"Deliverance of Mother Earth by Bioleaching: Solution for e-waste"

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ABSTRACT :

Bioleaching, the microbial extraction of metals, possesses the ability to be an environmentally friendly alternative to the traditional metallurgical processes. Carrying out the extraction under mild environmental conditions, bioleaching ensures reduced energy input and undesirable emissions but achieves the efficient recovery of metals. In this review, the latest bioleaching technologies, microbial diversity, and the integration of omics-based technologies for the optimization of the process are considered. Microorganisms including *Acidithiobacillus ferrooxidans*, *Leptospirillum ferriphilum*, and *Chromobacterium violaceum* have metabolic features allowing the dissolution of metals through the production of sulfuric acid or cyanide substances. Specifically, bioleaching represents an attractive methodology for the environmentally benign treatment of e-waste, which abundantly contains precious metals including gold and copper, even the amounts are higher than those contained in natural ores. Through microbial processes, metals are brought to solubilization under ambient pressure and temperature, ensuring the mitigation of ecological risks while recovering the valuable components for recirculation. Integration of genomics, proteomics, and metagenomics has broadened the understanding of microbial biofilm formation, resistance to oxidative stress, and tolerance of metals, resulting in the better engineering of strains for higher yields of recovery. Statistical approaches like Response Surface Methodology and designs of the Central Composite and Box-Behnken make possible the optimization of key parameters, like pH, temperature, pulp density, and size of the inoculum. Bioleaching processes are now applied beyond the classical copper and uranium extraction to the production of cobalt, nickel, the rare earths, and lithium from varied feedstocks like spent batteries and waste of industry. Novel technological innovations like the application of modular bioreactors and the in situ or space bioleaching exemplify the flexibility and the scalability of the technology. In general, bioleaching is one of the major technologies of the circular economy, transforming wastes into resources and contributing towards the sustainable metal recovery and the promotion of the green energy transition globally.

KEYWORD :

Bioleaching, e-waste, Sustainability, eco friendly metal extraction, proteomics, waste management, gold recovery ,microbe oriented, biohydrometallurgy

Introduction:

Every time we upgrade our smartphones, replace a laptop, or toss out that old TV remote that finally stopped working, we're contributing to what has become one of the most pressing environmental challenges of our time. It's easy to forget that behind every sleek new gadget lies a story of resource extraction, manufacturing, and eventually, disposal. But here's the thing that might surprise you: that old phone gathering dust in your drawer contains more gold per ton than most gold mines.We live in an age where technology evolves so rapidly that what seemed cutting-edge yesterday becomes obsolete tomorrow. Remember when computers were expected to last 4-5 years. Now, the average lifespan has shrunk to just 2 years, and it's getting shorter. This isn't just about technology advancing—it's about how we've structured our entire relationship with electronic devices around constant replacement rather than repair and longevity.What happens to your old electronics tells a deeply human story about global inequality and environmental justice. When you responsibly "recycle" your old phone in a developed country, there's an 80% chance it will end up on a ship bound for a developing nation. It might arrive at a sprawling e-waste processing site in Ghana, where children as young as 10 work alongside adults, burning cables to extract copper wire, breathing in toxic fumes that will affect their health for years to come**[1].**These informal recycling operations exist because formal recycling facilities—the kind with proper safety equipment and environmental controls—are expensive to build and maintain. So communities in countries like China, Nigeria, India, and the Philippines have built entire economies around processing the electronic waste that wealthier nations can't or won't handle responsibly.The 40 million metric tons of e-waste we generate annually isn't just a number—it represents millions of individual decisions to discard devices that contain a cocktail of both valuable and dangerous materials. When these devices end up in landfills or are processed unsafely, they release mercury that can damage developing brains, lead that accumulates in bones and organs, and dozens of other toxic compounds that persist in the environment for decades.But here's where the story takes an interesting turn: scientists have discovered that the same biological processes that have been quietly extracting metals from rocks for billions of years might hold the key to solving our e-waste crisis.Imagine if instead of using dangerous chemicals and high-temperature furnaces to extract metals from your old electronics, we could simply introduce the right bacteria and let them do the work. This isn't science fiction—it's bioleaching, and it's already happening in laboratories and pilot facilities around the world.

The microbes doing this work are remarkable creatures that have evolved to thrive in environments that would kill most life forms. Species like *Acidithiobacillus ferrooxidans* actually eat iron and sulfur, producing sulfuric acid as a byproduct. Others, like *Chromobacterium violaceum*, produce cyanide compounds that can dissolve gold and other precious metals. These bacteria don't see our electronic waste as trash—they see it as dinner.Behind every research paper on bioleaching is a scientist who became fascinated by the idea that microscopic organisms could solve massive human problems. Dr.researchers working with *Aspergillus niger*, a common bread mold, discovered they could coax it to extract gold from smartphone circuit boards with remarkable efficiency. Graduate students spend weeks or months fine-tuning conditions—pH, temperature, and nutrient levels—to get the best out of these microscopic biological miners.Anyone's ears perk up at the mention of *Paenibacillus* species, bacteria that were first isolated from soil. By sensitive cultivation, they've conditioned these microbes to extract not only some of the most ubiquitous metals such as copper and zinc, but also precious metals such as gold, silver, and palladium from graphics and computer memory cards. In one experiment, they recovered 87% of the zinc and 88% of the manganese—figures that would make the most conservative traditional mining operation green with envy.What makes bioleaching so fascinating is how much it resembles cooking—except the chefs are bacteria, and the recipe determines whether you can extract valuable metals or end up with biological soup. Temperature matters enormously: most of these bacterial miners work best between 25-30°C, just slightly above roomtemperature**[2].** Feed them too much electronic waste (high pulp density), and they become overwhelmed and stop working efficiently. Don't give them enough oxygen, and these aerobic organisms simply can't survive.The pH level is particularly critical. Most bioleaching happens in extremely acidic conditions—imagine working in an environment more acidic than lemon juice. At pH levels below 2.5, metals stay dissolved and available for extraction. Above that, they start to precipitate out of solution and become impossible to recover.The development of biological e-waste processing isn't just about finding a more efficient way to extract metals—it's about creating technologies that can be deployed safely in the communities currently bearing the brunt of our electronic consumption. Unlike traditional pyrometallurgical processing, which requires massive industrial facilities and produces toxic emissions, bioleaching can potentially be conducted at smaller scales with much lower environmental impact.This could mean that instead of shipping e-waste halfway around the world to be processed in unsafe conditions, communities could develop local biological processing capabilities that recover valuable materials while protecting worker health and environmental quality.From an economic standpoint alone, bioleaching is a sound idea. Conventionally processing e-waste is energy-consuming, taking place at high temperatures and with strong chemicals. Biological treatment occurs at ambient temperatures with microorganisms that, in effect, work for free—they simply require proper nutrients and conditions. Whereas naturally occurring gold-bearing ores may contain 0.5-13.5 grams of gold per ton of material, e-waste holds 10-10,000 grams per ton**[3].** We're actually discarding materials that are rich in more valuable metals than most mines, and we're doing it in such a manner that those metals can't be recovered at all safely. As we continue to generate ever-increasing amounts of electronic waste, the question isn't whether we'll need better processing technologies—it's whether we'll choose approaches that work with natural biological processes or continue to rely on energy-intensive, environmentally damaging methods.The microorganisms that researchers are studying today have been perfecting their metal-extraction techniques for billions of years.

They've evolved sophisticated mechanisms for dealing with toxic metals, extracting what they need, and thriving in harsh conditions. Perhaps it's time we learned from their expertise rather than trying to overpower nature with brute-force industrial processes. The destiny of e-waste processing could very well be biological—tiny, decentralized, ecologically friendly plants where specially grown microbial consortia convert our old technology back into the building blocks of tomorrow's inventions. It's a future where resource emerges from genetic waste, where biology and engineering are allied, and where the environmental price tag on our digital lives is slashed.This isn't just about better recycling—it's about reimagining our relationship with the materials that make our technological society possible, guided by the wisdom of organisms that have been solving similar problems since the dawn of life on Earth.

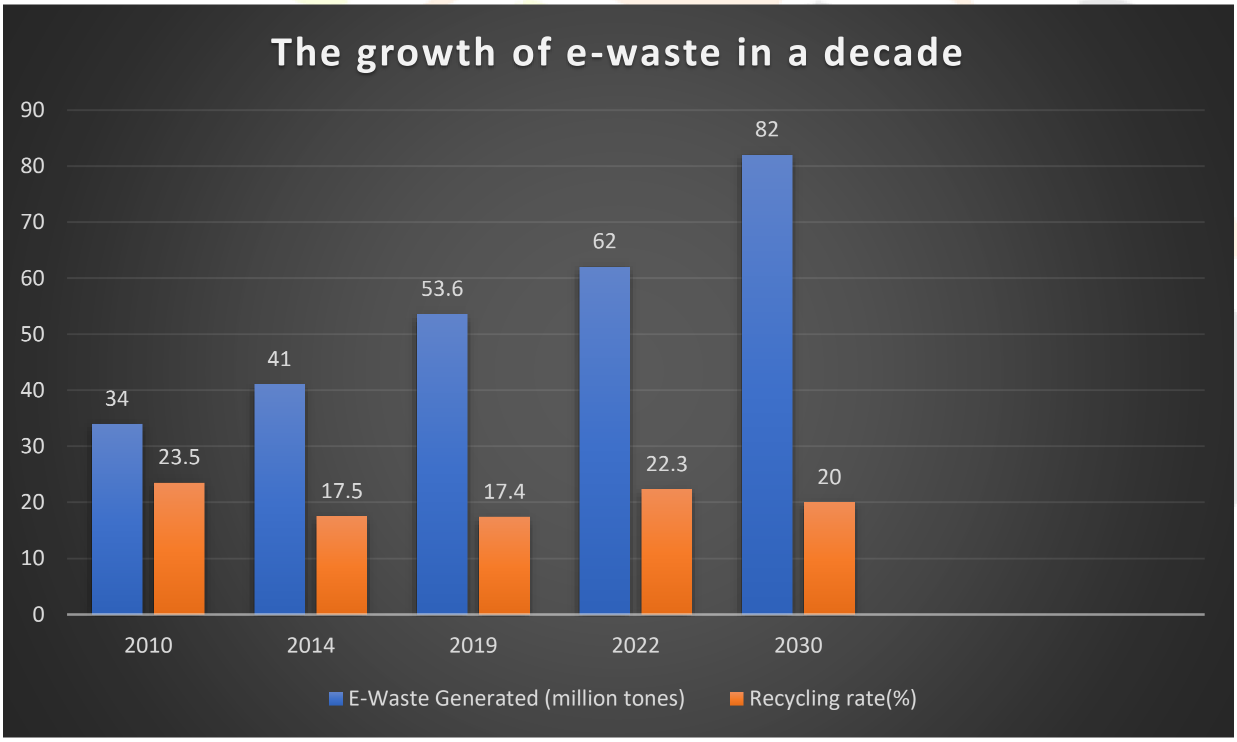

*Table 1:*Representation of E-waste generation in last decades**[4]**

Literature Review:

OMIC approach in bioleaching:

Contribution of genomics:

The use of OMICS technologies—such as genomics, metagenomics, proteomics, and metaproteomics—has opened up new possibilities for discovering previously unknown biomining microorganisms that are difficult to cultivate in the lab. These approaches also help us better understand the metabolic processes these microbes use during biomining, as well as how they adapt to their environments. Among all biomining microbes, *Acidithiobacillusferrooxidans* stands out as the most extensively studied and was actually the first biomining microorganism to have its genome fully sequenced by the Institute for Genomic Research (TIGR). To date, scientists have sequenced and made accessible 36 complete archaeal and 55 bacterial genomes relevant to biomining in the NCBI database.The use of OMICS has greatly increased the scope of our understanding of biomining biology. For instance, genomic studies have mainly aimed at improving the understanding of major metabolic pathways that drive fundamental processes such as iron and sulfur oxidation, quorum sensing (the mode by which bacteria sense and talk to each other), flagellar development, movement towards nutrients (chemotaxis), carbon fixation, nitrogen fixation, and general adaptation to extreme bioleaching conditions**[5].**

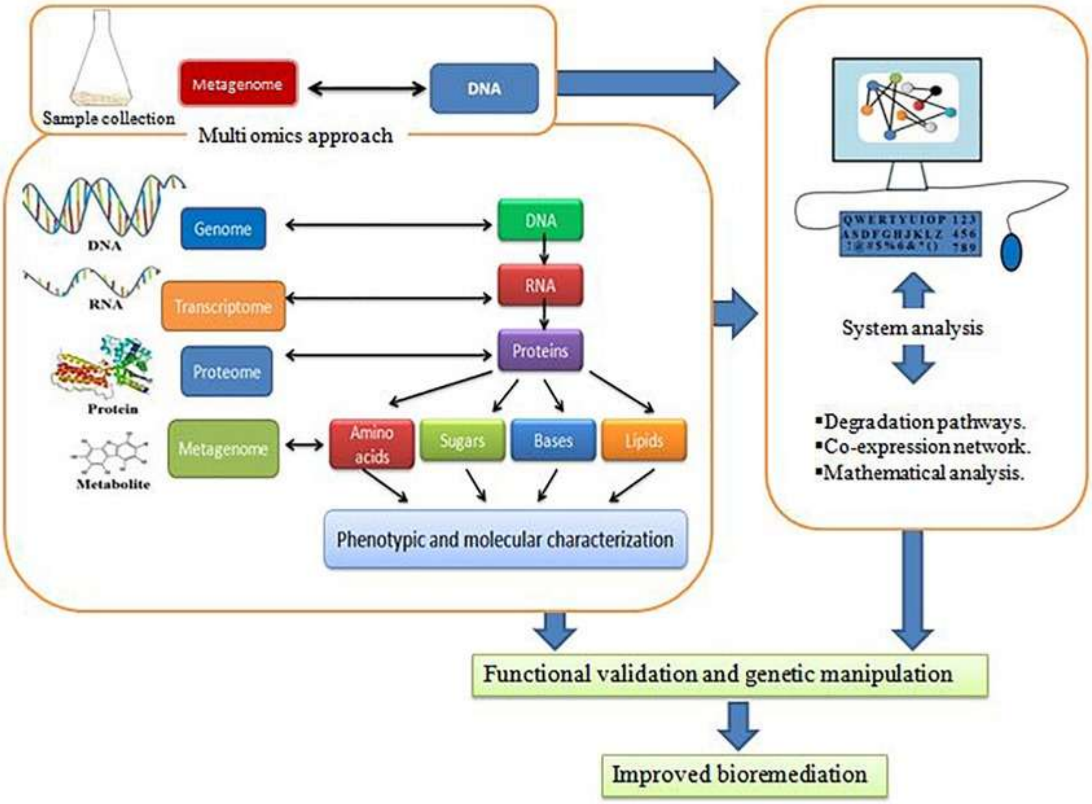

*Figure 1:Representationofmultiomicsapproachforimprovedbioremediation**[6]***

In a particularly significant study, Christel and coauthors (2018) reported a high-quality, closed genome of *Leptospirillum ferriphilum*, which provided valuable information regarding its metabolism. Integrating different OMICS strategies, scientists examined the genes and proteins that facilitate the growth and development of *Leptospirillum ferriphilum* on chalcopyrite under the conditions of bioleaching experiments.Lastly, systems biology plays an important role by integrating data from genomics, transcriptomics, and proteomics with computational tools. This allows scientists to turn raw data into meaningful biological insights, helping to identify the specific metabolic pathways that drive biomining processes.Researchers have been using shotgun sequencing of 16S rRNA genes to explore the microbial diversity in a manganese mining site and to understand how these microbes contribute to manganese solubilization. The results revealed that Proteobacteria dominate the microbial community, making up about 42.47%, followed by Actinobacteria at 23.99%. Likewise, in the Panasqueira tungsten mine, researchers investigated the chemical composition and microbial diversity of two tailing basins to determine the influence of local geochemistry on microbial community and function. Targeted 16S rRNA gene sequencing with the MiSeq platform revealed a range of microbes,*Pseudomonas*, Bacillus, Streptococcus, Acinetobacter, Rothia, Cellulomonas, and *Anaerolineaceae* family members**[7].** The microbial composition of Basin I was correlated with increased potassium and aluminum concentration, whereas Basin II was associated with arsenic, sulfur, and iron concentrations. Predictive analysis using PICRUSt software indicated a range of metabolic processes enabling these microbes to sustain themselves in such harsh environments.

OMIC TECHNOLOGIES	MOLECULAR TECHNIQUES	APPLICATIONS
Genomics and Metagenomic	PCR, qPCR, RT-PCR, DGGE, DNA microarray, Next Generation Sequencing, etc.	Microbial diversity study of bioleaching environment.
Proteomics	Chromatography, SDS PAGE, 2D PADE, Mass Spectroscopy, Protein microarray	In depth understanding of metabolic pathways bioleaching microbes.To study the genetic variation within the species. Proteomics, Chromatography, SDSPAGE, 2D PADE, Mass Spectroscopy, Protein microarray Identification and quantification of proteins involved in bioleaching processes

*Table 2:*ApplicationofOmicstechnologies inbioleachingfield**[8]**

Genome sequencing plays a crucial role in forming hypotheses about microbial metabolic pathways involved in bioleaching, including iron and sulfur oxidation and biofilm formation. *For example:* found thiosulfate–sulfurtransferase-like proteins-encoding genes in Acidithiobacillus ferrooxidans and associated with sulfur metabolism. *F*ound two operons, petI and petII, in A. ferrooxidans. The petII operon contains the ABC gene cluster (for the bc1 complex), co-transcribed with cyeA (cytochrome c), sdrA (a putative dehydrogenase), and hip. Expression analysis revealed that petI is induced under iron-replete growth, whereas petII is induced in both sulfur and iron media. It rebuilt metabolic pathways and found a gene cluster (rhd, tusA, dsrE, hdrC, hdrB, hdrA, orf2) that encodes three sulfurtransferases, in addition to sat and sdrA, which belong to a heterodisulfide reductase complex. With time, detailed models have been formulated for a number of, thermoacidophilic microorganisms, including Acidilobus saccharovorans Acidianus hospitalis, and A. ferrooxidans.There are even genome-scale models for Leptospirillum ferrooxidans, Acidithiobacillus thiooxidans, and mixed cultures like L. ferriphilum with Ferroplasmaacidiphilum. These genomic models have highlighted similarities in reduced inorganic sulfur compound (RISC) oxidation pathways between bacteria and archaea, while also revealing unique adaptations for survival in extreme mining environments.Comparative genomics has helped uncover genetic differences among strains of the same species**[9].** For example:

- *A. ferrooxidans* strains ATCC 53993 and ATCC 232707 share 2397 genes (78–90% of their genomes), but the ATCC 53993 strain shows greater copper resistance.
- In *A. thiooxidans*, 75–89% of the genome is shared among strains ATCC 19377, A01, and Licanantay.
- For *Sulfolobus* species, 18–28% of the genome varies between strains.

A broader genomic comparison of 20 bioleaching microorganisms has allowed scientists to predict metabolic and regulatory functions, often by studying close relatives found in bioleaching heaps. For example, Metallosphaerasedula carries genes for metal tolerance, autotrophic carbon fixation, and adhesion, along with a putative tetrathionate hydrolase gene for sulfur oxidation. Its genome also contains fox-gene-like clusters similar to those in *S. tokodaii* and *S. metallicus*.

Contribution of proteomics:

Proteomics represents a strong weapon for the investigation of proteins produced by cells under varying conditions or environmental stresses. Among the most frequent methods for this is 2D polyacrylamide gel electrophoresis (2D-PAGE) coupled with mass spectrometry (MS), and it has been broadly used to analyze protein expression in *Acidithiobacillusferrooxidans*under varying growth conditions.For instance, analyzed A.

ferrooxidans strain ATCC 19859 under growth on ferrous iron, metal sulfides, thiosulfate, and elemental sulfur. With 2D-PAGE, they detected changes in protein synthesis patterns in relation to the growth substrate**[10].** This is especially significant because in bioleaching, microbes are important for oxidizing iron (predominantly in the extracellular environment) and sulfur (in the periplasm). To learn more about sulfur oxidation metabolism in *A. ferrooxidans* , researchers performed high-throughput proteomic analysis on cells grown with thiosulfate. They found 131 proteins in the periplasmic fraction, with 86% predicted to have export signals. Nearly half of these proteins were linked to the cell envelope, transport and binding proteins, energy metabolism, and protein folding. Interestingly, 36% of the detected proteins were hypothetical—with no known function—highlighting how much remains to be discovered about this organism.Proteomic studies have also revealed how bioleaching microbes resist heavy metals. For example, when A. ferrooxidans ATCC 23270 was subjected to elevated copper concentrations, scientists observed the bacterium to adapt by over expressing the RND-type Cus efflux system (which effluxes toxic metals), expanding cysteine, histidine, and a putative disulfide isomerase production. Meanwhile, the bacterium suppressed some outer membrane proteins and ion transporters, presumably to decrease metal uptake. Copper stress also induced increased protein expression of proteins from the rus operon, implying a role in copper resistance. ferrooxidans DSM 14882 cells grown on Fe^{2+} with biofilm cells grown on pyrite over five days using shotgun proteomics. They had identified 1,157 proteins in total, of which 80 played a part in oxygen and metal homeostasis, ROS detoxification, redox control, and antioxidant synthesis.The researchers highlighted the role of globins in maintaining oxygen balance and protecting pyrite-grown biofilms from oxidative stress.Biofilm formation—mainly mediated by extracellular polymeric substances (EPS)—is also essential in bioleaching**[11].** Semi-quantitative shotgun proteomics comparing free-floating (planktonic) cells with 24-hour-old pyrite biofilms revealed several adaptations:Higher production of EPS, ABC transporters, effluxpumps, and stress-resistance proteins.Presence of proteins for both osmotic and oxidative stress resistance.Increased levels of EnvZ (osmolarity sensor protein) and an iron/2-oxoglutarate-dependent dioxygenase in biofilm cells.Proteins involved in glutathione (GSH) metabolism, which is important for RISC oxidation and oxidative stress defense. Higher periplasmic GSH levels in pyrite biofilms suggest enhanced sulfur oxidation and stress resistance.Increased biosynthesis of coenzymes and cofactors.This biofilm-focused study detected 1,319 proteins, of which 231 were hypothetical, again indicating significant potential for future discoveries.

Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is a mathematical and statistical technique for the optimization of complicated processes by multivariate analysis. Relative to the conventional optimization methods, RSM is cost-saving and time-efficient, as it demands fewer experimental runs for examining various factors and interactions collectively. RSM is widely utilized in the fields of biology, chemistry, food technology, environmental engineering, and other sciences. Through the use of designed experiments, RSM explores the inter-relationships among multiple independent variables to determine the best operating conditions for a process. RSM is accomplished by fitting mathematical models, usually linear or polynomial ones, to experimental results and checking the resulting model by statistical techniques**[12].**A significant strength of RSM is that it can study multiple parameters at various levels in parallel and also indicate their interactions. Throughout recent decades, RSM has evolved to become one of the most prevalent and successful experiment design tools, especially in chemical and environmental modeling, optimization, and simulation processes.

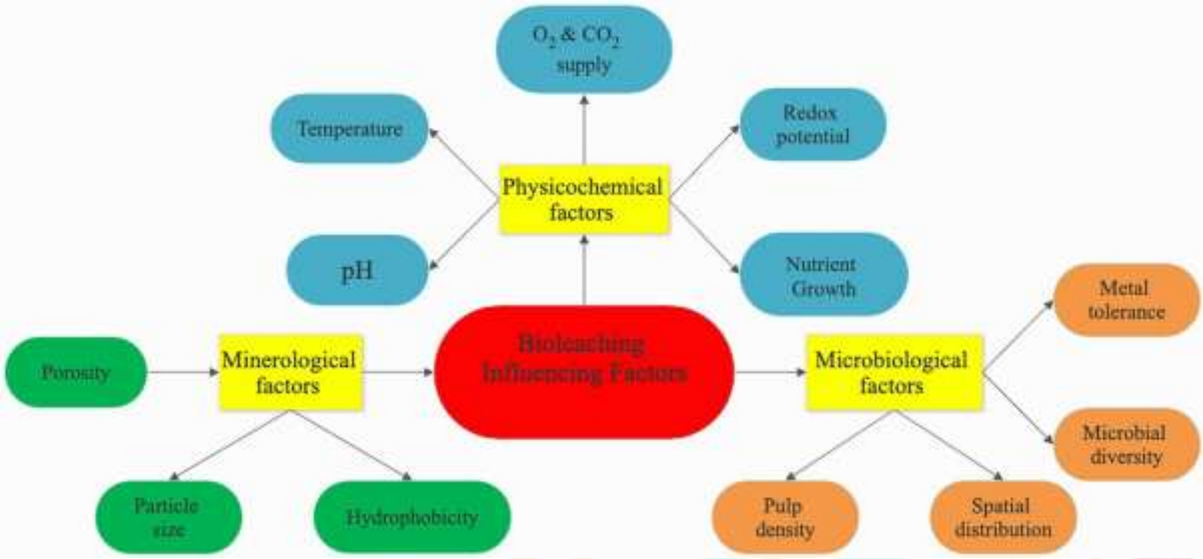

Figure 2：A reviewonbioleachingoftoxicmetalloids

Experimental Designs in RSM

Two of the most widely used designs in RSM are:

Central Composite Design (CCD) – Suitable for investigating variables at five levels.Box–Behnken Design (BBD) – Suitable for investigating variables at three levels.Both have been successfully applied in bioleaching optimization studies.The general RSM-based optimization process for bioleaching involves six key steps:Selection of independent variables that significantly influence system response (screening stage).Choosing the optimal experimental design (CCD or BBD).Running the experiments and recording results.Model fitting using regression techniques.Model confirmation using Analysis of Variance (ANOVA) and graphical analysis.Determination of optimal conditions for maximum recovery**[13].**

Screening of Independent Variables

Screening is crucial in RSM, as it focuses on identifying factors that significantly affect the process outcome. This is normally based on expert consultation, preliminary experiments, and a literature review. For bioleaching, typical important variables are initial pH, pulp density, temperature for growth, concentration of the substrate, and others—albeit the actual set of variables varies according to the type of waste and microbial strain utilized. Inadequate variable selection at this point may lead to inconsistent optimization outcome.

Optimal Experimental Design|

In the design stage, experiment points are defined according to the chosen method. Selecting the correct design is critical, as it affects the accuracy of the response surface and the predictive capability of the model.RSM commonly uses first-order and second-order polynomial models:
First-order model:y=b0+∑i=1kbixi+εy=b0+i=1∑kbixi+ε
where b0b0 represents the intercept, bibi are the linear coefficients, xixi are coded variables, and εε is the residual error. This model cannot be used to represent curvature.

Second-order model:y=b0+∑i=1kbixi+∑i=1kbiixi2+∑i=1k∑j=i+1kbijxixj+εy=b0+i=1∑kbixi+i=1∑kbiixi2+i=1∑kj=i+1∑kbijxixj+ε

which consists of quadratic and interaction terms and hence is capable of estimating curvature and saddle points**[14].**

Central Composite Design (CCD)

Proposed in 1951, CCD is one of the most widely used designs for second-order model fitting. It examines factors at five coded levels (−α, −1, 0, +1, +α). The number of experiments is represented by: N=2k+2k+C0N=2k+2k+C0 wherein kk is the number of factors, 2k2k represents runs along the factorial, 2k2k represents runs on the axes, and C0C0 represents center points. CCD models curvature efficiently without making experiments cumbersome.

Box–Behnken Design (BBD)
Developed in 1960, BBD combines two-level factorial designs with incomplete block designs. All factors are studied at three levels, and experimental points are located at the midpoints of edges of the process space. The key advantage of BBD is avoiding extreme experimental conditions, but it is unsuitable for cases requiring factor levels at maximum or minimum limits.
Running the Experiments & Obtaining Results
Once the experimental plan is set—often generated with statistical software—the experiments are performed, and results are recorded for model fitting.

Model Fitting to Experimental Data
Model fitting involves two stages:Coding the variables to transform real values into dimensionless quantities (−1, 0, +1) using:xi=zi−zi0Δzi*xi*=Δ*zizi*−*zi*0where zi*zi* is the real value, zi0*zi*0 is the central point, and Δzi*Δzi* is half the range of variation.Regression
analysis using the Least Squares Method (LSM) to estimate coefficients. Model quality is typically assessed using R^2, Adjusted R^2, and Predicted R^2.Adjusted R^2 accounts for the number of predictors, preventing artificial inflation from too many terms.Predicted R^2 assesses how well the model predicts new data, helping detect overfitting
Model Validation through ANOVA and Plots**[15].**

ANOVA pinpoints statistically significant factors and checks the model adequacy. F-statistic tests the ratio of variances; the higher the F-value, the more significant the model. p-value < 0.05 generally denotes significance at the 95% confidence level. Graphical outputs like contour plots and 3D surface plots facilitate visualization of optimal conditions and factor interactions.
Determining Optimal Conditions
Optimal operating conditions are determined by considering the fitted model in graphical and numerical terms. In the case of bioleaching, it's finding the optimum set of factors (e.g., initial pH, waste concentration, sulfur content, Fe^{2+} concentration) to achieve maximum metal recovery. Predictions from RSM must be validated through confirmatory experiments to ensure model reliability.
Limitations of RSM
Although widely adopted for modeling and optimizing bioleaching, RSM has some limitations:It is less effective when the true functional relationship is highly irregular or discontinuous.Accuracy depends strongly on the correct selection of initial factors and ranges.Model fitting assumptions (e.g., normally distributed residuals, independence) must be satisfied to avoid bias.
Application of RSM in Bioleaching Processes
Bioleaching offers an environmentally friendly way to recover valuable metals from various solid wastes, including soils, ores, e-waste, spent catalysts, sludge, and plant residues. In recent years, Response Surface Methodology (RSM), combined with Design of Experiments (DOE) tools such as Central Composite Design (CCD) and Box–Behnken Design (BBD), has been widely used to optimize operational parameters for improving heavy metal removal efficiency**[16].**
Biorecovery from Soils and Ores
Bioleaching of ores and soils using RSM has been extensively reported as a greener alternative to conventional chemical leaching. Most microorganisms applied in these studies are acidophiles, which generate ferric iron (Fe^{3+}) and sulfuric acid through sulfur oxidation. Both compounds contribute to lowering pH — a critical factor for efficient metal solubilisation .Studies show that an optimum pH of 2 or lower promotes the oxidation of iron and sulfide ions and enhances microbial activity. In bioleaching by fungi, the carbon sources (glucose, sucrose)

are responsible for organic acid generation, which enhances metal recovery yields.Sun et al. (2022) maximized Ni, Cu, and Co recovery — with minimal Mg and Fe dissolution — from high-Mg nickel sulfide ore by A. ferrooxidans, *Ferrimicrobium acidiphilum*, and *Leptospirillum ferrooxidans***[17].** CCD optimization of particle size, acid dosage, pulp density, and inoculation resulted in 89.4% Ni, 36.8% Cu, 84.1% Co recovery, with particle size as the most significant variable for Ni, Cu, and Co recovery. It utilized a two-step RSM optimization of the biodesulfurization of A. caldus from sulfide ores. Based on Plackett–Burman screening, particle size, shaking speed, and inoculum size were identified as significant factors. Maximum conditions enhanced desulfurization effectiveness by 8.1% after 5 days.Tested a mixed microbial consortium for uranium recovery, finding an optimal 91.4% U extraction via BBD analysis of pH, Fe^{2+} concentration, solid-liquid ratio, and inoculationusing *A. ferridurans*, achieved 95.5% U recovery from low-grade ore under optimized pH, particle size, temperature, aeration, and irrigation.Selvi&Aruliah (2018) demonstrated bioleaching-enhanced electrokinetic remediation (BEER) for Zn-contaminated soils using *Serratia marcescens*, achieving 93.8% Zn removal versus 72.9% for bioleaching alone**[18].**

Biorecovery from E-wastes

E waste bioleaching is affected by numerous parameters—pH, pulp density, substrate Bioleaching, the microbial extraction of metals, possesses the ability to be an environmentally friendly alternative to the traditional metallurgical processes. Carrying out the extraction under mild environmental conditions, bioleaching ensures reduced energy input and undesirable emissions but achieves the efficient recovery of metals. In this review, the latest bioleaching technologies, microbial diversity, and the integration of omics-based technologies for the optimization of the process are considered. Microorganisms including *Leptospirillumferriphilum*, and *Chromobacteriumviolaceum*have metabolic features allowing the dissolution of metals through the production of sulfuric acid or cyanide substances. Specifically, bioleaching represents an attractive methodology for the environmentally benign treatment of e-waste, which abundantly contains precious metals including gold and copper, even the amounts are higher than those contained in natural ores. Through microbial processes, metals are brought to solubilization under ambient pressure and temperature, ensuring the mitigation of ecological risks while recovering the valuable components for recirculation**[19].**

Integration of genomics, proteomics, and metagenomics has broadened the understanding of microbial biofilm formation, resistance to oxidative stress, and tolerance of metals, resulting in the better engineering of strains for higher yields of recovery. Statistical approaches like Response Surface Methodology and designs of the Central Composite and Box-Behnken make possible the optimization of key parameters, like pH, temperature, pulp density, and size of the inoculum. Bioleaching processes are now applied beyond the classical copper and uranium extraction to the production of cobalt, nickel, the rare earths, and lithium from varied feedstocks like spent batteries and waste of industry. Novel technological innovations like the application of modular bioreactors and the in situ or space bioleaching exemplify the flexibility and the scalability of the technology. In general, bioleaching is one of the major technologies of the circular economy, transforming wastes into resources and contributing towards the sustainable metal recovery and the promotion of the green energy transition globally., temperature, agitation, and inoculum load. RSM is particularly useful for the optimisation of these parameters use BBD with *Aspergillusniger e*nzymes for the recovery of metals from rejected cell phone PCBs. Optimisation of glucose oxidase (GOx), Fe^{2+} concentration, pulp density, and shaking speed resulted in 100% Cu, 70% Ni, 40% Pb, and 100% Zn recovery maximized recovery of Indium and Strontium from smartphone touchscreens by using *A. ferrooxidans* with 100% In and 5% Sr recovery at optimal ferrous sulfate concentration, pulp density, sulfur content, and pH. obtained 97% Cu and 74% Ni recovery from computer PCBs by employing *A. niger* under optimal pH, pulp density, inoculum density, and processing time.Optimized Au and Ag bioleaching using *Pseudomonas balearica*, finding yields of 73.9% Au and 41.6% Ag, with glycine concentration and pulp density strongly influencing recovery. Recovered 99.2% Li, 50.4% Co, and 89.4% Ni from spent lithium-ion batteries using *A. ferrooxidans and A. thiooxidans*. Higher sulfur concentration and lower pH favored Li recovery.

Biorecovery from Spent Catalysts

Spent refinery catalysts can contain precious metals like Pt, Mo, Ni, and V. Bioleaching in such matrices generally varies with pH, pulp density, particle size, and aeration rate. extracted Pt from refinery catalysts by using A. niger-produced oxalic acid. pH adjustment greatly enhanced the production of oxalic acid and Pt recovery, which was 37% recovery under optimized BBD conditions**[20]**maximized A. thiooxidans-catalyzed Mo, Ni, and Al leaching from hydrocracking catalysts using CCD. The best particle size (60.7 µm), pulp density (0.9% w/v), and aeration (209 $mL \cdot min^{-1}$) recovered 87% Mo, 37% Ni, and 15% Al in 7 days. likewise employed CCD to optimize Al, Ni, Mo, and V recovery from refinery catalysts with respect to pH, sulfur content, and pulp density as the key variables.

Continued – Biorecovery from Spent Catalysts

Optimisation studies have shown that pH is often the most influential factor in recovering metals such as aluminium (Al), molybdenum (Mo), nickel (Ni), and vanadium (V) from spent catalysts. In one such RSM study, optimum conditions were found at pulp density 1%, sulfur concentration 1.5%, and pH 1.5, giving high recovery rates: Ni 93%, Al 44%, Mo 34%, and V 94%.Motaghed et al. (2014) explored platinum (Pt) and rhenium (Re) biorecovery from spent refinery catalysts using *Bacillus megaterium*. By using the CCD method, they optimized initial concentration of glycine (0–15 g/L) and pulp density (1–10%). Statistical analysis indicated that glycine concentration had a far more significant effect on Re recovery compared to pulp density. The optimal results were obtained by 12.8 g/L glycine and 4% pulp density, with the recovery of 15.7% Pt and a remarkable 98% Re recovery. It used *Aspergillusniger* to recover cobalt (Co), Mo, and Ni from spent catalysts.Inoculums percentage (012%), pulp density (0–4 g/L), and agitation speed (100–160 rpm) — and optimised them via CCD**[21].** Their best settings — pH 5, 31.8 °C, pulp density 2 g/L, 115 rpm shaking, and 12% inoculum — achieved 71% Co, 69% Mo, and 46% Ni recovery.

3.4. Biorecovery from Sludge and Plant Residues

Industrial sludges, such as electroplating sludge, contain both valuable and toxic metals. Given the environmental challenges, bioleaching provides a sustainable route for resource recovery. It recovered Ni, Cu, Zn, and Cr from electroplating sludge using sulfur-oxidizing bacteria. Using CCD, they optimised pulp density (1–5%), temperature (15–55 °C), initial pH (0.6–1.6), and shaking speed (105–165 rpm). Their best conditions 2% pulp density, 45 °C, pH 0.8, and 150 rpm agitation — achieved 100% Ni, 96.5% Cu, 100% Zn, and 76.1% Cr recovery . It optimised bioleaching for Zn removal from paint sludge with *A. thiooxidans*. Key factors included temperature, shaking speed, pH, and particle size. The highest efficiency (22% Zn removal) was achieved at 32 °C, 120 rpm, 1 mm particle size, and pH 4.2, Simultaneous Cu, Cr, Zn, and Ni recovery from sewage sludge using pure cultures of *A. ferrooxidans*, *A. thiooxidans*, and a mixed culture. Using BBD optimisation with variables like initial pH, solids concentration, and ferrous/sulfur ion concentrations, they found the mixed culture most effective, achieving 98.54% Cu, 57.99% Cr, 60.06% Ni, and 95.60% Zn recovery under optimal settings. (2018) introduced a sulfur-oxidising fungal strain (*Aspergillus* sp. SMHS-3) capable of processing toxic refinery spent sulfidic caustic waste. Using BBD to optimise pH, thiosulfate concentration, sucrose concentration, and Mo concentration, they achieved a 1.2× increase in sulfur decomposition rate. ANOVA confirmed the model's strong predictive power ($p < 0.0001$).It recovered Cu and Fe from converter slag using *A. ferrooxidans*. Optimisation via CCD for initial pH (1.5–3), Fe^{2+} concentration (0.5–9 g/L), and pulp density (5–50 g/L) led to 95–100% Cu recovery at pH 1.8, pulp density 1.4 g/100 mL, and Fe^{2+} 7.3 g/L**[22].**

Treatment of E-Waste by the Fungus:

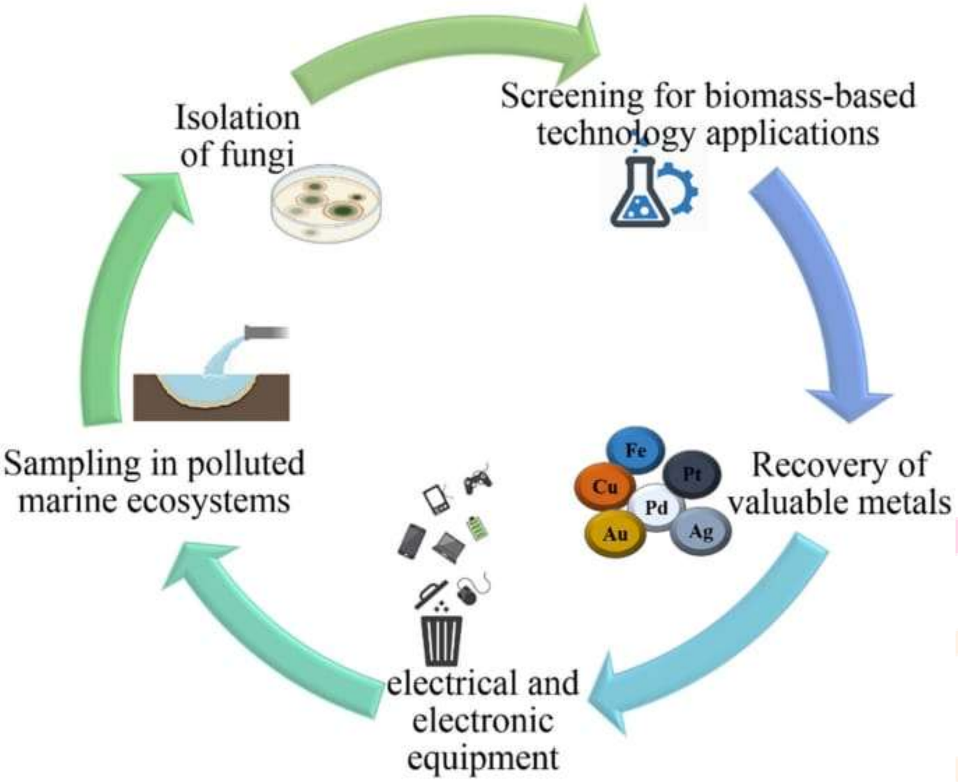

*Figure*3: Utilisation of metals from E-waste by the help of Fungus

Fungal bioleaching refers to the solubilization and mobilization of metals from solid substrates through the biochemical activity of fungi. This process occurs primarily via organic acids generated during fungal fermentation, which promote ligand-induced dissolution of metals from solid matrices. Commonly produced acids include citric, oxalic, gluconic, and other carboxylic acids. Filamentous fungi such as *Aspergillus* spp., *Fusariumgraminearum*, *Trichodermaharzianum*,and *Aspergillusfumigatus* are widely studied for these capabilities due to their adaptability to diverse growth substrates and metabolic flexibility.These fungi can be cultivated under relatively simple fermentation conditions and on low-cost media derived from agricultural or industrial residues**[23].** While mycotoxins, which are produced in some species, have the ability to taint agricultural commodities and be harmful to animal and human health, this issue is not relevant to industrial bioleaching.Industrial processes are conducted under contained, non-food-grade conditions where the aim is to utilize the positive metabolic yield of the fungi. Fungal Cell Structure and Function in Bioleaching Fungal cells are eukaryotic and consist of specific structural elements cell wall, plasma membrane, cytoplasm, mitochondria, endoplasmic reticulum, and Golgi apparatus each with their role in maintaining the cell's life, growth, and reproduction. In bioleaching, these structures indirectly support acid production, enzyme secretion, and complexation processes critical for metal solubilization.Metabolites in Bioleaching: Organic Acids and Siderophores The primary bioleaching agents are low-molecular weight organic acids synthesized via heterotrophic metabolism. Oxalic, citric, gluconic, malic, succinic, pyruvic acids, and others are responsible for metal dissolution through: Acidolysis proton-promoted dissolution. Complexolysis and chelation production of soluble metal-organic complexes. Redoxolysis oxidation–reduction processes. Bioaccumulation incorporation of metals into fungal biomass. Organic acids vary in their leaching effectiveness according to acidity (pKa value) and functional groups. For instance, oxalic acid (pKa 1.23) is stronger than formic acid (pKa 3.75) and lactic acid (pKa 3.86) and thus more effective in leaching Fe from minerals like kaolin. Oxalates can form sparingly soluble or highly soluble salts depending on the cation present, pH, and competing ligands**[24].**

pH Regulation:

pH serves as both a process indicator and a controlling factor for acid secretion. Filamentous fungi often drastically reduce environmental pH when grown in unbuffered media, which can be both beneficial for metal solubilization and detrimental to fungal metabolism if excessively low**[25].**

Agitation Rate:

Moderate stirring (100–300 rpm) generally enhances acid secretion, whereas higher agitation (500–800 rpm) influences enzyme activities, shifting metabolic fluxes from citrate accumulation toward downstream metabolites such as oxoglutarate.

Pulp Density:

Increasing solids concentration from 1% to 2% (w/v) reduces medium volume and reactor size by up to 50%, lowering operational costs. Industrial bioleaching operations often employ ≥10% pulp density for efficiency.

Amino Acids and Metal Complexation:

Certain amino acids like glycine, histidine, and alanine can complex with metals and facilitate dissolution. For instance, while histidine promotes faster initial dissolution of gold, glycine achieves greater overall recovery upon extended leaching.

Applications and LimitationsFungal bioleaching systems are effective for a wide range of metals, including Zn from ZnO, Fe and Al-bound phosphorus, and other metals in ores or contaminated soils. The genera *Aspergillus* and *Penicillium* are notable for their tolerance to metal toxicity and adaptability to varying pH levels, often outperforming bacterial systems in speed and environmental resilience.Nevertheless, despite numerous laboratory successes, industrial-scale fungal bioleaching has not yet been commercialized. Challenges include maintaining optimal growth conditions at scale, managing low pH inhibition, and ensuring consistent metabolite production in large bioreactors**[26].**

Methodology:

Bioleaching: An overview

Bioleaching, also known as biomining, is a process that uses microorganisms to extract valuable metals from low-grade ores. In simpler terms, certain naturally occurring microbes can break down minerals, making it possible for metals to dissolve in water. Once this happens, we can separate the metals from the material by washing it with water.For example, in the case of copper extraction, copper sulfide (CuS_2) can be broken down by microbes into copper sulfate ($CuSO_4$), which dissolves in water, leaving behind the unwanted solid material that is then discarded.A related process called bio-oxidation is slightly different — here, microbes oxidize mineral compounds containing metals of interest, but the metals remain in solid form, concentrated in the deposits.

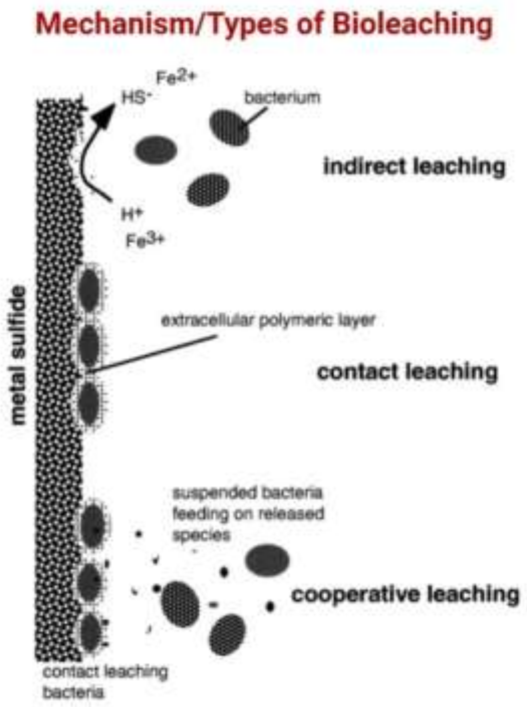

*Figure 4:*Mechanismoftypeof bioleaching

Other terms such as bio-extraction, biomining, and bio-recovery are also used to describe this natural way of mobilizing metals from solid materials, whether through the action of microorganisms, certain parasites, or even plankton-like communities. Overall, biomining is a sustainable method increasingly used in the mining industry to recover metals efficiently and economically**[27].**

Methods in Mineral Recovery:

The way we recover metals from mineral-rich rocks plays a crucial role in bioleaching. Interestingly, scientists only discovered these microbial methods relatively recently. In 1947, Thiobacillusferrooxidans was first isolated from coal mine drainage, and since then, it has been found in most natural and artificial leaching sites.These microbial methods are especially useful for extracting metals from low-grade ores without polluting the air. Along with *T. ferrooxidans*, other acid-loving (acidophilic) bacteria involved in bioleaching include T. thiooxidans, Leptospirillumferrooxidans, and members of the genus Sulfolobus.T. ferrooxidans is a tiny, rod-shaped, gram-positive bacterium. It grows in acidic conditions (pH 1.5–2.5), optimally at 10–30 °C, and can withstand temperatures up to 37 °C. It obtains energy by oxidizing ferrous iron to ferric iron and by oxidizing reduced sulfur to sulfuric acid (H_2SO_4) with oxygen as the terminal electron acceptor. It utilizes carbon dioxide (CO_2) as its source of carbon. Other members of the Thiobacillus genus are also involved in bioleaching, including T. thiooxidans, T. acidophilus, and T. organoparus. Most economically significant minerals are metal sulfides (MS) that are very insoluble. Microorganisms can demobilize metals from these ores using two main strategies:

1. **Direct Leaching** In this method, the microorganisms directly attack the ore mineral. T. ferrooxidans becomes attached to mineral particles, and enzymes on its cell wall initiate oxidative reactions that cause the metal sulfide crystal structure to disintegrate**[28].** The oxidation happens in two main steps:

1. $CuS + 0.5\ O_2 + 2H^+ \rightarrow Cu^{2+} + S^0 + H_2O$
2. $S^0 + 1.5\ O_2 + H_2O \rightarrow H_2SO_4$

Here's how it works: When copper sulfide minerals are oxidized, *T. ferrooxidans* produces copper ions (Cu^{2+}) and elemental sulfur as a byproduct. This sulfur forms a coating on the remaining mineral surface — a barrier that prevents further leaching. That's where T. thiooxidans comes in — it breaks down this sulfur layer, producing more H_2SO_4 and exposing fresh mineral for continued extraction.
Cooperation Between Microbes
Microbial teamwork is vital. For example, *Leptospirillumferrooxidans* is even more acid-tolerant than *T. ferrooxidans*, growing at pH as low as 1.2, thriving on iron sulfide (FeS_2), and tolerating temperatures up to 40 °C. Similarly, certain Sulfolobus species — a group of archaeawork in extreme conditions (pH 1–3 and temperatures 50–90 °C), further aiding in the breakdown of ores.By using a combination of direct and indirect leaching methods, we can efficiently extract metals from ores that would otherwise be uneconomical to process**[29].**

2.IndirectLeaching:

In indirect leaching, the microorganisms don't attack the metal ore directly. Instead, they produce powerful oxidizing agents, such as ferric iron (Fe^{3+}) or sulfuric acid (H_2SO_4), which dissolve the metals so they can be extracted.For this process, a strongly acidic environment is essential. Acid-loving microbes like *Thiobacillusferrooxidans* play a key role here. They gain energy by oxidizing ferrous iron (Fe^{2+}) or sulfide minerals, which leads to the production of ferric sulfate ($Fe_2(SO_4)_3$) — a potent metal-dissolving chemical.Here's how it works step-by-step:Oxidation of pyrite (FeS_2):
$FeS_2 + 3.5\ O_2 + H_2O \rightarrow FeSO_4 + H_2SO_4$
Conversion of ferrous sulfate to ferric sulfate:$2FeSO_4 + 0.5\ O_2 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O$
The ferric sulfate produced can then attack various copper sulfide minerals, such as:Chalcopyrite ($CuFeS_2$):
$CuFeS_2 + 2Fe_2(SO_4)_3 \rightarrow CuSO_4 + 5FeSO_4 + 2S^0$
Chalcocite (Cu_2S):$Cu_2S + 2Fe_2(SO_4)_3 \rightarrow 2CuSO_4 + 4FeSO_4 + S^0$

Bornite (Cu_5FeS_4):$Cu_5FeS_4 + 6Fe_2(SO_4)_3 \rightarrow 5CuSO_4 + 13FeSO_4 + 4S^o$

This is called indirect leaching because the ferric sulfate does the dissolving — the process itself does not require oxygen or direct microbial contact with the minerals. However, microbes are still essential because they continually regenerate ferric sulfate and create the acidic conditions needed for efficient leaching.Additionally, T. ferrooxidans can oxidize the elemental sulfur (S^o) produced as a byproduct into more sulfuric acid:

$2S^o + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$

The sulfuric acid has two functions: It maintains the pH low, which is suitable for T. ferrooxidans and inhibits the hydrolysis breakdown of ferric sulfate:

$Fe_2(SO_4)_3 + 2H_2O \rightarrow 2Fe(OH)SO_4 + H_2SO_4$

It can dissolve other copper minerals directly, such as copper carbonate hydroxide (malachite):

$Cu_3(OH)_2(CO_3)_2 + 3H_2SO_4 \rightarrow 2CuSO_4 + 2CO_2 + 4H_2O$

In brief, indirect leaching is a collaborative effort — microbes produce the chemical "attack agents" and the acidic environment, while the chemicals themselves perform the heavy-duty work of dissolving the metals.

Microorganisms involved in bioleaching:

Microorganisms Involved in Heap Bioleaching

Heap leaching primarily involves two major groups of microorganisms: iron-oxidizing chemolithotrophs and sulfur-oxidizing chemolithotrophs . These microbes can fix carbon dioxide from the atmosphere and utilize it as a carbon source**[30]**. They grow autotrophically in mining environments by using ferrous iron or reduced sulfur compounds as electron donors and oxygen as the terminal electron acceptor. Many of the organisms used in metal

Microorganism	**Parameters**	**pH**	**Particle Size**
T.ferrooxidans	Particle size,pulpdensity,and Fe concentration	2.0	210-250um
-	Review	-	-
A.ferrooxidans	Energy source,initialpH,Pulp density and temperature	1.0-2.5	-74um
A.ferroxidans	Thermal pretreatment	1.5	-
-	Review	-	-
Ind.bacteria	Chemical vs biological bioleaching	4.0	-841um
Ind.bacteria	Initial pH and temperature	4.0,7.0,9.0	-74um
Ind.bacteria	Pulp density	4.4	-841um
-	Review	-	-
Ind.bacteria	Bacterial attachment	4.20	-74um
L.ferriphilum,Acidithiobacilluscaldus	Bacterial attachment	2.0	-149um
Ind.bacteria	Feasibility	3.2	-
-	Review	-	-
Ind.bacteria	Temperature	2.43	-841um
A.ferroxidans	Feasibility assessment	1.75	-
A.niger	Strain variations	3.5	-

A.niger	Manganese supplement	6.8	-74um
A.niger	Growth medium	3.5	-

*Table 3:*Microorganisms involved in bioleaching**[31]**

solubilization are thermophilic, thriving at elevated temperatures.The type of microbial community and mineral decomposition rate depend largely on temperature and pH. Microorganisms display distinct temperature preferences:Mesophiles: grow optimally at 30–35 °CModerate thermophiles: optimal range 45–55 °CExtreme thermophiles: thrive above 70 °C

Representative Bioleaching Microorganisms

One of the first acidophilic iron- and sulfur-oxidizing bacteria to be isolated was *Acidithiobacillusferrooxidans*, first by Temple and Colmer in 1951. Subsequent biomining species of significance have been documented, including *Leptospirillumferrooxidans*, *Acidithiobacillus thiooxidans*, and *Acidithiobacilluscaldus*. *Acidithiobacillus*species are rod-shaped, Gram-negative, non-spore-forming bacteria that can survive in both aerobic and anaerobic environments. Common genera are: thiooxidans,ferrooxidans,caldus, *acidophilus*,*concretivorus*,*albertis*, *prosperus* These microorganisms oxidize elemental sulfur in order to produce sulfuric acid, reducing pH levels to approximately 1.0 suitable for leaching activities. *A. ferrooxidans* can convert ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) at ~40 °C, reducing pH to 1.8–2.0 [32]. It is often considered a model organism in biomining studies. While *A. thiooxidans* is mesophilic, it is highly acid-tolerant, surviving at pH as low as 0.5 [42,Other key acidophiles include:*Ferroplasmaacidiphilum*: grows optimally at 33–45 °C, pH 1.7–1.3 .*Leptospirillumferrooxidans*: an obligate chemolithotroph and major iron oxidizer with an optimal pH of 1.5–1.8.*L. thermoferrooxidans*: thrives at ~45 °C.*L. ferriphilum* and *Sulfobacillus* spp.:moderately thermophilic, active at40–60 °C *Metallosphaerasedula*: thermophiles capable of oxidizing various minerals at 68 °C and 80–85 °C, respectively, with optimal pH ranges of ~1.3–1.7 and 1.0–4.5 thermophilic archaea (*Acidianusbrierleyi* and *A. infernus*): grow at 70–90 °C, pH 1.5–2.0

Fungal Bioleaching Agents
In fungal-assisted biomining, Aspergillus and Penicillium species are among the most effective and widely used **[51–53]**.Microbial Consortia for Enhanced BioleachingLiao et al.(2019) demonstrated that microbial consortia can significantly improve the bioleaching of low-grade sulfide ores. They proposed a staged inoculation strategy:Iron-oxidizing bacteria introduced during the initial and middle stages.Sulfur-oxidizing bacteria added towards the final stage .Cyanogenic Microbes for Precious Metal Recovery [33]
Microbial recovery of precious metals from electronic waste (e-scrap) is attracting increasing interest. Cyanide-producing (cyanogenic) bacteria such as *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Chromobacteriumviolaceum*, and *Pseudomonas plecoglossicida* have been reported to leach gold by producing cyanide ions during metabolism Thermophilic microbes and fungi also secrete organic acids (citric, lactic, gluconic, oxalic) and enzymes that contribute to metal solubilization . For example, a mixed culture of *A. thiooxidans* and *Leptospirillumferrooxidans* extracted 62% of copper from ores within 30 days [34].Bioleaching Microorganisms in heap leaching, two main types of microorganisms are usually involved:

1. Iron-oxidizing chemolithotrophs
2. Sulfur-oxidizing chemolithotrophs

These microbes can fix carbon dioxide from the air and use it for energy. They grow in mining environments by using iron or reduced sulfur compounds as electron donors and oxygen as the electron acceptor. Most of the microbes used in bioleaching are thermophilic (heat-loving).Temperature and pH Preferences. The type of microorganisms involved in mineral breakdown depends on temperature and pH:Mesophiles – grow best at 30–35 °CModerate thermophiles – grow best at 45–55 °CExtreme thermophiles – thrive at 70 °C or higherKey

Bacterial Groups

Acidithiobacillusferrooxidans Initially discovered in 1951 by Temple and *Colmer.Oxidizessulfur* and iron, reduces soluble ferrous iron to ferric iron, and decreases the pH to approximately 1.8–2.0.Rod-shaped, Gram-negative, non-spore-*former.Survives* even without oxygen (anaerobic).A model microbe used in biomining studies. *Acidithiobacillusthiooxidans* Mesophilic but with very high acid tolerance, surviving at pH levels as low as 0.5. Specializes in oxidizing sulfur to form sulfuric acid, decreasing pH to close to 1.0, enhancing leaching conditions. *Acidithiobacilluscaldus* and other *Acidithiobacillus*species Cover A. acidophilus, A. *concretivorus*, A. *albertis*, and A. *prosperus*. All have the ability to oxidize elemental sulfur and sustain a very low pH for bioleaching. *Leptospirillumferrooxidans* Strict iron oxidizer, strongly acid tolerant (pH 1.5 – 1.8). Optimal temperature at about 30–45 °C; L. *thermoferrooxidans* proliferates at 45 °C, and L. *ferriphilum* grows at 40–60 °C [35]. *Ferroplasmaacidiphilum*Thrives in the pH range 1.3–1.7 and at temperatures 33–45 °C. *Sulfolobusmetallicus* and *Metallosphaerasedula*Thermophilic archaea that can oxidize minerals at elevated temperatures: S. *metallicus:* ~68 °C, pH 1.3–1.7 M.*sedula:* 80–85 °C, pH 1.0–4.5 *Acidianusbrierleyi* and *Acidianusinfernushydrothermophilic* archaea with growth at 70–90 °C, pH 1.5–2.0. Fungal Bioleaching Agents Aspergillus and Penicillium species are strong fungal *bioleachers.*They produce organic acids like citric, oxalic, gluconic, and lactic acid, along with enzymes that help dissolve minerals.Microbial Consortia StrategyA 2019 study suggested that bioleaching works best when:Iron oxidizers are introduced during the initial and middle stages of leaching.Sulfur oxidizers are added in the final stage to maintain acidity and continue mineral breakdown.

Microbes in E-Waste Recycling

Microbes are now being used to extract precious metals like gold and copper from electronic waste (e-scrap).Cyanogenic bacteria (e.g., *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Chromobacteriumviolaceum*, *Pseudomonas plecoglossicida*) produce cyanide ions during metabolism, which dissolve gold.Mixed cultures of *A. thiooxidans* and *Leptospirillumferrooxidans* have been shown to extract 62% of copper from e-waste in 30 days.

Result:

Practical review: copper and uranium bioleaching

Copper Bioleaching and Recovery

Copper is a highly valuable metal known for its excellent thermal conductivity and ductility. It is widely used in electricity, construction, transportation, and many other industries. Because the demand for copper remains consistently high but naturalsuppliesare limited, bioleaching has become a popular and efficient way to extract copper from low-grade ores. Countries such as the United States, Australia, Canada, Mexico, South Africa, and Japan all use this method, with the U.S. alone producing about 10% of its copper through bioleaching.

Types of Copper Ores for Bioleaching

The main copper ores commonly used in bioleaching are:

Covellite(CuS),Chalcocite(Cu_2S),Chalcopyrite ($CuFeS_2$)Chalcopyrite is particularly interesting because, besides copper (around 26%), it also contains significant amounts of iron, zinc, and sulfur.

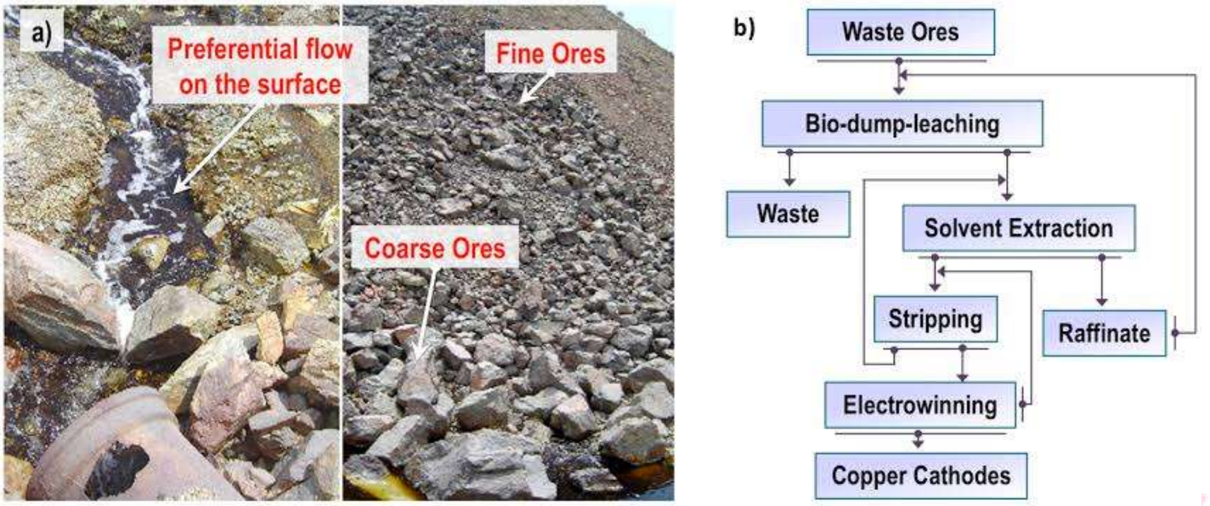

Figure 5:(a)Copperremediationusing *Thiobacillusthiooxidans*(b)Flowchart forcopper cathode production from the waste ores

How Copper Bioleaching Works

Thebacterium *Thiobacillusferrooxidans* (alsocalled *Acidithiobacillusferrooxidans*) plays a key role. This microbe oxidizes the insoluble chalcopyrite ($CuFeS_2$) into soluble copper sulfate ($CuSO_4$). Sulfuric acid (H_2SO_4) is produced as a byproduct, which keeps the environment acidic—perfect for microbial growth and activity.

The main chemical reactions involved are:

For chalcopyrite oxidation:
2CuFeS2+8.5O2+H2SO4→2CuSO4+Fe2(SO4)3+H2O2CuFeS2+8.5O2+H2SO4→2CuSO4+Fe2(SO4)3+H2O

For covellite oxidation:
CuS+2O2→CuSO4CuS+2O2→CuSO4

The bacteria act in two ways: directly oxidizing the copper sulfides, and indirectly by converting ferrous sulfide (FeS) present in many ores into ferric ions, which further dissolve the copper minerals.

Copper Recovery Techniques

Once copper sulfate is formed, copper can be recovered from the solution through:Solvent extraction, which isolates copper chemically, or
Cementation with scrap iron, where copper replaces iron according to:
CuSO4+Fe0→Cu0+FeSO4CuSO4+Fe0→Cu0+FeSO4**[36].**

Dump Leaching for Copper Recovery

For copper content less than 0.5%, smelting is not effective, and hence the recovery takes place by dump or heap leaching. For dump leaching, crushed ore is stacked more than 100 feet high on an impermeable base. Water is continuously passed over the heap. While the water percolates through the heap, pyrite (FeS_2) oxidation acidifies and supplies ferric sulfate to the surroundings, which promotes the development of *T. ferrooxidans* in the heap. The water that leaves the pile gets copper ions. It is gathered into a launder (basin), with scrap iron being added to bring about the precipitation of the copper out of the solution:

Cu2++Fe0→Cu0+Fe2+Cu2++Fe0→Cu0+Fe2+
The iron-rich solution (Fe^{2+}) is transferred to shallow oxidation ponds, where *T. ferrooxidans* oxidizes ferrous iron back to ferric iron (Fe^{3+}), regenerating sulfuric acid by oxidizing sulfur compounds. Some ferric iron precipitates out as iron hydroxide, but the acidic ferric sulfate solution is pumped back to the top of the dump to

continue the cycle.In essence, the dump functions like a continuous flow reactor where bacteria attached to ore particles steadily solubilize copper**[37].**

Modern Copper Bioleaching by Heap Percolation

Today, large-scale copper bioleaching mostly uses heap percolation. Here's how it works:The ore is crushed to about 1 cm size, treated with diluted sulfuric acid, and sometimes agglomerated into small, mechanically strong balls.These are stacked into heaps with enough space (voids) to allow oxygen and carbon dioxide to flow through.Acid solution is sprayed or dripped over the heap repeatedly. This helps dissolve the copper minerals while bacteria inside the heap grow and do their work.The copper-rich solution that percolates through the heap is collected and sent to a solvent extraction unit, which purifies and concentrates the copper.Finally, the copper is recovered from the solution by electrowinning, producing high-purity metallic copper.The entire bioleaching process can take two months or more, depending on conditions.

URANIUM BIOLEACHING:

Uranium Bioleaching

Uranium (U) bioleaching is widely practiced in countries such as Canada, the United States, and India, among others. It is an effective way to recover uranium from low-grade ores (containing only 0.01–0.5% U) and even from low-grade nuclear wastes.Since uranium is the key fuel for nuclear power generation, microbial recovery from otherwise unusable ores can help address global energy shortages. Although bioleaching cannot solve nuclear safety or waste disposal concerns, it can significantly improve the economic efficiency of nuclear power by enabling the commercial use of low-grade uranium resources and wastes.Recovering uranium from radioactive waste is especially valuable because it reduces waste disposal problems, which is one of the biggest drawbacks of nuclear energy**[38].**

How Uranium Bioleaching Works

Bacterial uranium leaching is most successful in geological formations where uranium exists in the tetravalent oxide form (UO_2), which isinsoluable and present naturally in ores.Although *Thiobacillusferrooxidans* (now *Acidithiobacillusferrooxidans*) cannot directly oxidize UO_2, it aids indirect oxidation. Here's how:Uranium ores often contain iron sulfide (FeS_2) alongside UO_2.*T. ferrooxidans* oxidizes ferrous iron (Fe^{2+}) in FeS_2 to ferric iron (Fe^{3+}).This ferric iron then chemically oxidizes UO_2 to the soluble hexavalent form — uranium sulfate (UO_2SO_4) — which can be leached out.

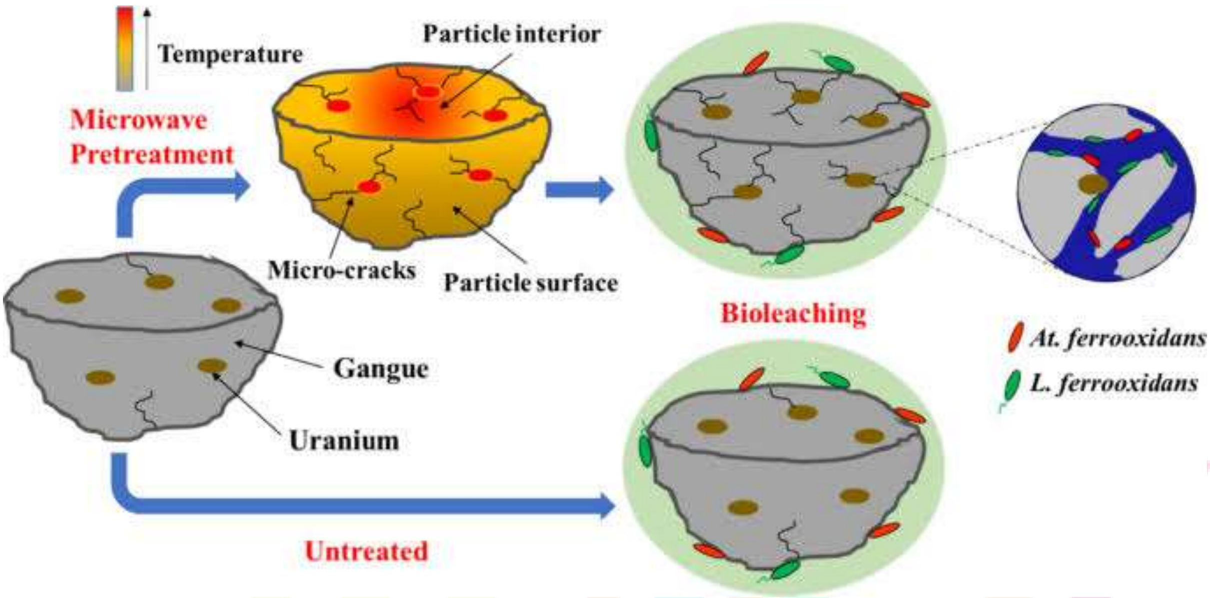

Figure 6:Processof Uraniumbioleaching

Reaction:

$UO_2 + Fe_2(SO_4)_3 + 2H_2SO_4 \rightarrow [UO_2(SO_4)_3]^{4-} + 2FeSO_4 + 4H^+$
Optimal Conditions for Uranium Extraction
Temperature: 45–50 °C
pH: 1.5–3.5
Incoming air with about 0.2% CO_2
Uranium Recovery Process
Once uranium is converted to its soluble form in the leach solution:

1. It is extracted into an organic solvent (commonly tributyl phosphate).
2. The dissolved uranium is then precipitated and purified using ion-exchange chromatography.

Using bioleaching, uranium recovery rates can range from 30% to as high as 90%.Economic and Geological Considerations. The success of uranium bioleaching depends on:The mineral composition and type of geological deposit**[39].**Whether a natural drainage system exists (such as a fault line with an impermeable basin) — this makes in situ leaching economical.However, in most cases, heap leaching produces higher uranium recovery than in situ methods.

Advantages and Applications of bioleaching:

Advantages:

Cost-effective processing of low-grade ores – particularly in heap and dump configurations, significantly reducing capital expenditure (CAPEX) and operating expenditure (OPEX) compared with conventional metallurgical processes.Operation under mild physicochemical conditions – ambient pressure and relatively low temperatures minimize energy requirements relative to pyrometallurgical smelting routes.Reduced atmospheric emissions – negligible release of sulphur dioxide (SO_2), nitrogen oxides (NO_x), and particulate matter compared to roasting or smelting.Compact plant footprint and simplified infrastructure – suitable for modular heap-leach installations and decentralized processing.Compatibility with in situ and heap-leaching methodologies – minimizes ore handling, haulage, and surface disturbance compared with full comminution–milling–smelting flowsheets.Treatment of refractory gold ores via bio-oxidation – facilitates exposure of encapsulated gold, substantially enhancing subsequent cyanidation recovery rates.Reduction in chemical oxidant requirements –

microbial metabolism continuously regenerates ferric iron (Fe^{3+}) and sulfuric acid (H_2SO_4) as effective lixiviants.Lower production of secondary hazardous wastes – and simplified downstream metal recovery compared to conventional chemical leaching systems.Reprocessing of mine tailings and beneficiated wastes – enabling recovery of residual metals while simultaneously reducing environmental hazards associated with the residues.Application in "urban mining" – facilitates metal recovery (e.g., Cu, Au, and critical elements) from secondary resources such as printed circuit boards (PCBs) and spent batteries**[40].**

Lower greenhouse gas and pollutant emissions – providing a smaller overall emissions profile than pyrometallurgical processes and contributing to industrial decarbonization objectives.Enhanced occupational safety – due to operation under non-flammable, low-temperature, low-pressure conditions.Industrial maturity and scalability – with bio-heap leaching accounting for a significant proportion of global copper production; stirred-tank bioleaching primarily reserved for high-value feedstocks.Flexibility for integration into hybrid process flowsheets – such as chemical–biological two-stage oxidation, thereby improving reaction kinetics and mineral selectivity.Process optimization potential – through control of microbial consortia composition, pH, redox potential, and aeration regimes to selectively target specific mineral phases.Reduced particulate and acoustic emissions – compared with high-temperature roasting and other intensive thermal operations.Suitability for deployment in remote or off-grid locations – owing to relatively simple mechanical and process equipment requirements.Mitigation of toxic emissions from informal e-waste recycling – providing an environmentally benign alternative to manual burning or acid-leaching practices.Contribution to circular economy objectives – by transforming waste streams and by-products into economically recoverable metal resources.Lower long-term reagent consumption – due to in situ biological regeneration of lixiviants (Fe^{3+} and H_2SO_4) sustaining leaching reactions over extended operational periods.

Applications:

Copper from chalcopyrite and other Cu-sulfides (heap/tank) — overcoming passivation, including chloride-assisted systems.Bio-oxidation pre-treatment of refractory gold ores (BIOX®-type) to unlock cyanidation or alternative lixiviants. Uranium from low-grade ores (heap and in-situ leaching variants). Zinc from sphalerite concentrates/ores. Nickel & cobalt from sulphide ores (e.g., pentlandite) via acidophilic consortia in stirred tanks/heaps. Nickel & cobalt from laterites via reductive/indirect bioleaching (e.g., using heterotrophs or spent media). In-situ bioleaching of base-metal deposits (borehole sections), with field optimization studies. Mine tailings remediation & metal recovery (Cu, Zn, As, Au pre-treatment). Removal of arsenic from highly contaminated mine tailings. Coal depyritization/desulfurization to lower SOx emissions. Metals recovery from waste printed circuit boards (WPCBs) and other e-waste via acidophiles/fungi. Critical metals (Ni, V, Mo, Co) from spent hydrodesulfurization (HDS) refinery catalysts. Li-ion battery "black mass" (Li, Co, Ni, Mn) — biological lixiviants/consortia. Rare-earth elements (REEs) from spent fluorescent-lamp phosphors. REEs (and Sc) from bauxite residue (red mud). Phosphorus recovery from sewage sludge / sludge ash via sulfur-oxidizer-generated acidity. REEs from end-of-life Nd-Fe-B magnets and other REE-bearing wastes (emerging bio-routes). Metals from municipal solid-waste incineration (MSWI) fly ash and related ashes (bio-mobilization). Indium/tin from LCD/ITO wastes via microbial leaching strategies. General heap biomining of low-grade ores at industrial scale (e.g., Escondida) — copper production via bioheaps**[41].**

Economical Section:

A thorough techno-economic evaluation of bioleaching for metal recovery from metallurgical by-products, such as basic oxygen steelmaking dust (BOS-D) and goethite, highlights the multifaceted financial dynamics underpinning this process. The analysis integrates both capital and operational expenditure, advanced scenario modeling, and robust financial indicators to capture the full economic landscape of large-scale bioleaching adoption.Capital and Operational ExpenditureCapital expenditure (CAPEX) constitutes the dominant fixed cost and encompasses procurement and installation of critical process equipment—reactors, piping, control and sensor units, electrical systems, as well as construction and site development. In a benchmark 2024 study, the

CAPEX for a modern aerated and stirred bioreactor plant (comprising twelve 450 m^3 reactors) was estimated at approximately $119.8 million. This high upfront cost is justified by the plant′s ability to process considerable annual tonnages of by-products under tightly controlled and automated conditions, with modular scalability offering operational resilience and maintenance flexibility.Operational expenditure (OPEX), calculated at $5.9 million annually for the benchmark plant, includes recurring costs such as energy (notably for aeration, mixing, and especially electrowinning—up to 3338 kWh/t), water ($2.47/$m^3$), reagents (acids, salts, and microbial growth substrates), maintenance, and personnel. Energy requirements for electrowinning are particularly influential; thus, plant location and electricity pricing can markedly affect financial outcomes. Personnel costs were doubled relative to prior studies to reflect the higher labor demand for managing twin reactor sets, reinforcing the importance of careful human resource planning**[42].**Revenue Streams and Scenario AnalysisBioleaching revenue depends on the value and extraction yield of target metals. The financial analysis typically models multiple product scenarios: (1) extraction of all recoverable elements, (2) selective recovery of high-value metals (e.g., copper, lithium, or rare earth elements), and (3) secondary revenue from selling de-zincified residues as iron resources. This diversified approach cushions against fluctuations in individual metal prices and maximizes plant utility.For example, profitable copper extraction from goethite was achieved at higher pulp densities (≥5%) in both bioreactor designs, producing a net present value (NPV) exceeding $1.27 billion and an internal rate of return (IRR) of 65% over 20 years. Notably, financial modeling predicted a payback period of just one year—demonstrating compelling investment viability. This success was contingent on high process efficiency, robust yields, favorable market pricing, and the ability to process significant throughput.

Discounting, Inflation, and Financial Modeling .To ensure robust economic assessment, the financial model incorporates a 10% discount rate and a 3.5% annual inflation rate, aligning with best practice and industrial precedent. Dynamic modeling using tools such as GoldSim supports multi-year cash flow projections, sensitivity analyses, and stress-testing of critical variables (metal price, reagent/energycost,yield variability). Literature Insights and Sustainability Considerations. Recentreviews emphasize that bioleaching′s financial feasibility is highly sensitive to fluctuations in global metal markets, local energy/water costs, and regulatory incentives for secondary resource valorization.The flexibility of multi-reactor configurations, coupled with advances in automation and process monitoring, improve operational uptime and cost control.While CAPEX remainssubstantial, OPEX optimization through yield improvements, waste minimization, and resource recycling (e.g., water, iron residue) can substantially magnify profitability and environmental sustainability The scientific literature demonstrates that, when modeled properly and executed at scale, bioleaching can be not only technically effective but also exceedingly profitable for selective metallurgical residues—especially for copper and other high-value elements. Financial success fundamentally depends on strategic plant design, judicious choice of feedstock and target metals, and process optimization for maximum yield and lowest unit costs. Forthcoming regulatory changes and sustainability imperatives will further accentuate the value proposition for bioleaching as a future-proof metallurgical pathway**[43].**

Discussion:

Future Aspects:

1. Urban Mining Revolution: Turning E-Waste Mountains into Metal Treasure Troves

Smartphone in a drawer, possibly a dead laptop collecting dust, or perhaps a mess of out-of-date cables. Take that and multiply it by billions of people across the globe, and you start to see just how big our electronic waste problem is. But here's where things get interesting—these unwanted devices are, in fact, urban mines, with greater concentrations of precious metals than some natural ore deposits. Recent breakthrough research has identified that selectively chosen microbial communities, specifically acidophilic (acid-loving) bacteria, can successfully "digest" the intricate matrix of printed circuit boards and leach valuable metals with remarkable efficiency. The star players in these researches are *Leptospirillum*-dominant cultures in collaboration with diverse *Acidithiobacillus*species—microorganisms that have been basically enlisted as biological prospectors. The figures are truly staggering: under lab conditions optimized for maximum recovery, these microbial consortia have recovered 98% copper and 82% nickel from smartphone PCBs. What's most impressive about this is that conventional pyrometallurgical treatment (essentially high-temperature smelting) of e-waste not only requires lots of energy but also produces toxic vapors and tends to lose precious metals in slag. Bioleaching, on the other hand, runs at room temperature and can be calibrated to selectively extract certain metals without touching others. But the excitement here is scalability. Scientists are currently developing modular bioreactors that would be deployable in city centers, basically establishing local metal recycling plants that convert regional streams of e-waste into useful commodity metals**[44].** Envision a world in which there is one biological refinery in each major city, recycling yesterday's technology into raw materials for tomorrow.

2. The Quest for Rare Earth Elements: Awakening Sleeping Giants in Industrial Waste

Rare earth elements—despite their name—aren't actually that rare in Earth's crust. The challenge is that they're typically dispersed in very low concentrations and are notoriously difficult to separate from each other. China now leads in the production of REE not because they have exclusive deposits, but because they've been willing to accept the environmental implications of traditional extraction and separation methods. That is where bioleaching is creating new avenues altogether. One of the most promising developments is a two-stage biological process for treating coal fly ash—the fine powder that coal-burning power stations produce. Historically viewed as waste, fly ash actually holds large amounts of REEs, but they're trapped in silicate matrices which are very hard to dissolve. Step forward *Paenibacillusmucilaginosus*, a microbe that has an almost magical talent for dissolving silicate minerals—a process termed bio-desilication. In recent studies, researchers have used this microbe as the first stage of treatment, essentially pre-conditioning the fly ash by breaking down the silicate framework that encases the REEs. Once liberated from their silicate prison, the rare earth elements become accessible to downstream leaching processes.What's particularly elegant about this approach is that it converts what was previously considered a waste disposal problem—coal fly ash—into a domestic source of critical materials. Those nations which have traditionally relied on importing REE's can perhaps attain an enhanced resource security by exploiting their own industrial waste streams. The consequences go beyond merely extraction. Emerging bioengineering technologies are under development that combine selective bioleaching with innovative separation technologies, such as designer ligand polymers that can discriminate between chemically similar rare earth elements. This might result in cleaner, more sophisticated processing streams that yield battery-grade REEs with much fewer harsh chemicals and large waste streams of the traditional variety**[45].**

3. Deep Earth Bioleaching: Mining at the Frontier of the Possible

Traditional open-pit mining is becoming increasingly problematic—not just environmentally, but economically. The shallow, high-grade deposits that built the mining industry are largely depleted, forcing companies to go deeper, process lower-grade ores, and deal with increasingly complex geology. This is where in-situ bioleaching represents a paradigm shift.Imagine being able to extract copper from ore bodies located a full kilometer underground without ever bringing the rock to the surface. Recent modeling studies and field-oriented analyses have demonstrated that this isn't science fiction—it's an emerging reality. The concept involves creating controlled underground biological reactors where engineered microbial communities can flourish at depths previously considered inaccessible to biological processes. The technical hurdles are truly formidable. Down here, you're working with high pressures, low oxygen levels, messy groundwater chemistry, and the logistical embarrassment of trying to manage biological systems in a setting that humans can hardly reach. But scientists have had to come up with advanced methods for oxidant delivery, pH control, nutrient flow management, and in-situ monitoring of microbial activity using networks of subsurface sensors.What makes this particularly compelling is the potential for dramatically reduced environmental impact. Instead of creating massive open pits that permanently alter landscapes, in-situ bioleaching could extract metals through a network of carefully placed wells, leaving the surface largely undisturbed. The process also promises to access ore bodies that would be economically unfeasible to mine through conventional methods.Field trials are already underway in several locations, with researchers carefully mapping how microbial communities adapt to deep subsurface conditions and optimizing the geochemical parameters needed to maintain stable biological activity at depth. Early results suggest that with proper management, these deep biological systems can operate continuously for years, potentially revolutionizing how we think about mineral extraction**[46].**

4. Space Bioleaching: Preparing for Humanity's Multi-Planetary Future
When NASA researchers discuss a permanent human presence on the Moon or Mars, one of the hardest questions isn't a rocket, let alone life support—it's resources. It costs too much to send things from Earth; a permanent space presence is going to need In-Situ Resource Utilization (ISRU)—basically, surviving off the planet itself. This is where experiments with the BioRock on the International Space Station have given some of the most intriguing results in recent bioleaching research. These were not ivory-tower exercises; they were practical tests of whether biological metal recovery could succeed in the harsh environment of space. The results were remarkable. Microbial communities successfully extracted rare earth elements and other valuable materials from basalt samples—a rock type that's abundant on the Moon and Mars—while operating under microgravity conditions. This proved that bioleaching isn't just theoretically possible in space; it actually works, opening the door to biological resource processing facilities on other worlds.But the implications go far beyond space exploration. The extreme conditions of space—radiation exposure, temperature fluctuations, limited resources—are forcing researchers to develop ultra-robust, self-sustaining biological systems. These advances are feeding back into terrestrial applications, leading to more resilient bioleaching processes that can operate in harsh environments on Earth.Future lunar or asteroid mining operations might rely on biological systems that can be shipped in compact, dormant forms and then activated with local resources. Picture a scenario where a small biological starter culture, launched from Earth, could be used to establish a full-scale metal processing facility on an asteroid, turning space rocks into the raw materials needed for further space exploration and development**[47].**

5. The Lithium Revolution: Powering the Energy Transition
As the planet moves quickly toward renewable power and electric cars, lithium is the oil of the 21st century. Yet classical lithium mining—especially from brines—is water-hungry, time-consuming, and regionally concentrated. Meanwhile, the piles of discarded lithium-ion batteries higher and higher by the year are both an environmental problem and an enormous untapped reservoir. A seminal 2024 paper proved that *Acidithiobacillus*bacteria was able to mobilize lithium efficiently from a range of lithium-bearing minerals, such as jadarite, spodumene, and lepidolite. This is important because these minerals contain extremely large lithium deposits that have proven hard to be processed economically conventionally. The novelty of this research is how it unites primary resource extraction with recycling of batteries. The same biological mechanisms that can release lithium from hard rock ores may be redesigned to extract lithium more gently from recycled batteries. This would result in integrated biorefinery schemes in which plants treat both new lithium ores and recycled battery components with equivalent biological strategies. The environmental benefits are convincing. The conventional lithium extraction from hard rock needs to be processed at high temperatures using strong chemicals, whereas brine extraction may require months or years and tends to drain the local water resource. Bioleaching is done at ambient temperatures with less toxic chemicals and can be a closed-loop system that consumes less water and generates little waste[48]. Initial economic studies indicate that bio-based lithium extraction can be as cost-competitive as traditional processes, especially with the inclusion of environmental costs. With ongoing demand for batteries and tightening environmental controls, biological lithium processing may be the go-to option for green supply chains.

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