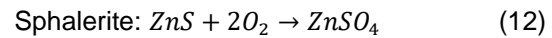
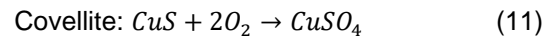
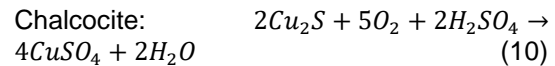
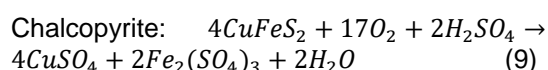
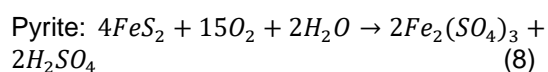
3.1 Direct Mechanism

In such phase, *T. ferrooxidans* can rapidly degrade metal oxidizing agents to soluble metal sulfates, in accordance with Equation (7).



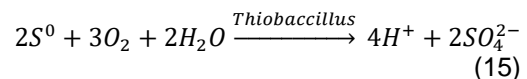
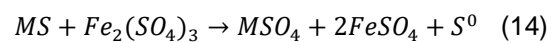
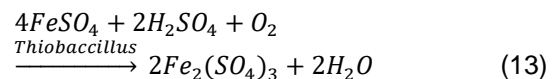
A solid phase could become liquid by this process, for which further processing can be used to recover the metal, because the metal sulfides exist in an insoluble state and the metal sulfate (MSO_4) is Often water soluble.

The mechanism might hypothetically run endlessly till all the MS had been changed into product(MSO_4).The following reactions (Equations 8-12) are examples of direct mechanism.

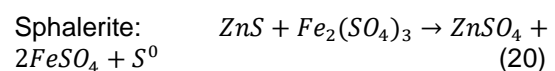
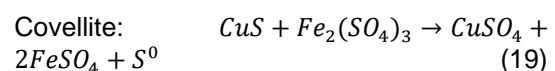
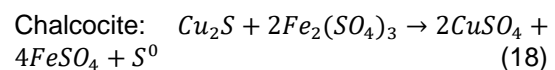
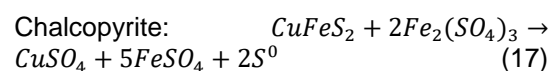
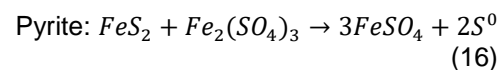


3.2 Indirect Mechanism

Sulfur metals being dissolved by ferric ions is a case of either an indirect process. Equation (14) is created chemically without the intervention of microorganisms, in contrast to Equation (13), which is the consequence of *Thiobacillus* action. According to Equation(15), *Thiobacillus* also decomposes elemental sulfur.



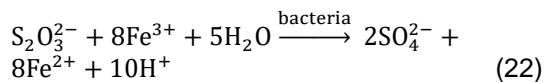
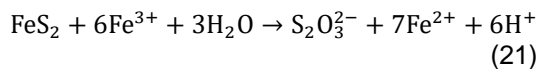
As a result, ions continuously diffuse between processes in a cycle between 13 and 15, and formation of H^+ during the sulfate oxidation increases the overall efficiency. Here are a few examples of chemical oxidation procedure: (Equations 16–20).



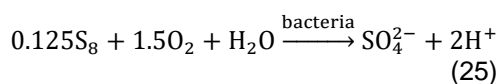
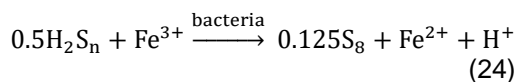
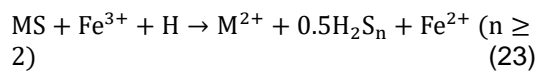
The paradigm of direct and indirect metals leaching remains subject to discussion while considering the theory of a direct mechanism into account. Modern methodologies for extracellular polymeric compounds, new theories, and the examination of breakdown products produced during bioleaching mechanism have been discovered recently in investigations.

No evidence has been found to support a direct enzymatically mediated mechanism, despite repeated indirect mechanisms being proposed in latest researches [46–47]. Phases in the oxidation of galena were recognized as copper sulphate and polysulfide, along with chalcopyrite, sphalerite, orpiment, hauerite, and realgar. FeS₂, MoS₂, and WS₂ are three examples of alkali metals sphalerites that require the use of thiosulphate as a bridge in order to be affected by iron (III) ions throughout an oxidation reaction. In the second stage, which entails the dissolution of metal by ferrous ions and/or protons, polysulfide and elemental sulphur served as the two main sulphur intermediates. Equations 21 and 25 below describe the two mechanisms [46–47].

Thiosulphate mechanism (FeS₂, MoS₂, WS₂):



Polysulphide mechanism (for ZnS, CuFeS₂, PbS):



The main difference between these two processes is that a metallic sulfide will only be degraded using proton and ferrous ion. Microorganisms resuscitate ferric ions or protons and then condense them at the interface between the sulfur dioxide surface and water or the sulfur dioxide surface and bacteria cell to facilitate metal leaching mechanisms [47].

T. ferrooxidans, *T. thiooxidans*, and *L. ferrooxidans* are the three microorganism species that are interconnected in this model. The exopolymer layer surrounding the cells where the natural action actually occurs acts as the determining factor [47].

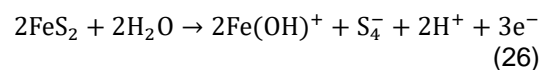
The term "contact leaching" was first employed by Tributsch [48] to characterize the direct

leaching method in which bacteria are thought to react to the sulfur dioxide surface. In the contact leaching scenario, sulphur dioxide is treated to enhance the dissolution process. The idea that the biofilm reacts with the sulphur directly through enzymatic processes is unsupported by any data [46,48–49]. In cooperative leaching [49], the bacteria that cause contact leaching stimulate the surrounding electrolyte's bacterial load to extract sulphur species that are both aqueous and particulate.

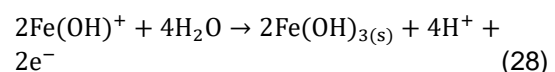
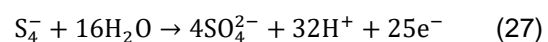
Blight et al. [50] studied the bio-oxidation of pyrite using a modified bioleaching system that included *T. ferrooxidans*. Their prepositions were: (i) iron species act as intermediaries; the iron is oxidized at the equivalent of cell possessiveness and consolidates at the sulfide/oxide interface; (ii) when the microorganism attached itself to the oxide/sulfide intersection, cell duplication developed, extracellular polysaccharides were produced, and iron oxyhydroxides were formed; and (iii) sulfur species inculcate through the boundary layer.

The proposed stoichiometry is as follows (Equations 26-31).

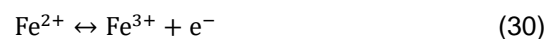
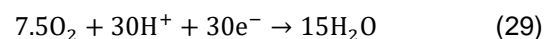
At the oxide/ sulphide interfaces:



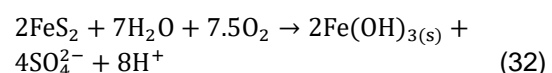
At the bio-film:



The biofilm's reductive reaction:



Overall stoichiometry:



3.3 Dump Leaching

The collection of uncrushed trash piles in dumps is a large element of dump leaching [51]. It is used for the low-grade ores used in mineral

extraction. The massive rocks are reduced to manageable shards and moved to dumps by bombing in the pit. Millions of metric tons of useless minerals are kept at these landfills. To create the ideal circumstances for both the eradication of bacteria that would oxidize the metal and also its recovery, acidified water is placed on the surface and permeates the wastes. Dump recirculating seems to have been a common approach for extracting copper ores [52].

3.4 Heap Leaching

Heap leaching as well as dump leaching have similarities since larger minerals are broken down into globules and microscopic fragments in moving containers loaded with polluting fluids. As a result, the parent rock is being prepared for the bacteria. The purified parent rock is circulated on pads that correspond to penetrating polymer drain routes, enhancing the discharge with the mineral-encompassing mixture from the underside of the parent rock. Also, it has air for the optimum potential microbial development [53]. Using this overview of a leaching procedure, gold ores are pre-treated and metal sulfide is piled in to eliminate the trapped gold from sulfide minerals [54]. These procedures also involve the gravity-based recovery of minerals [55,56].

3.5 Factors that Influence Bioleaching Process

The effectiveness of bioleaching is greatly influenced by microorganisms and the chemical makeup of the substrate that must be treated, which contains metals(ore, concentrate). It is crucial to choose the right mixture of the dissolving agent and the pretreatment material in order to achieve an optimal metal percentage yield with this technique, and also to create the perfect conditions for bacterial growth. Hence, a wide range of biological and macrobiotic elements may have an impact on the activities of the bacterium and the effectiveness of bioleaching.

The variables can be classified into groups including physicochemical parameters, microbiological parameters, metal containing substance features, and method or circumstance types. The bulk of these parameters have lately undergone evaluation by several writers as shown, Temperature [57–58], pH [59–64], redox potential [65–66], oxygen content and availability

[67–69], and other physicochemical factors are among the parameters.

Similar to how hydrogen ion absorption determines pH, the ORP of an electrode pair is determined by the propensity of electron transport between chemical species and electrodes. In view of this, ORP describes how easily electrons are migrated to or from species in the mixture. The bio-oncology of iron (II) to iron (III) during bioleaching changes the ORP. It is possible to observe the movement of microbes owing to oxidizing ions like iron (III) or oxygen have a positive relationship with ORP [70]. By increasing the redox potential as a result of biological activity, it is possible to extract metal from solid wastes or ores more easily [71]. The microbiological features of bioleaching are also important since they have a significant impact on the process' performance. These parameters include, metal tolerance [72-73], microbial activity [72-73], microbial diversity [74-77], population density [78-80] and microbial oxidation ability [81–84]. The microbiological parameters of the bioleaching are extremely important since they have a big impact on how well the process works. These factors include microbial diversity, population size, and the capacity of microorganisms to metabolize metals and alloy tolerance [81–84]. This group takes into account how the leaching technique used has affected the environment, for instance, whether the process is completed in one or two steps [85–86], the quantity of treated solid [87-90], or the way it operates (column, heap, or bioreactor activities) [91–102] All of these elements that could potentially have an impact on the process are being optimized in an effort to enhance metal recovery. However, because the experimental parameters will vary depending on the metal-controlling substance and the bioleaching system [103–108], optimization must be done for each system and waste.



Fig. 2. Copper Harbor, Keweenaw Co., MI, USA

Table 3. Biological metal ions assessment and treatment of numerous liquid and solid wastes. Wang et al. [108]

	Mixtures or microbes	Pollution source	Efficiency/Capacity	Effective mechanism	Reference
Mixtures of microbes	<i>Leptospirillum ferriphilum</i> CS13, <i>Acidithiobacillus caldus</i> S2, <i>Sulfobacillus acidophilus</i> S5,	Electroplating sludge	Removal rate of various heavy metals was over 99%	Bioleaching	[109]
Mixtures of microbes and sludge	Water treatment sludge	Electroplating wastewater	Cu ²⁺ and Cr ⁶⁺ adsorption capacities are about 1.7 and 3.5 mg g ⁻¹	Bio-reduction and Bio-sorption	[110]
	Mixture of <i>Candida lipolytica</i> and sewage sludge	Electroplating wastewater	6.66 mg g ⁻¹	Bio-reduction and Bio-sorption	[111]
	Sulfate-reducing bacteria (43.3% of Desulfovibrio) enriched sludge	Electroplating effluent	100% within 3 days	Biological precipitation	[112]
Main bacteria strains	<i>Acidithiobacillus ferrooxidans</i>	Dewatered metal plating sludge	Metal solubilization: 97% of Zn, 96% of Cu, 93% of Ni, 84% of Pb, 67% of Cd and 34% of Cr in 20 days	Bioleaching	[113]
Single culture	<i>Arthrobacter</i> sp. X34 <i>Bacillus</i> sp. Y9-2 <i>Rahnella</i> sp. Y9602	Simulated groundwater	0 73% precipitated in 5 days 95% precipitated in 5 days	Bio-mineralization	[114]
Mixtures of microalgae and bio-resource	<i>Chlorella vulgaris</i> and calcined eggshells	Acid mine drainage	99.7% of Fe, 99.5% of Cu, 99.9% of Zn, 99.8% of Mn, 100% of As, and 100% of Cd in 6 days	Bio-sorption	[115]
Mixtures of microbes	Sulfate-reducing bacteria	Underground mine drainage	90.5% of Cd, 89.3% of Zn	Bio-mineralization	[116]
Mixtures of microbes	<i>Bacillus subtilis</i> ITSUKMW1, <i>Acinetobacter junii</i>	Synthetic Cr ⁶⁺ solution	99% reduction of Cr ⁶⁺ (100mgL ⁻¹) in 64 h	Bio-reduction	[117]

	Mixtures or microbes	Pollution source	Efficiency/Capacity	Effective mechanism	Reference
	VITSUKMW2, and <i>Escherichia coli</i> VITSUKMW3				
Mixtures of microbes	<i>Ferrovum</i> , <i>Thiomonas</i> , <i>Gallionella</i> , <i>Leptospirillum</i>	Sb-rich mine water	90% of total Sb, 80% of Sb (III)	Biological precipitation and bio-transformation	[118]
Immobilized fungi Mixtures	<i>Cladosporeum</i> <i>perangustum</i> , <i>Penicillium commune</i> , <i>Paecilomyces</i> <i>lilacinus</i> , <i>Fusarium</i> <i>equiseti</i>	Tannery wastewater	100% of Cr (VI), 99.92% of total Cr, 95.91% total Pb, 100% of Pb (II)	Bio-sorption	[119]
Mixed microbes	<i>Stenotrophomonas sp.</i> WY601, <i>Proteus</i> <i>mirabilis</i> , <i>Microbacterium</i> <i>paraoxydans</i> , <i>Bacterium FX2</i> , <i>Bacillus tequilensis</i>	Tannery sludge	90% of Cr (VI) within 65 h (single culture of WY601)	Bioreduction	[120]
Mixed plants and Endophytic bacteria	<i>Pantoea sp. strain</i> TYRI15, <i>Microbacterium</i> <i>arborescens</i> TYSI04; <i>Bacillus endophyticus</i> PISI25, <i>Bacillus</i> <i>pumilus</i> PIRI30, <i>Bacillus sp</i>	Textile effluent	97% of Cr, 89% of Fe, 88% of Ni, 72% of Cd	Bio-accumulation And phytoextraction	[121]

3.6 Recent Advancement in Reactors for Bioleaching

The design of bioleach reactors poses a sizable number of difficulties for the process engineer. These include the need to add a lot of oxygen (and some carbon dioxide) to the process water, keep a fast-settling mineral slurry suspended, and keep it at a constant temperature throughout the process (typically by removing heat produced by the exothermic oxidation reactions). All of these processes must be done while a "fourth phase" of the biomass is present, which may be sensitive to high shear rates, and in a vessel that can survive the acidic, corrosive, and abrasive environment that bioleaching bacteria prefer. The choice of suitable building materials for the bioleach reactor has become more difficult with the introduction of thermophiles. In terms of metallurgy, bioleaching is a slow process with typical residence lengths of 4 to 6 days as opposed to a flotation cell or even an autoclave, where retention times are measured in minutes or hours. Therefore, a large process volume is needed, and bioleach reactors are some of the biggest high-intensity process vessels ever designed. The difficulty for the design engineer is to produce a fairly consistent environment in the bioleach reactor so that the circumstances for biomass growth and oxidative activity are ideal across the reaction space. The bioleach reactor must be a mechanically agitated, stirred tank due to the physical and chemical criteria of the bioleach process [122]. Without mechanical agitation, air-stirred reactors are unable to meet the process's prerequisite of air transfer. The stirred-tank reactor is the only kind of reactor that has been deployed in actual reality, despite several other cutting-edge designs being put out [123]. The process volume can be reduced by placing the reactors in a sequence, going from large to small, according to process modeling utilizing fundamental chemical reaction engineering concepts [124]. All commercial bioleach plants have been designed with a set of equal volume reactors, often with two to three main reactors in parallel feeding into a series of two to three secondary reactors in a pair. However, practical limitations (such as the shared characteristics of spares) make this ideal impractical.

Given the great yields in immersed leach metal extraction, the switch from shaking flasks to bioreactors was thoroughly investigated fairly early. More than 80% of the total zinc was recovered from the sulfide zinc concentrate, and

it was found that tank leaching was more efficient at treating ore concentrates. The construction and cost of operation of tank leaching are higher than those of dump, heap, or in situ leaching procedures. However, the rate of metal extraction is substantially higher, and this method is already being effectively used to the bioleaching of refractive gold ores.

3.7 Merits of Bioleaching

3.7.1 Cost-effectiveness

Bioleaching is always simple and, as a reason, less expensive to manage and control than conventional techniques simply because only a small number of employees are necessary for operating different biochemical facilities.

3.7.2 Environment friendliness

Comparatively speaking, the process is less damaging to the environment than existing separating methods. Given the significance of controlling costly emissions of sulphur dioxide during mining, this would be profitable for the industry. Due to the concerned microorganisms' spontaneous development as well as the fact that surface mining or its surroundings can only have a little impact, less landscape degradation results. The mine's atmosphere is perfect for the bacteria's growth, which is then nourished and rebuilt.

3.7.3 Concentration of ore

If the minerals in the ore are not crucial for other processes, they can sometimes be obtained through bioleaching. Just by substantially substituting the extensive crushing and grinding, it is possible to perform bioleaching, which in a conventional procedure results in prohibitive energy consumption and expense.

3.8 Demerits of Bioleaching

3.8.1 Cost-effectiveness

Compared to smelting, the bioleaching process is quite slow. This yields minimal benefit and causes a notable shortage of resources for modern organisms.

3.8.2 Environmental degradation

Eventually, the process produces poisonous compounds. Sulfuric acid and Hydrogen ion ions

can leak into the groundwater as well as the surface water when they are produced, making them acidic and harming the ecosystem. During acid mine sewage, heavy ions like arsenic, iron, and zinc are released.

3.8.3 pH

As the pH of this mixture rises as a result of diluting with freshwater resources, these ions are considered to as "Yellow Boy" contamination because they appear quickly. The biodegradation solution should be carefully thought out in consideration of the possibility of a microbiological failure. Bioheap dumping, unlike other methods, cannot be entirely stopped once it has begun since leaching would continue in connection with rains and natural microorganisms.

4. CONCLUSION AND FUTURE PROSPECTS

Since bioleaching avoids a large portion of the carbon footprint and energy expenditures involved with conventional mining, it is seen as a "green technology" that will gain importance in the years to come. A potential method for reducing the environmental damage done by mining deposits, sulphur atmospheric emissions, and acidic wastewater is bioleaching, which utilizes natural bacteria to leach metals from deficient ores. One may argue that it is an improvement over the typical mining method, which worsens environmental destruction. Bio-mining has investigated low-grade ores and metallurgical waste as potential sources of metals. Because bio-mining not only offers functional transparency but also has cheap operating costs, little capital expenditure, and quick construction timeframes, it is projected that its commercial use would increase soon. They also share the same benefit as green plants being that they remove carbon dioxide from air, thereby reducing the difficulty that is known to be the cause of climate change. The mining sector claims that biotechnology has improved instruments for successful metal recovery. It is clear that the research done on the particular group of microorganisms adapted to their colonies that encourage bioleaching is mostly centered on institutes, academic divisions, and universities. If bio-mining technology is to be commercially viable, cooperation and collaboration between these research institutions and mining companies are very essential. Government agencies must actively promote the

technique to mining firms so that they would employ it to promote the ecosystem and a brighter future. In the future, the extraction and recovery of several metal won't require the use of biotechnology.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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