

## Technical Article

# Bio-Hydrometallurgy of Electronic Waste: Extraction of Precious Metals by Cyanogenic Microorganisms and Influencing Factors

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## Abstract

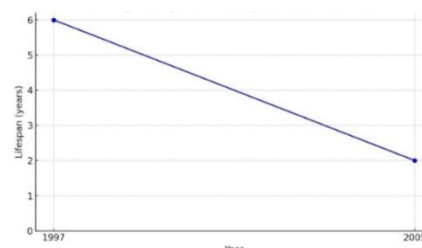
Extracting of precious metals from electronic waste (e-waste), such as waste printed circuit boards (WPCBs), contributes to environmental sustainability while offering high recovery rates and significant profitability. If improperly disposed of, E-waste contains heavy and toxic metals that can leach into groundwater and rainwater, eventually entering the human food chain. On the other hand, e-waste is also a rich source of precious metals, particularly gold (Au). One of the most effective methods for recovery from e-waste is bio-hydrometallurgy, which utilizes microorganisms to enhance recovery efficiency, ensure environmental compatibility, and reduce energy consumption. The indirect bioleaching method is employed in the case of precious metal extraction from e-waste. This process requires bacteria or fungi capable of producing cyanogenic compounds, including bacterial species such as *Chromobacterium violaceum*, *Bacillus megaterium*, *Pseudomonas fluorescens*, and *Pseudomonas plecoglossicida*, as well as fungal species such as *Marasmius oreades*, *Clitocybe* sp., and *Polysporus* sp. Several factors influence bioleaching efficiency by cyanogenic microorganisms, including pulp density, pH, waste particle size, temperature, dissolved oxygen (DO), cyanide concentration, and production rate. Among these, pulp density, pH, DO, and cyanide content are more critical than waste particle size and temperature.

**Keywords:** E-Waste Recycling, Precious Metal Extraction, Bio-Hydrometallurgy, Bioleaching.

## 1. Introduction

Electronic waste (e-waste) refers to discarded electrical and electronic devices that have reached the end of their functional lifespan [1]. With rapid advancements in technology and the continual upgrading of electronic devices, particularly computers, the volume of e-waste has been increasing over time. This rise is partly due to the decreasing lifespan of such devices. For instance, while the average lifespan of a computer was approximately six years in 1997, by 2005, it had declined to just two years (Fig. 1.) [2]. E-waste contains valuable and heavy metals [3], posing a significant environmental threat as they contaminate soil and water [3]. Therefore, e-waste recycling in an environmentally sustainable manner is crucial. Proper e-waste recycling mitigates environmental pollution and contributes to the economy, as these discarded materials contain substantial amounts of precious and heavy metals, making their recovery financially worthwhile [3]. Research indicates that e-waste contains a significantly higher concentration of precious metals than natural ores. Some studies have reported that the number of valuable metals in

e-waste can be up to ten times greater than in mined ores. For example, one ton of e-waste can contain between 10 grams and 10 kilograms of gold (Au), whereas a ton of Au ore typically contains only 0.5 to 13.5 grams of Au [4]. Cyanogenic bacteria facilitate the bioleaching of Au from electronic waste, and their efficiency can be enhanced through biological oxidation [5]. Studies have shown that a single ton of mobile phone waste contains approximately 350 grams of Au, 1380 grams of silver (Ag), 210 grams of palladium (Pd), and 130 kilograms of copper (Table. 1). In 2018, a study conducted by the United Nations University (UNU) reported that around 40 different types of metals could be extracted from discarded smartphones, with the amount of Au recovered being 25 to 30 times higher than that found in mined ores [6].



**Fig. 1.** The average Lifespan of a Computer (1997 vs 2005) [2].

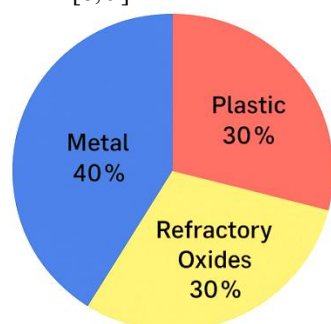
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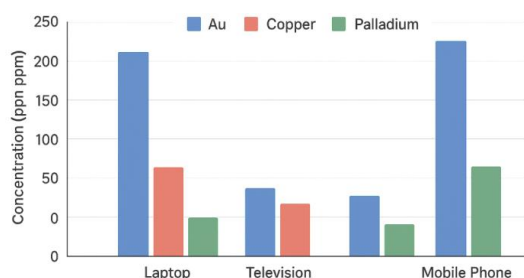
**Table. 1. Metal content in one ton of mobile phone waste [4].**

Copper (Cu)	Palladium (Pd)	Silver (Ag)	Gold (Au)
130	0.21	1.38 kg	0.35 kg

E-waste generally comprises 40% metal, 30% plastic, and 30% refractory oxides (Fig. 2.) [7]. Various metals, including base metals such as cobalt, copper, cadmium, chromium, iron, magnesium, nickel, vanadium, zinc, molybdenum, and palladium, as well as precious metals like gold, silver, platinum, and lead, can be recovered from electronic waste [8, 9].

**Fig. 2. E-waste contents [7].**

According to research conducted by the United Nations Environment Program (UNEP), more than 50 million tons of e-waste are generated globally each year, yet only 10% of it is recycled [10]. In addition to valuable and essential metals like copper and Au, e-waste contains toxic and hazardous metals such as mercury, arsenic, cadmium, selenium, and hexavalent chromium [11, 12]. These pollutants pose severe environmental risks and contribute to the depletion of the Earth's finite metal resources. The composition of printed circuit boards (PCBs) varies depending on their application. For example, laptop PCBs contain approximately 250 ppm of gold, 20% of copper, and 110 ppm of palladium. In television PCBs, copper constitutes 10%, with palladium and gold at 110 ppm and 20 ppm, respectively. Meanwhile, mobile phone PCBs contain around 350 ppm of gold, 13% of copper, and 210 ppm of palladium (Fig. 3.) [13].

**Fig. 3. The concentration of Au, Cu, and Pd in PCBs [13].**

Two common methods for recovering metals from e-waste are the hydrometallurgical and pyrometallurgical processes. However, these methods have notable drawbacks. E-waste often contains significant impurities, requiring large volumes of chemical solvents in hydrometallurgical processing, which increases costs. Moreover, some solvents with high selectivity, such as cyanide, are environmentally hazardous. On the other hand, pyrometallurgical methods require high energy consumption to heat and process these materials. Furthermore, these traditional approaches fail to achieve satisfactory metal recovery rates [14].

As a result, an alternative and more efficient technique known as bioleaching has been developed for e-waste recycling. Bioleaching is a cost-effective and high-yield approach that utilizes microorganisms to extract metals from waste materials [15]. This method is considered one of the most environmentally friendly strategies for e-waste recycling [3].

In bioleaching, instead of conventional leaching solutions, microorganisms and fungi are used to produce chemical compounds such as sulfates, cyanides, and organic or inorganic acids, which facilitate metal dissolution. This dissolution occurs selectively, allowing for targeted metal recovery [13]. Microorganisms and fungi can transform metals from their solid state into liquid [10]. It is important to note that microbial and fungal bioleaching efficiency varies based on factors such as temperature, the type and concentration of metals in the waste, pH levels, and other environmental conditions, which will be discussed further in subsequent sections [16].

In addition to preventing environmental degradation, recycling e-waste is crucial in reducing carbon footprints. The metals recovered from electronic waste contribute lower carbon emissions than traditional mining processes [17].

## 2. Recycling of E-Waste

E-waste contains significant amounts of heavy metals, which not only contribute to environmental pollution but also lead to the loss of precious metal deposits. These toxic and non-biodegradable metals contaminate soil and water when improperly disposed of. Therefore, e-waste recycling is essential from environmental and economic perspectives [3]. The rapid growth of e-waste generation has been accelerating over time [18]. It has been reported that approximately 2.5 million tons of e-waste (both domestically produced and imported) are generated annually in China [19], while global e-waste production reaches around 50 million tons [10]. The sheer volume of e-waste, even without considering its toxicity and environmental impact, poses a major challenge for proper disposal.

According to reports from the U.S. Environmental Protection Agency (EPA), only 18% of e-waste is recycled, while the remaining portion is either incinerated or disposed of in landfills [20].

E-waste landfills occupy vast amounts of land and introduce toxic metals into the soil, contaminating groundwater and rendering the surrounding areas unsuitable for agriculture. However, e-waste can also serve as a secondary source for extracting valuable and precious metals through recycling [21]. Au, known for its excellent conductivity and stability in various conditions, is widely used in the electronics industry [22]. The amount of Au in e-waste makes it a potentially valuable economic resource and a sustainable alternative to traditional Au mining. Recycling precious metals from e-waste in an environmentally friendly manner generates financial benefits and removes toxic metals from the environment [12].

One of the traditional methods for metal extraction and recycling is pyrometallurgy. This process involves heating e-waste at temperatures ranging from 400 to 700°C in an inert atmosphere [23]. While pyrometallurgy is efficient and cost-effective, it poses environmental challenges due to the emission of harmful greenhouse gases. Another approach is hydrometallurgy, which, although environmentally safer than pyrometallurgy, is not economically viable due to its high operational costs. Electronic devices contain various metals, some of which are particularly interesting for recycling. Given this diversity, the recycling process must be selective. However, hydrometallurgical methods are not inherently selective when applied to e-waste and require large amounts of chemical solvents, making them financially impractical [3]. Considering the need for economic feasibility, selective recycling, and environmental sustainability, bio-hydrometallurgy—using microorganisms to recover metals—emerges as the most cost-effective and eco-friendly alternative to conventional methods. This innovative approach represents a revolutionary advancement in e-waste recycling, offering high efficiency and sustainability [3]. Although natural bioleaching has existed on Earth for millions of years, its industrial applications have only been explored in recent decades. Since then, humans have increasingly utilized microorganisms for various metallurgical processes, leveraging their natural abilities for metal extraction. One of the most critical components of e-waste is printed circuit boards (PCBs). PCBs account for approximately 3–6% of the total weight of e-waste [24] and typically consist of 30% metallic and 70% non-metallic components [25]. Based on their structural composition, PCBs are classified into two main categories: FR-4 and FR-2. FR-4 PCBs are manufactured using epoxy resin reinforced with glass fibers, providing high thermal resistance and excellent water resistance. In contrast, FR-2 PCBs

are phenol-based polymeric materials, featuring a copper layer on a cellulose fiber or glass-reinforced substrate. FR-2 PCBs are commonly used in household electronic appliances [23], while FR-4 PCBs are preferred for more compact and sophisticated electronic devices [13].

Another classification of PCBs is based on the concentration of precious metals, dividing them into low-grade, medium-grade, and high-grade categories. As PCB performance improves with higher metal concentrations, medium- and high-grade PCBs dominate the market and are more commonly targeted for recycling [13]. Each type of PCB contains different metal concentrations. For example, laptop PCBs contain approximately 20% copper, 250 ppm Au, and 110 ppm palladium. In contrast, mobile phone PCBs have higher metal concentrations, with 13% copper, 350 ppm Au, and 210 ppm palladium. Meanwhile, low-grade PCBs, such as those in televisions, contain around 10% copper, 20 ppm Au, and 110 ppm palladium [21].

As these values indicate, all PCBs contain trace amounts of Au, with variations depending on the type of board. Consequently, PCBs have garnered significant attention as a primary target for bio-recycling of Au [23].

### 3. The Best Way of Recovery from WPCBs

As discussed in the previous section, pyrometallurgical and hydrometallurgical treatment methods can recover some metals despite their drawbacks, such as high energy consumption in pyrometallurgy [13], although bio-hydrometallurgical methods significantly improve the efficiency of metal recovery [14]. Bio-hydrometallurgy utilizes microorganisms that can naturally convert metals from a solid form into a dissolved state [14]. This process selectively dissolves metals based on their electrochemical properties, separating them from other materials [3]. Microorganisms produce specific chemical compounds that act as partial solvents, facilitating selective metal dissolution. According to Ilias et al. [26], instead of using conventional leaching agents, bio-mining techniques leverage microbial-produced chemicals such as sulfate ions, cyanides, and organic or inorganic acids for metal extraction. The primary difference between hydrometallurgy and bio-hydrometallurgy lies in the source of the leaching solution. Pre-prepared chemical solutions are used in hydrometallurgy, whereas in bio-hydrometallurgy, microorganisms naturally generate these solvents. The efficiency of these microbes varies depending on environmental conditions. For optimal performance, the selected organisms must be highly adaptable to electronic waste, and their population should be carefully regulated to prevent depletion [13].

Bio-hydrometallurgy is primarily used for recovering precious metals like Au, with cyanide being a well-known solvent for Au extraction. Due to its toxicity and the harmful byproducts associated with conventional cyanide production, microbial-based cyanide generation presents an environmentally friendly alternative [4].

Over the past decade, bio-hydrometallurgy has emerged as one of the most promising methods for recycling electronic waste [12]. The dissolution of Au by cyanide, produced by microorganisms, involves an anodic and a cathodic reaction [12]. Heterotrophic microorganisms produce cyanide through oxidative processes, which is utilized to recover Au from printed circuit boards (PCBs). Cyanide in static conditions can also dissolve certain metals [13, 27]. Sulfuric acid is one of the primary inorganic acids observed in microbial solutions. It is secreted by sulfur-oxidizing microorganisms such as *Thiobacillus* species, which play a crucial role in metal leaching [28].

Although “microorganisms” is frequently used in bio-hydrometallurgical contexts, fungi also play an essential role in this process. Some of the commonly used fungi include *Penicillium simplicissimum*, *Penicillium chrysogenum*, and *Aspergillus niger*, among others [29, 30].

Specialized equipment is required for bio-hydrometallurgical processes to achieve high efficiency. This includes stainless steel and rubber-coated vessels that resist acidic environments. Additionally, metal reactions with acidic solutions generate gases that must be carefully managed to ensure process safety [31].

#### 4. Bio-Hydrometallurgy and Bioleaching

Bio-hydrometallurgy is implemented industrially through two main techniques (Fig. 4): (a) bioleaching and (b) oxidation [15]. Bioleaching involves microorganisms that dissolve target metals such as copper, nickel, and gold, generating a solution rich in metal ions, which can then be extracted using various recovery methods [32]. In contrast, oxidation functions inversely to bioleaching, where microorganisms separate unwanted mineral compounds from the solid matrix, dissolving them into a solution. This process results in a mixture of undesired cations and the target metal, which can be isolated [1].

Although bio-hydrometallurgical methods are predominantly used for extracting Cu, Ni, Au, and Ag—especially from electronic waste—their applications extend to other metals, metalloids, and non-sulfide ores [15]. Advantages of Bioleaching include being Eco-friendly and safe, processing an abundance of naturally occurring microorganisms, no secondary pollution, such as dust or gas emissions, low capital investment requirements, reduced energy consumption, lower operational

costs, simple technological implementation, suitability for low-grade ores and waste materials, and facilitates waste recycling [3]. Types of Bioleaching include indirect bioleaching, direct bioleaching, and complex bioleaching [23].

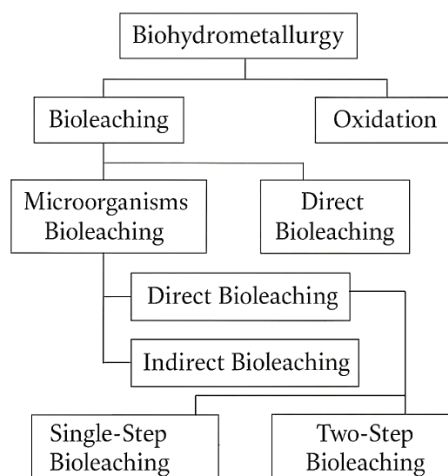


Fig. 4. The Bio-hydrometallurgy process [15].

##### 4.1. Indirect Bioleaching

Indirect bioleaching involves a two-step process where bacteria are cultured separately from the waste material. These bacteria produce a leaching solution that selectively dissolves metals. This prepared solution is then applied to the electronic waste to extract metals [33]. This method is particularly effective for recovering precious metals, such as Au, from toxic waste materials like certain PCBs, where direct microbial growth is not feasible. Thus, the culture medium and reaction environment remain separate [23].

##### 4.2. Direct Bioleaching

Direct bioleaching can be classified into single-step bioleaching and two-step bioleaching. In this approach, microorganisms interact directly with the waste material. The key difference between single-step and two-step methods lies in the bacterial environment [23].

###### 4.2.1. Single-Step Direct Bioleaching

In single-step bioleaching, microorganisms grow in a nutrient medium, and once they reach the exponential growth phase, bioleaching begins [34]. However, heavy metals in electronic waste can hinder microbial growth, leading to lower reaction rates and population decline. Challenges of this method include:

- Inhibited microbial growth due to metal toxicity

- Longer adaptation periods are required for microorganisms to tolerate metal ions, delaying leaching
  - Adsorption of metal cations onto microbial cells, decreasing metal recovery efficiency [35].
- Due to these challenges, the two-step method is often preferred.

#### 4.2.2. Two-Step Direct Bioleaching

To overcome the limitations of the single-step method, two-step bioleaching involves cultivating microorganisms separately from the electronic waste. This separation minimizes toxicity and allows a larger microbial population to develop faster [34]. Once microbial growth peaks, the culture is introduced into the leaching environment [36].

This method is more efficient than single-step bioleaching [37] and is particularly advantageous for large-scale waste processing [23]. Generally, direct bioleaching is considered superior to indirect bioleaching due to its lower pH, which enhances metal dissolution [38].

However, direct bioleaching is ineffective in certain cases, such as extracting copper from PCBs, and acid supplementation may improve indirect bioleaching efficiency [13].

#### 4.3. Fungal Bioleaching

In addition to bacteria, fungi have also been utilized in bioleaching for metal recovery. Prominent fungal species studied in this field include *Penicillium simplicissimum*, *Penicillium chrysogenum*, and *Aspergillus niger* [29, 30].

While fungi offer certain advantages in bioleaching, they also present limitations such as greater sensitivity to nutrient availability, higher production costs, and slower processing times. However, fungi also exhibit unique benefits such as ability to grow in higher pH environments, enhanced performance on alkaline materials, accelerated bioleaching rates, and increased acid secretion [39].

Fungal acids act similarly to organic acids, facilitating metal extraction by exchanging hydrogen ions with metal cations. This reduces heavy metal toxicity and enhances microbial growth and leaching efficiency [40].

According to Watling et al. [41], *Sulphobacillus* species exhibit lower metal tolerance than other microorganisms. In high-metal-concentration environments, fungi serve as a viable alternative. Similarly, Plumb et al. [42] explored bioleaching using *Acidithiobacillus* and found it effective in conditions unsuitable for highly thermophilic microorganisms.

This fungus thrives at pH levels of 3–3.5 and temperatures between 45°C and 83°C, making it

highly effective in leaching sulfide minerals such as pyrite, arsenopyrite, and chalcopyrite [43].

Among inorganic acids used in bioleaching, sulfuric acid—secreted by sulfur-oxidizing microorganisms like *Acidithiobacillus* species—is a key agent [44].

### 5. Factors Influencing Bioleaching

Various parameters in bio-hydrometallurgy significantly impact the efficiency of bioleaching. Modifying these factors can either enhance or inhibit the process, and in some cases, completely halt it. Extensive experimental research has identified these factors as critical to optimizing bioleaching performance.

The primary variables affecting precious metals extraction include pulp density (solid-to-liquid ratio), pH, particle size of e-waste, temperature, dissolved oxygen (DO), and cyanide production rate. These parameters influence both biological aspects, such as bacterial growth and survival, and chemical reactions, with some affecting both simultaneously [45].

#### 5.1. PH Level

pH is a vital factor in bioleaching's biological and chemical aspects. Heterotrophic bacteria thrive in alkaline conditions, whereas autotrophic bacteria operate effectively in acidic environments [13].

A 2010 study observed increased pulp density also elevates pH levels [46]. However, higher pulp density does not necessarily enhance bioleaching efficiency despite this rise in pH. An overly dense solution increases toxicity, leading to bacterial mortality, which counteracts any potential benefits of an elevated pH.

The interaction between Au and cyanide generates hydroxide ions ( $\text{OH}^-$ ), further elevating pH [47]. Multiple studies have shown that the optimal pH range for maximum Au bioleaching efficiency with heterotrophic bacteria is between 10 and 11.

#### 5.2. Waste Particle Size

The particle size of electronic waste directly influences the bioleaching process. Smaller particles provide a larger surface area for microbial interaction, which enhances the leaching rate. However, excessive fragmentation can increase particle collisions, causing mechanical stress that may damage bacterial cells [10].

Maintaining an optimal particle size is crucial to balancing bioleaching efficiency while minimizing bacterial damage. Li et al. [48] confirmed that bioleaching improves with reduced particle size due to increased surface exposure [47].

However, compared to other parameters, particle size has a relatively lower impact on overall efficiency [35].

### 5.3. Pulp Density

Pulp density, representing the solid-to-liquid ratio in the leaching solution, determines the proportion of electronic waste in the bioleaching system [49]. During extraction, toxic metals in the waste can negatively impact bacterial viability.

Increasing pulp density reduces microbial populations and suppresses their oxidation activity [50].

Waste printed circuit boards (WPCBs) containing Au also house metals like Cu, Ni, Fe, and Zn [51]. These metals exhibit a higher affinity for cyanide, binding to it and reducing its availability for Au dissolution [49]. Research by Li et al. [49] identified 0.33 (v/v) WPCBs as the optimal pulp density for Au extraction using *Pseudomonas fluorescens*. Furthermore, Shin et al. demonstrated a linear correlation between pulp density and bioleaching efficiency, where higher pulp density results in lower extraction rates [40].

### 5.4. Temperature

Temperature significantly affects bioleaching efficiency, depending on the type of microorganism involved. While increasing temperature generally enhances the process, each microbial species has a specific tolerance range. Exceeding this threshold can lead to bacterial inactivation or death [52].

- Mesophilic bacteria function optimally at 25–30 °C.
- Thermophilic bacteria operate effectively at 40–45 °C [13].
- Fungi generally exhibit higher temperature tolerance compared to bacteria.

Most bioleaching reactions occur within a temperature range of 20–60 °C, with cyanide-producing bacteria typically operating at the lower end of this spectrum. Examples include:

- *Chromo-bacterium violaceum*: optimal growth and leaching at 35–37 °C [53].
- *Pseudomonas fluorescens*: active between 25–30 °C [54].
- *Pseudomonas plecoglossicida*: functions at 20 °C [55].

### 5.5. Dissolved Oxygen (DO)

Dissolved oxygen (DO) is a crucial factor in bioleaching, as the aerobic bacteria used in the process require oxygen for survival and metabolism. Additionally, oxygen plays a key role in the cyanide—Au reaction [47]. To prevent oxygen depletion, which could inhibit bioleaching, DO levels must be maintained using incubator shakers or by adding H<sub>2</sub>O<sub>2</sub> [56].

Oxygen supply in incubator shakers is regulated by rotational speed (rpm):

- Low rpm results in inadequate oxygen levels.
  - Excessively high rpm generates turbulence, leading to particle-bacteria collisions that can damage microbial cells [13].
- Biolchini et al. [57] determined that an optimal rotational speed between 120 and 145 rpm enhances bioleaching efficiency. Adding 0.04% H<sub>2</sub>O<sub>2</sub> has improved the Au bioleaching rate, increasing recovery from 10.8% to 11.31% at pH 11 [58]. A major factor contributing to DO depletion is copper's tendency to react with cyanide. Since Cu binds to cyanide, it indirectly reduces available oxygen, negatively impacting Au dissolution. To counteract this effect, copper levels should be minimized before initiating bioleaching [58].

### 5.6. Cyanide Production

Cyanide plays a fundamental role in the extraction of precious metals, and certain bacteria, such as *Chromo-bacterium violaceum* and *Pseudomonas fluorescens*, are capable of cyanide biosynthesis [59]. Cyanide production by *C. violaceum* highly depends on environmental conditions [47].

The key factors influencing cyanide synthesis include:

- PH
- Temperature
- Dissolved oxygen (DO)
- Glycine concentration

A sufficient glycine concentration is essential for cyanide production [47]. However, excessive glycine levels can inhibit bacterial growth [13]. Additionally, cyanide synthesis requires a pH above 10 for optimal performance [12].

Since cyanide production raises pH levels, it can negatively impact other bacterial populations. To prevent this, cyanide biosynthesis is typically conducted as a separate stage in a two-step bioleaching process [12]. Adding specific salts, such as NaCl and MgSO<sub>4</sub>•7H<sub>2</sub>O, at low concentrations has been found to act as catalysts, enhancing *C. violaceum*'s cyanide production [47].

### 6. Conclusion

Bio-hydrometallurgical precious metals extraction from e-waste demonstrates significantly higher efficiency than pyrometallurgical and hydrometallurgical methods. The primary motivations for recycling e-waste include the high gold content in these materials and the prevention of environmental contamination by heavy metals leaching into groundwater and soil. Bioleaching, which utilizes biological agents to dissolve target metals into solution, is applied indirectly for gold extraction. Since electronic waste contains heavy metals that can inhibit microbial growth, the microorganisms are first cultivated separately and

introduced into the leaching solution during their exponential growth phase. These microbes, including *Chromo-bacterium violaceum*, *Bacillus megaterium*, *Pseudomonas fluorescens*, and *Pseudomonas plecoglossicida*, play a critical role by producing cyanide, which facilitates gold dissolution through an electrochemical reaction. Maintaining optimal conditions is essential for maximizing bioleaching efficiency. A high pH, preferably between 10 and 11, is required for these cyanide-producing bacteria to thrive. The process is typically conducted at temperatures ranging from 20 to 60 °C.

Additionally, pulp density must be carefully regulated, as an excessive solid-to-liquid ratio can be lethal to bacteria, while a lower density prolongs the process and reduces yield. The optimal pulp density has been reported as 0.33 (v/v). Dissolved oxygen (DO) is another critical factor, as it directly influences cyanide reactions with gold. Oxygen depletion halts the bioleaching process entirely. Therefore, maintaining sufficient DO levels is necessary to ensure continuous and efficient gold recovery.

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