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Microbial Bioleaching: Harnessing *Acidithiobacillus* for Sustainable Extraction of Iron and Copper from Mineral Ores

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ABSTRACT

Microbial bioleaching offers a promising and sustainable alternative to traditional mining methods, which often involve energy-intensive processes and harmful chemicals like cyanide, leading to environmental degradation. This study investigates the use of *Acidithiobacillus ferrooxidans* (Strain: NCIM 5370) to recover valuable metals from low-grade ores and mining waste, minimizing ecological impact. The primary objective is to evaluate the efficiency of these microorganisms in solubilizing metal ores under controlled conditions, optimizing growth parameters to maximize metal extraction, and assessing the environmental benefits in comparison to conventional methods. Bioleaching, one of the key mechanisms in bio-mining, employs microorganisms such as *Acidithiobacillus ferrooxidans* to oxidize sulfides and ferrous iron, creating acidic conditions that facilitate the extraction of metals in an environmentally friendly manner. These microorganisms thrive in extreme conditions, converting insoluble metal compounds into soluble forms, thus enabling efficient recovery. Another mechanism, bioremediation, addresses environmental damage caused by mining activities, including acid mine drainage and heavy metal contamination. By utilizing bacteria, fungi, or plants to neutralize or immobilize toxins, bioremediation offers a sustainable solution to industrial waste management and environmental restoration. This research also explores the scalability of bioleaching for industrial use and investigates potential enhancements through microbial optimization. The findings support the integration of bioleaching into sustainable mining practices, offering a viable alternative to conventional methods. By bridging lab-scale results with real-world applications, this study advances the adoption of eco-friendly metal recovery technologies.

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Keywords: Bioleaching, Acidithiobacillus ferrooxidans, Iron, Copper, Metal Recovery

INTRODUCTION

Traditional mining is one of the oldest mining methods which dominated metal extraction until the early 20th century. These processes relies on manual tools and energy-intensive smelting, are a major contributor to environmental degradation, frequently resulting in deforestation, soil erosion, pollution, and significant greenhouse gas emissions.[5] Although metals like iron and copper are found in high-grade deposits, these reserves are rapidly depleting due to extensive mining.[10] Consequently, the industry has become increasingly reliant on recovering metals from low-grade ores, necessitating a critical shift towards more sustainable and resource-efficient recovery technologies capable of handling these complex materials.[2,26,27,31]

Among these new methods, biomining has emerged as a promising eco-friendly technique that harnesses microorganisms to recover valuable metals from low-grade ores and mine wastes.[9] Unlike smelting, which requires high temperatures, bioleaching occurs under ambient conditions, reducing both energy consumption and emissions. This technique specifically leverages the natural metabolic processes of specialized microorganisms to dissolve and recover metals, providing a sustainable low-energy alternative to conventional pyrometallurgical and hydrometallurgical techniques.[4,14,19,21] A key player in this field is *Acidithiobacillus ferrooxidans*, an acidophilic, chemolithoautotrophic bacterium,[5,12,19,22] that plays a crucial role by oxidizing sulfides and releasing metals in an environmentally friendly manner.[7,11] This biological approach significantly reduces toxic emissions, lowers energy use, and offers a scalable pathway for sustainable metal recovery, addressing global demand while minimizing ecological damage.

Acidithiobacillus ferrooxidans (Strain: NCIM 5370) is of particular interest because of its resilience in acidic, metal-rich environments and its adaptability to industrial settings. Understanding its physiology and optimizing parameters like pH, temperature, pulp density, and oxygen availability are crucial to maximizing recovery rates.

This study aims to evaluate and explore the role of microorganisms, particularly *Acidithiobacillus ferrooxidans*, in sustainable extraction of metals from low grade metal ores. By bridging laboratory results with industrial-scale implementations, this approach not only addresses the growing global demand for metals but also offers a pathway toward environmentally responsible and resource-efficient metal recovery.

Characteristic Features of *Acidithiobacillus ferrooxidans* (NCIM 5370):[5,16,19,22]

Table 1: Traits of *Acidithiobacillus ferrooxidans*

Features	NCIM 5370 (or) Comparable Strains
Genome Size	3.0 to 4.2 Mb
G+C Content	57.6 to 58.9%
Optimum Growth pH	1.5 to 2.5

Optimum Temperature	28ºC to 35ºC
Metal Tolerance	Up to 50 g/L Ni, 55 g/L Cu
Main Energy Sources	Fe^{2+} (ferrous iron), S^0 (elemental sulfur), RISCs (reduced inorganic sulfur compounds)
Unique Features	Chemotaxis, quorum sensing, robust outer membrane, extensive resistance genes

These attributes allow the organism to survive and perform bioleaching in the acidic, metal-rich environments commonly found in mining wastes.[12,19,22] Furthermore, strain-specific genome islands and gene clusters have been identified, which include genes for arsenic, mercury, copper, and multi-metal resistance proteins, as well as those associated with sulfur and iron oxidation and general adaptation mechanisms.[5,12,19]

Mechanisms of Microbial Bioleaching:

Acidithiobacillus ferrooxidans plays a crucial role in two major biochemical cycles crucial for metal solubilization – ferrous iron oxidation and reduced sulfur compound oxidation.[1,12,15,19]

Iron Oxidation – It proceeds through a well-characterized electron transport chain.

1. **Downhill pathway (Energy Generation):**
   $Fe^{2+}(outside) \rightarrow Rusticyanin(Rus) \rightarrow Cyc1 \rightarrow Cytaa_3 \rightarrow O_2$
   Electron flow drives ATP synthesis via proton motive force.
2. **Uphill pathway (Reducing power Generation):**
   $Fe^{2+} \rightarrow Cyc2 \rightarrow Rus \rightarrow CytA_1 \rightarrow bc1\ complex \rightarrow quinone\ pool \rightarrow NDH1 \rightarrow NAD^+$
   Provides NAD(P)H for CO_2 fixation and reductive biosynthetic reactions.
3. The *rus* and *pet* operons encode the protein complexes central to these pathways, including cytochromes, bc1 complex, and rusticyanin.[5,10,12,19,25]

Sulfur Oxidation – It occurs through multiple, overlapping enzymatic systems:

- Sulfide-quinone reductase (SQR)
- Sulfur dioxygenase (SDO)
- Tetrathionate hydrolase (TetH)
- Thiosulfate quinone oxidoreductase (TQO)
- Hetero disulfide reductase (HDR)

Electrons released during the aerobic oxidation of elemental sulfur and thiosulfate are transferred to the quinone pool, then through bc1 complexes, and finally to terminal oxidases or NADH complex I.[10,25]

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Direct and Indirect Bioleaching:[1,10,19]

Table 2: Direct and Indirect Bioleaching		
Mechanism	**Direct Leaching**	**Indirect Leaching**
Definition	Bacterial cells attach to mineral surfaces, catalysing oxidation via cell-bound enzymes.	Planktonic bacteria oxidize Fe^{2+} to Fe^{3+}, which then chemically oxidizes sulfide minerals.
Microbial Action	Attachment and surface enzymatic attack	Fe^{3+} regeneration in solution
Chemical Reactions	$FeS_2 + 3.5O_2 + H_2O \rightarrow FeSO_4 + H_2SO_4$	$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$
Industrial Applications	Surface biofilm, heap, or tank leaching	Heap/tank leaching, e-waste recovery, uranium extraction

Both mechanisms often operate simultaneously, especially under industrial conditions.

Role of Chemotaxis, Quorum Sensing and EPS:

Acidithiobacillus ferrooxidans utilizes chemotaxis to move toward favourable mineral substrates and employs quorum sensing to coordinate biofilm formation. The secretion of extracellular polymeric substances (EPS) strengthens cell adhesion to mineral surfaces and creates microenvironments that concentrate leaching agents, thereby enhancing metal solubilization.[12,19]

Optimized Growth and Bioleaching Parameters:[5,9,13,16,18,19,25]

Table 3: Optimized Growth and Bioleaching Parameters		
Parameter	**Optimum Range**	**Influence on Bioleaching**
pH	1.5 to 2.5	Low pH enhances Fe^{2+} oxidation and prevents jarosite precipitation.
Temperature	28ºC to 35ºC	Supports maximum enzymatic activity; higher temperatures may denature proteins.
Fe^{2+}/S^0 Concentration	Fe^{2+} up to 120mM; S^0 as substrate	Promotes microbial growth and energy generation; excess may cause inhibition.
Oxygen	1.5 to 4 mg/L (DO)	Essential for aerobic oxidation of Fe^{2+} and reduced sulfur.

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Carbon dioxide	Atmospheric (~0.04%) or 7-8% enriched	Sole carbon source for autotrophic metabolism; enrichment improves biomass yield.
Pulp Density	1 to 10% (w/v)	Higher density increases metal yield but may hinder oxygen and nutrient diffusion.
Particle Size	30 to 50 µm	Greater surface area enhances microbe–mineral contact; too fine may reduce permeability.
Heavy Metal Tolerance	Up to 55 g/L Cu Up to 8 g/L Fe	Enables bioleaching of metal-rich ores and wastes.

By sub-culturing and adapting microorganisms to defined pulp densities or metal concentrations, their biomass accumulation is enhanced, lag phases are minimized, and extraction rates are significantly improved.[26]

Comparative Environmental Impact:[14,19,20,27]

Table 4: Environmental Impact

Method	CO_2 Emissions	Pollutant Generation	Water Demand	Scalability	Recovery from low-grade ores
Pyrometallurgy	High	High	High	Medium	Poor
Hydrometallurgy	Moderate	Moderate	High	Medium	Moderate
Bioleaching	Low	Low	Low	High	Excellent

METHODOLOGY

Sample Collection –

The microbial culture used in this study was procured from the National Collection of Industrial Microorganisms (NCIM), Pune. The isolate was identified as *Acidithiobacillus ferrooxidans*, an acidophilic microorganism known to thrive in highly acidic environments, typically at a pH of around 2.[5,14,23]

Isolation of Microorganisms –

Microorganisms were isolated using serial dilution and plating on 9K medium, which is selective for *Acidithiobacillus ferrooxidans* and related species.[28] Plates were incubated at 30°C under acidic conditions (pH 2). Colonies with distinct morphology were sub-cultured to obtain pure isolates. *Acidithiobacillus ferrooxidans* appear as pink, rod-shaped colonies indicating gram negative bacillus.[14,24]

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Figure 1: ***Acidithiobacillus ferrooxidans*** **(Strain NCIM 5370)**

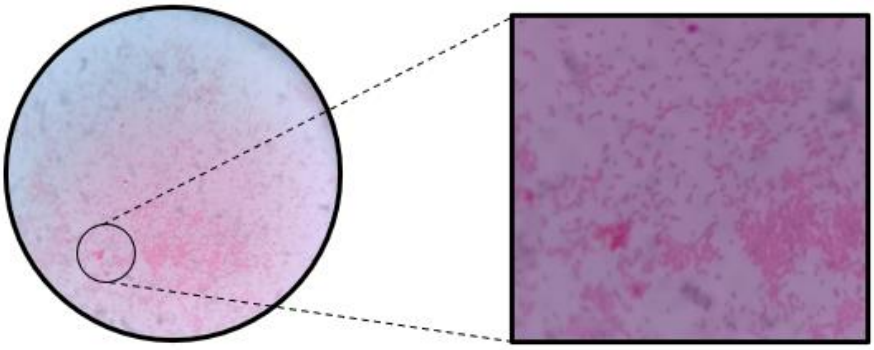

Figure 2: Microscopic view of ***Acidithiobacillus ferrooxidans*** **(40x)**

Bioleaching Process –

Low-grade iron and copper ores were finely granulated into thin powder form. This process exposes more mineral particles to microorganisms, like *Acidithiobacillus ferrooxidans*, which thrive on direct contact with sulfide minerals like pyrite (FeS_2) or chalcopyrite ($CuFeS_2$).[6,23] The study was conducted in 250ml conical flasks containing 100ml of sterile 9K medium supplemented with 5ml of pre-cultured organism.[7,14,30] Flasks were inoculated with actively growing cultures of *A. ferrooxidans* and incubated at 35°C under constant shaking (150 rpm).[5,8,23]

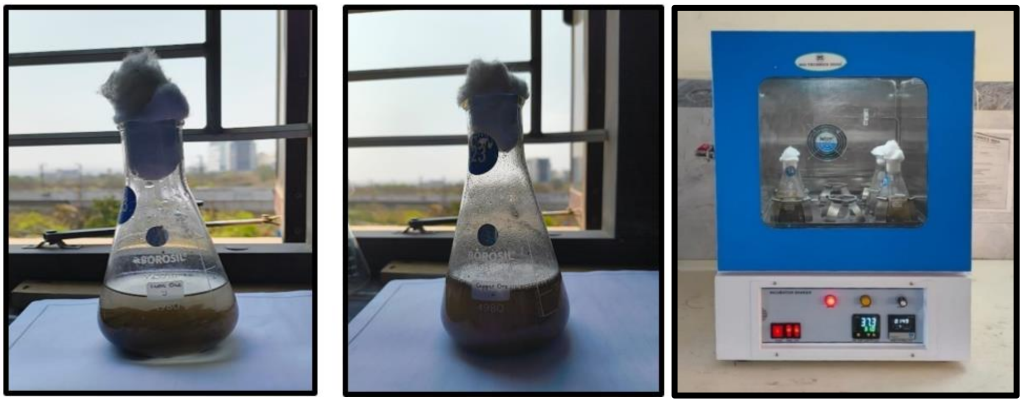

Figure 3: Inoculation and Bioleaching Process

Recovery of Metals –

Following bioleaching, the microbial biomass and unreacted solids were separated by centrifugation, and the clarified leachate was analysed for dissolved metal content using spectrophotometer. Metal recovery was achieved through chemical precipitation.[2,28] For iron, the pH was adjusted to 3.5-4.0 using $Ca(OH)_2$ or NaOH to precipitate Fe^{3+} as $Fe(OH)_3$, which was filtered, dried, and converted to Fe_2O_3 by calcination. For copper, the leachate was adjusted to pH 4-5 and treated with Na_2S or H_2S to precipitate Cu^{2+} as CuS, which was collected and further processed by smelting to obtain pure copper.

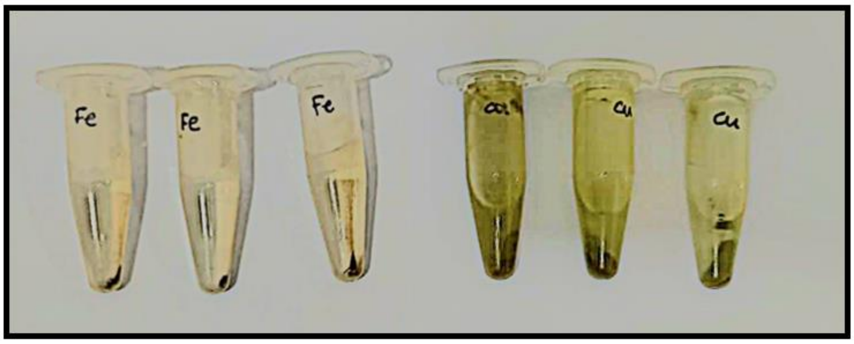

Figure 4: Extracted Metals from Metal Ores

RESULTS

The growth of *Acidithiobacillus ferrooxidans* was monitored for two weeks, with a pH maintained between 1.4 and 2.0. The optical density (OD) at 570 nm was measured to assess bacterial growth, revealing a progressive increase over time. The results indicate a typical bacterial growth curve with an initial phase, followed by an exponential phase, and a gradual stabilization towards the end of the observation period. This growth pattern is characteristic of microbial adaptation and metabolic activity, providing key insights into the efficiency and sustainability of *A. ferrooxidans* in bioleaching applications.

Table 5: Absorbance at 570nm	
Number of Days	**Optical Density (OD)**
2	0.10
4	0.15
6	0.46
8	0.72
10	0.79
12	0.86
14	0.94

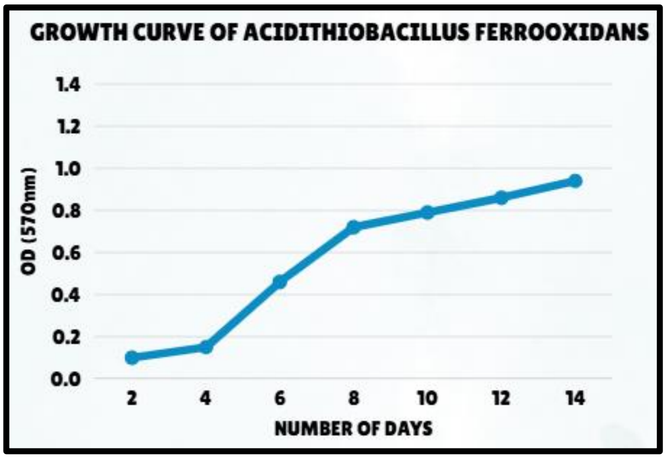

Figure 5: Growth Curve at 570nm

Overall, the results confirm that *Acidithiobacillus ferrooxidans* successfully adapted to the experimental conditions and exhibited sustained growth. The observed trends align with typical bacterial growth dynamics, where environmental factors like pH, oxygen availability, and substrate concentration influence growth rates.

In this study, we investigated the bioleaching potential of *Acidithiobacillus ferrooxidans* for extracting iron and copper from metal ores for seven days using UV-Vis spectroscopy. The absorbance was measured at specific wavelengths: 480 nm for iron and 750 nm for copper. The results indicate a progressive increase in absorbance for both metals over time.

Table 6: Absorbance of Metals		
Number of Days	**IRON (480nm)**	**Copper (750nm)**
1	0.173	1.875
2	0.292	1.916
3	0.344	1.971
4	0.395	2.021

5	0.472	2.078
6	0.502	2.105
7	0.576	2.129

Figure 6: Absorbance of Iron and Copper on Day 7

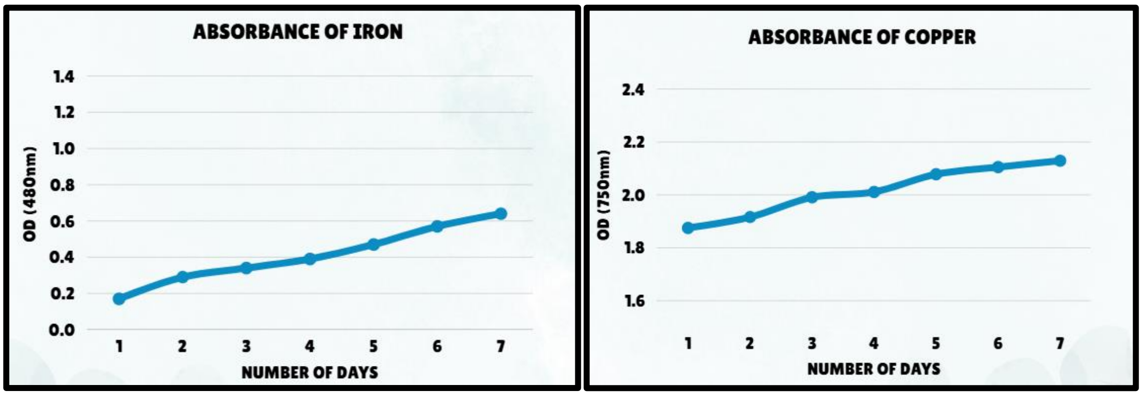

Figure 7: Absorbance of Iron and Copper

DISCUSSION

The present study demonstrated the bioleaching potential of *Acidithiobacillus ferrooxidans* for extracting iron and copper from ores, showing a steady increase in metal solubilization over the seven-day period. Copper exhibited higher absorbance values than iron, reaching 2.129 at day seven, while iron increased more gradually to 0.576. This trend reflects the efficiency of *A. ferrooxidans* in facilitating copper dissolution, consistent with its known metabolic preference for oxidizing ferrous ions and catalyzing sulfide mineral breakdown.

Our findings align with earlier work on electronic waste, where up to 98-99% copper dissolution was achieved in 21 days, indicating that even moderate inoculum sizes can achieve high recovery over time. Studies on scrap TV circuit boards showed that iron supplementation improved copper recovery from 35%

to 89%, emphasizing the role of Fe^{2+} cycling. Compared to fungal bioleaching with *Aspergillus niger* (68% Cu recovery), bacterial systems, including ours, demonstrated faster and more efficient copper dissolution.[3,17,29]

Overall, the study reinforces the efficiency of *A. ferrooxidans* for copper-specific bioleaching and highlights opportunities for optimization through longer incubation, iron supplementation, or integration with supportive substrates, supporting its potential as a sustainable alternative to conventional metal extraction.

CONCLUSION

Microbial bioleaching, exemplified by *Acidithiobacillus ferrooxidans* NCIM 5370, offers a sustainable, cost-effective, and scalable alternative to conventional mining for extracting valuable metals from low-grade ores and mining residues. This approach leverages well-understood biochemical mechanisms and has demonstrated efficacy in both laboratory and industrial settings, including complex waste streams such as e-waste and spent batteries. Recent advances in strain engineering, process optimization, real-time monitoring, and hybrid circular strategies have further enhanced the efficiency and applicability of bioleaching.

Although there are challenges, such as slow metal extraction from resistant minerals like chalcopyrite, acid mine drainage, and the need for careful process control, bioleaching is increasingly being adopted. Growing regulatory, economic, and environmental pressures make microbial bioleaching a promising method for sustainable metal recovery, supporting resource security and environmentally responsible mining within a circular economy.

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