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Review

Bioleaching of Metals from E-Waste Using Microorganisms: A Review

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Abstract: The rapid and improper disposal of electronic waste (e-waste) has become an issue of great concern, resulting in serious threats to the environment and public health. In addition, e-waste is heterogenous in nature, consisting of a variety of valuable metals in large quantities, hence the need for the development of a promising technology to ameliorate environmental hazards associated with the indiscriminate dumping of e-waste, and for the recovery of metal components present in waste materials, thus promoting e-waste management and reuse. Various physico-chemical techniques including hydrometallurgy and pyrometallurgy have been employed in the past for the mobilization of metals from e-waste. However, these approaches have proven to be inept due to high operational costs linked to the consumption of huge amounts of chemicals and energy, together with high metal loss and the release of secondary byproducts. An alternative method to avert the above-mentioned limitations is the adoption of microorganisms (bioleaching) as an efficient, cost-effective, eco-friendly, and sustainable technology for the solubilization of metals from e-waste. Metal recovery from e-waste is influenced by microbiological, physico-chemical, and mineralogical parameters. This review, therefore, provides insights into strategies or pathways used by microorganisms for the recovery of metals from e-waste.

Keywords: microorganisms; e-waste; bioleaching; bioprocess parameters; metals; biorecovery

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1. Introduction

The development of electronic and electrical industries and the widespread use of electronic technologies result in the production of more and new electronic devices [1,2]. When these products reach their service life, a huge amount of electronic waste (e-waste) is accumulated. It was estimated that more than 50 million metric tons of e-waste was generated in 2019 with a projection of 74 million metric tons in 2030 [3]. However, so far, about 9.3 metric tons of e-waste has been collected and valorized, representing 17.4% of the overall amount of the e-waste generated [4–6]. In South Africa, based on United Nation's global e-waste surveillance report, about 360,000 tons of e-waste is generated yearly, and the Gauteng province is responsible for about 55% of it, since it is the economic hub of the country [7]. E-waste is categorized into subgroups depending on the metallic composition, type, origin, age, manufacturer, and constituents' parts. The United Nations categorized e-waste into six groups, namely temperature exchange equipment (e.g., refrigerators), screens and monitors, lamps, large equipment (e.g., large household appliances), small equipment (e.g., toasters and tools), and small information technology/telecommunication equipment (e.g., cellular phones and routers) [8] (Figure 1). However, printed circuit boards (PCBs) are indispensable in many devices, where they connect different electronic components. PCBs could be one-sided, double-, or multi-layer-sided, based on their configuration and alignment [9]. Each year, 0.5 Mt of PCBs are generated, and the global PCB production increases by 4% on average. Similarly, central processing units in computers contain a relatively high proportion of precious metals [2].

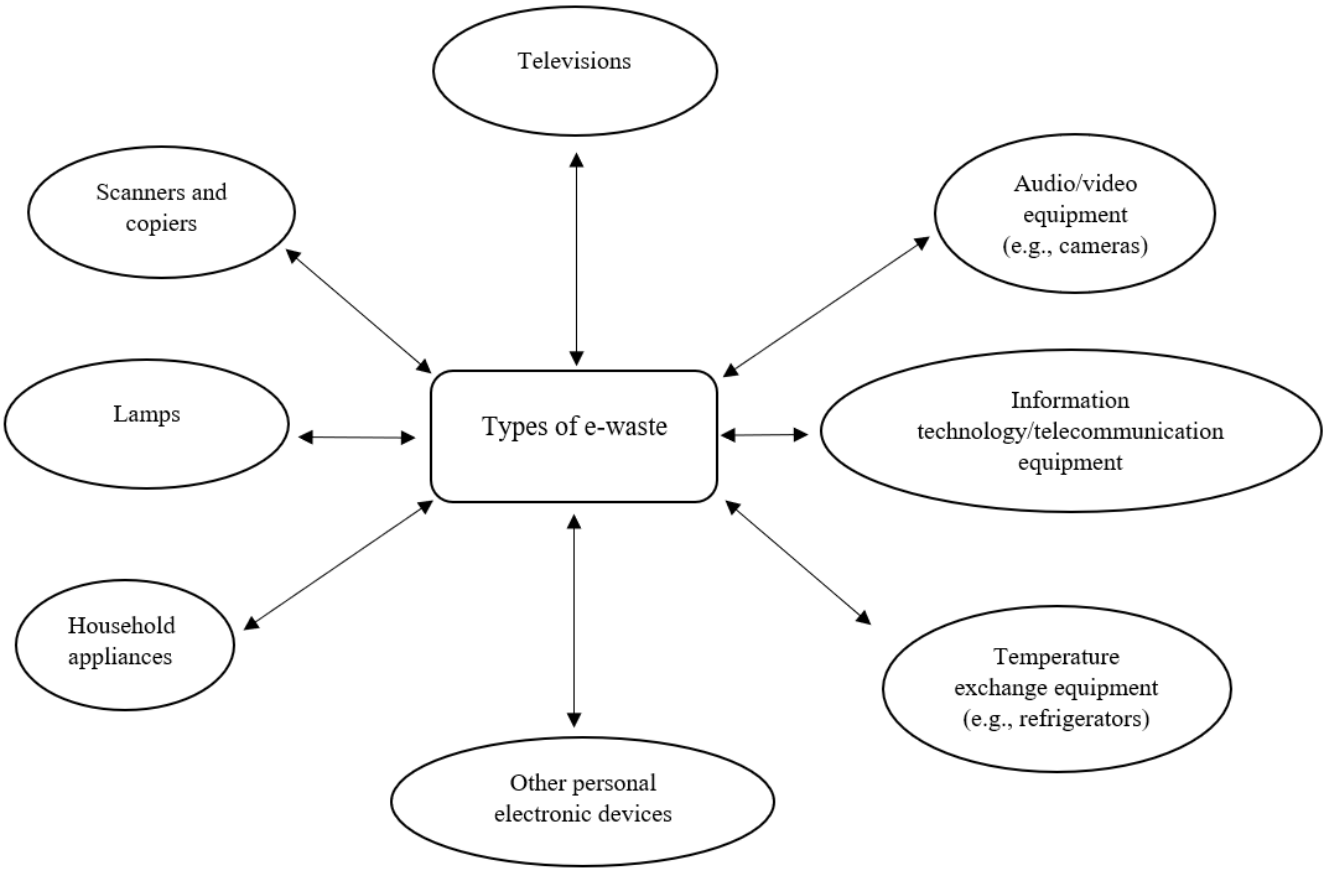

Figure 1. Various types of e-waste.

The composition of e-waste is very heterogeneous and includes a variety of hazardous and/or non-hazardous substances including polymers, glass fiber, flame retardants, and ferrous and non-ferrous metals, which, if improperly managed, can be toxic to humans and the environment [10,11]. In addition, e-waste contains several precious metals (such as Au, Ag, and Pt group metals), base metals (Al, Co, Cu, Ni, Zn, and Fe), rare earth metals (e.g., In, Nd, and Ta), and others (e.g., Be, Cd, Cr, Hg, Pb, Sb, Sn, and Ti) in larger quantities than those in some ores [12,13]. These metals are used in various applications such as electronics, computers, automotive, jewelry, dentistry, and aviation industries owing to their superb electrical conductivity and chemical resistance. According to some statistics, there is about 10–1000 g gold/ton of e-waste, found to be 17 times when compared with mineral ore [14,15]. Metal recovery from e-waste (secondary source) supports the conservation of primary resources (ore) and prevent environmental degradation while contributing to transition to a circular economy. E-waste has been treated as a secondary ore in urban mining due to the presence of precious metals of which the concentrations are higher than that of a primary ore. As a result, the development of a new technology for the recovery of metals from e-waste is urgently needed to improve the added value of the waste materials. Various methods, including mechanical separation, pyrometallurgical, and hydrometallurgical processes, are employed for the removal of metals from e-waste [16–18]. However, these techniques are characterized by environmental hazards, high operational costs, high metals loss, and the release of secondary by-products, as well as the consumption of huge amounts of energy and chemicals [19–21]. An alternative approach to ameliorate the afore-mentioned challenges is the use of microorganisms to mobilize the metals from e-waste [22].

Bio-hydrometallurgy (also known as bioleaching) is referred to as a green and sustainable technology that utilizes the metabolic activities of microorganisms (fungi, bacteria, archaea) for the solubilization of metals from low-grade ores, mineral concentrates, or

e-waste [1,6,23] (Figure 2). In other words, it is a process of the conversion of non-soluble metallic compounds present in the solid matrix into soluble form under the influence of microorganisms [2,6]. It is considered a simple, effective, inexpensive, and eco-friendly method with less energy requirements [20,24]. Furthermore, bioleaching generates less secondary waste and permits the ease of metal recovery during the recovery process. However, the longevity of leaching time and low metal yields are crucial factors that hamper the large-scale application of bioleaching. Microbes secrete certain metabolites (lixiviants), which aid the solubilization and mobilization of insoluble metals from e-waste. The leaching efficiency is determined mainly by the physiology of the microorganisms and the mineralogical contents of e-waste [25]. Therefore, the present review focuses on the mechanisms employed by microorganisms for the recovery of metals from e-waste. In addition, bioprocess parameters influencing the efficacy of metal bioleaching coupled with techniques used for the commercial mobilization of metals from e-waste is discussed extensively.

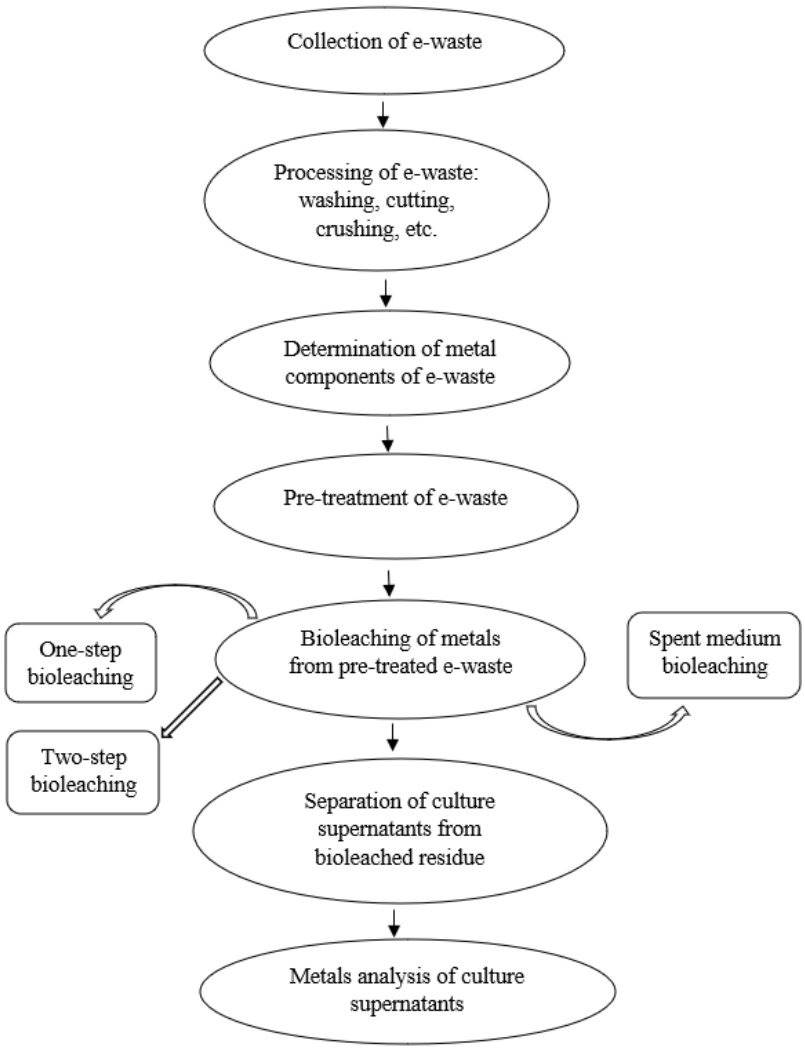

Figure 2. Illustrative diagrams showing procedures for the bioleaching of metals from e-waste.

2. Environmental and Health Effects of E-Waste

An improper disposal of e-waste in landfills or other dumpsites poses serious threats to the environment and public health [26]. E-waste consists of an array of toxigenic organic and inorganic pollutants (such as polybrominated diphenyl ethers, polychlorinated biphenyls, brominated flame retardants, dioxins, heavy metals, etc.), which are hazardous upon exposure to ecosystems, flora and fauna. These substances are released directly or indirectly in form of heavy metals, acids, and lethal chemicals. Direct exposure occurs

via the inhalation of fine and coarse particles, skin contact with hazardous substances or the ingestion of contaminated dust [27]. Human contact with e-waste renders certain effects by increasing in concentration as it travels up the food chain, leading to various health challenges such as DNA damage, birth defects, cardiac failure, cancer, skin dermatitis, etc. [27,28]. Furthermore, an improper disposal of e-waste causes the release of dust particles or toxins (such as dioxins) into the environment, resulting in air pollution and damage to the respiratory system [29]. In addition, the incineration of e-waste emits toxic fumes and gases, which pollutes the surrounding air. Hazardous substances present in e-waste leak through the soil and further contaminate surface and underground water, leading to the acidification and toxification of water. This kills marine and freshwater organisms, disturbs biodiversity, harms ecosystems, as well as disrupts the ecological set-up of soil [6,27].

3. Bioleaching of Metals from E-Waste

Microorganisms play a crucial role in the extraction of metals from e-waste, a technology known as bioleaching [30]. Microorganisms that are involved in the bioleaching of metals from e-waste include iron and sulfur-oxidizing bacteria, cyanogenic bacteria, and fungi [31,32]. Generally, three groups of microbes, including chemolithotrophic bacteria, heterotrophic bacteria, and fungi are known as microbial candidates for the recovery of metals from e-waste. These microorganisms and their leaching efficiencies are discussed in detail below.

3.1. Bioleaching by Chemolithotrophic Microorganisms

These microbes are also known as iron and sulfur-oxidizing bacteria or acidophilic bacteria. They are involved in the oxidation of ferrous ion to ferric ion, or elemental sulfur to sulfuric acid during metal bioleaching ((Equations (1) and (2)) [33–35]. The biogenic ferric iron and sulfuric acid serve as oxidizing agents (lixiviants) for the mobilization of base metals from the solid matrix via acidolysis and redoxolysis bioleaching mechanisms (Equations (3)–(5)) [36].

$$2FeSO_4 + H_2SO_4 + 1/2\ O_2 \rightarrow Fe_2(SO_4)_3 + H_2O \quad (1)$$

$$S^0 + H_2O + 3/2O_2 \rightarrow H_2SO_4 \quad (2)$$

$$H_2SO_4 + MeS \rightarrow H_2S + MeSO_4 \quad (3)$$

$$H_2SO_4 + MeO \rightarrow H_2O + MeSO_4 \quad (4)$$

$$Fe_2(SO_4)_3 + MeS + H_2O + 3/2O_2 \rightarrow Me^{2+} + {SO_4}^{2-} + 2FeSO_4 + H_2SO_4 \quad (5)$$

In addition, the microbes consume atmospheric CO_2 as a carbon source, and inorganic compounds such as ferrous ion (Fe^{2+}), elemental sulfur, and/or reduced sulfur compounds (polysulfide, H_2S, ${S_2O_3}^{2-}$, and S_8) as an energy source. This group of microorganisms is unique among other classes of microbes most frequently studied due to their potential to facilitate metal dissolution from e-waste via a series of bio-oxidation and bioleaching reactions [37–39].

Based on their desired temperature, chemolithotrophic bacteria could be mesophilic (28–37 °C), moderately thermophilic (40–60 °C), and extremely thermophilic (60–80 °C). Examples include *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, *Leptospirillum ferriphilum*, *Sulfobacillus thermosulfidooxidans*, *Thermoplasma acidophilum*, etc. [9,40]. Most of these microbes can tolerate lower pH values and higher concentrations of metals such as silver, uranium, and molybdenum [41,42]. However, members of the genus *Acidithiobacillus* are prominent and well-studied organisms for the bioleaching of metals due to their outstanding tolerance to heavy metal toxicity and low nutrient requirements for metal solubilization [43,44] (Table 1). For instance, Arshadi and Yaghmaei [45] inves-

tigated the potential of a pure culture of *Acidithiobacillus ferrooxidans* in the mobilization of metals from discarded PCBs collected from Pars Charkhesh Asia, which is a recycling unit in Tehran, Iran. Maximum recovery efficiencies of copper (90%) and nickel (88%) were recorded. The bioleaching of waste PCB sludge using *Acidithiobacillus ferrooxidans* resulted in the recovery of copper (76%), nickel (74%), and zinc (72%) at optimal conditions of $FeSO_4 \cdot 7H_2O$ (60 g/L), initial pH 0.5, and an incubation period of 6 d [46]. Kadivar et al. [47] extracted metals from discarded mobile phone PCBs in the presence of *Acidithiobacillus thiooxidans*. The bioleaching process resulted in 98% (copper) and 82% (nickel) at 30 °C (160 rpm) within 72 h. Arshadi et al. [48] recorded 100% copper, 100% iron, and 54% nickel during the biorecovery of metals from electronic waste combination at optimal conditions of pulp density (15 g/L), inoculum size (10%, *v*/*v*), temperature (30 °C), and agitation speed (130 rpm). In addition, *Acidithiobacillus thiooxidans* and *Acidiphilum acidophilum* achieved 75% (copper) and 100% (copper), respectively, from the bioleaching of computer PCBs within 9 d and 2.5 h, respectively [49,50].

Table 1. Efficiencies of some chemolithotrophic bacteria (pure culture) in the bioleaching of metals from e-waste.

Bacteria	Metal Source	Recovery Efficiency (%)	Bioleaching Conditions	Reference
Acidithiobacillus ferrooxidans	PCB	Cu (80%)	pH 2.0; temperature 30 °C; incubation time 2d	[51]
Acidithiobacillus thiooxidans	Spent mobile phone PCB	Cu (98%); Ni (82%)	160 rpm; 30 °C; 72 h	[47]
Acidithiobacillus ferrooxidans	OLED touch screens of mobile phone	In (100%); Sr (5%)	Initial pH 1.1; 29 °C; 140 rpm; 15 d	[52]
Thiobacillus ferrooxidans	Nickel–cadmium batteries	Cd (100%); Ni (96.5%); Fe (95%)	pH 2.0; temperature 30 °C; incubation time 93 d; pulp density 0.2 g/L	[53]
Acidithiobacillus ferrooxidans	Nickel–cadmium batteries	Cd (100%)	pH 1.5; temperature 30 °C; incubation time 28 d	[54]
Acidithiobacillus ferrooxidans	Mobile phone PCB	Cu (99%); Ni (99%)	170 rpm; temperature 30 °C; initial pH 1.0; pulp density 9.25 g/L; Fe^{3+} concentration 4.17 g/L; incubation time 55 d	[55]
Acidithiobacillus ferrooxidans	Nickel ion batteries	Co (65%)	pH 2.5; temperature 30 °C; incubation time 20 d; pulp density 5 g/L	[56]
Acidithiobacillus thiooxidans	Computer PCBs	Cu (75%)	Pulp density 0.7%; 9 d	[49]
Acidithiobacillus ferrooxidans	Nickel ion batteries	Co (99.9%)	pH 3.0; temperature 35 °C; incubation time 6 d	[57]
Acidithiobacillus ferrooxidans	Mobile phone PCB	Cu (95.92%); 93.53% (Al); 92.58% (Zn); 65.27% (Ni); 95.33% (Sn)	Temperature 20–35 °C; waste PCB concentration 5%; inoculation volume 5% (*v*/*v*)	[58]
Acidiphilium acidophilum	Computer PCBs	100% (Cu)	Incubation time 10 d; H_2O_2 concentration 30%	[50]
Acidithiobacillus ferrooxidans	Mobile phone PCB	95%–100% (Cu)	Incubation temperature 30 °C; 130 rpm; pulp density 7.5 g/L; 48 h	[59]
Acidithiobacillus ferrooxidans	Computer PCBs	92% (Cu)	pH 1.8; pulp density 35 g/L; 30 °C; 170 rpm	[19]

The utilization of mixed cultures or a consortium of chemolithotrophic organisms yields better results in comparison to a single microbial species [39] (Table 2). Arslan [60] used *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* for the extraction of metals from discarded PCBs while investigating the effect of bioprocess parameters on the bioleaching process. Peak recovery efficiencies of 94% (copper), 89% (nickel), 88% (zinc), and 59% (aluminum) were recorded at optimum conditions of 10% (*v*/*v*) inoculum volume, 10% pulp density, 10 d leaching time, 1.8 initial pH, 30 ± 2 °C initial temperature, 125 µm particle size, and 180 rpm agitation speed. In addition, a cocktail of *Leptospirillum ferriphilum*

and *Sulfobacillus thermosulfidooxidans* was employed for the bioleaching of copper from waste PCBs [1]. About 94% of copper was recovered from 100 g/L PCB concentrates in 9 d. Ghassa et al. [61] achieved recovery efficiencies of 99.7% (nickel), 99.9% (copper), and 84% (lithium) during the bioleaching of metals from lithium-ion batteries in the presence of a consortium of the *Ferroplasma* sp., *Sulfobacillus* sp., *Leptospirillum ferriphilum*, and *Acidithiobacillus caldus* at 45 °C (130 rpm). Mixed cultures of adapted acidophilic bacteria consisting of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* were inoculated into a growth medium under the conditions of $FeSO_4$ (36.7 g/L), sulfur (5 g/L), pH 1.5, particle size (<75 µm), and pulp density (40 g/L) during the bioleaching of metals from spent lithium-ion laptop batteries [62]. Maximum recovery efficiencies of 89.4% (nickel), 99.2% (lithium), and 50.4% (cobalt) were recorded. Similarly, improved bioleaching of metals from waste PCBs of computers by *Acidithiobacillus ferrooxidans* and *Acidiphilium acidophilum* at a pulp density of 7.5 g/L within 18 d has been reported [63]. Maximum leaching efficiencies of 96% (copper), 94.5% (zinc), 75% (nickel), and 74.5% (lead) were recorded.

Table 2. Efficiencies of some chemolithotrophic bacteria (mixed culture/consortium) in the bioleaching of metals from e-waste.

Bacteria	Metal Source	Recovery Efficiency (%)	Bioleaching Conditions	Reference
Acidithiobacillus ferrooxidans, *Leptospirillum ferriphilum*, *Acidithiobacillus caldus*, *Acidithiobacillus thiooxidans*, *Sulfobacillus* sp., and *Ferroplasma* sp.	Cell phone PCB	98%–99% (Cu)	Pulp density (7%, 10%, and 15% *w*/*v*); incubation time 12 d	[64]
Acidithiobacillus ferrooxidans and *Acidithiobacillus acidophilum*	Waste PCB	96% (Cu); 94.5% (Zn); 75% (Ni); 74.5% (Pb)	Pulp density 7.5 g/L; pH 2.5; 170 rpm; 30 °C; 18 d	[63]
Acidithiobacillus ferrooxidans and *Acidithiobacillus thiooxidans*	Waste PCB	Cu (86%); Zn (100%); Ni (100%)	Pulp density 15 g/L; 180 rpm; 30 °C; 25 d	[65]
Acidithiobacillus ferrooxidans, *Ferroplasma acidiphilum*, and *Leptospirillum ferriphilum*	Desktop computer motherboards	80.5% (Cu)	Pulp density 5%; 170 rpm; 45 °C; pH 1.6; Fe^{3+} concentration 9 g/L; graphite 2.5 g/L; 5 d	[66]
Acidithiobacillus ferrooxidans, *Leptospirillum ferrooxidans*, and *Acidithiobacillus thiooxidans*	Mobile phone PCB	97.3% (Cu); 55.8% (Al); 79.3% (Ni); 66.8% (Zn)	Fe^{3+} concentration 9 g/L; pulp density 10%; inoculum volume 10% (*v*/*v*); pH 1.8	[67]
Acidithiobacillus caldus, *Leptospirillum ferriphilum*, *Sulfobacillus* sp., and *Ferroplasma* sp.	Lithium-ion batteries	Co (99.9%); Ni (99.7%); Li (84%)	45 °C; 130 rpm	[61]
Acidithiobacillus ferrooxidans and *Acidithiobacillus thiooxidans*	Spent lithium-ion laptop batteries	89.4% (Ni); Co (50.4%); Li (99.2%)	$FeSO_4$ 36.7 g/L; Sulfur 5 g/L; pH 1.5; particle size < 75 µm; 40 g/L pulp density	[62]

3.2. Bioleaching by Heterotrophic Microorganisms

Heterotrophic microbes (bacteria and fungi) consume organic carbon sources and water as electron and energy sources [6]. In addition, this group of organisms can tolerate a broader pH range and complex metals, making them employable in the treatment of moderate alkaline waste materials [39].

3.2.1. Bioleaching by Heterotrophic Cyanogenic Bacteria

Heterotrophic bacteria produce by-products that facilitate the mobilization of metals from e-waste. This is typical of cyanogenic bacterium that secretes hydrogen cyanide (HCN) as a secondary metabolite to mobilize precious metals from e-waste, a phenomenon known as cyanogenesis [22]. Under alkaline conditions, cyanogenic organisms synthesize cyanide in the presence of glycine (precursor) between the exponential and stationary phase via oxidative decarboxylation. This reaction is catalyzed by HCN synthase. The metabolite dissolves precious metals from e-waste through the formation of a soluble metal–cyanide complex, which facilitates the extraction of metals [68]. However, low yields of cyanide by the organisms and toxicity of e-waste to the cyanogenic bacteria affect the mass recovery

of metals from waste materials [2]. Cyanide production by microorganisms is influenced by temperature, pH, dissolved oxygen concentration, and glycine [69]. The dissolution of precious metals by cyanide consists of anodic (6) and cathodic (7) reactions, which are summarized in Equation (8) illustrated below:

$$4Au + 8CN^- \rightarrow 4Au\,(CN)_2{}^- + 4e^- \quad (6)$$

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad (7)$$

$$4Au + 8CN^- + O_2 + 2H_2O_4 \rightarrow Au\,(CN)_2{}^- + 4OH^- \quad (8)$$

Cyanogenic microbes are efficiently employed to leach valuable metals and metalloids such as gold, silver, platinum, palladium, and titanium from e-waste. Prior to the bioleaching of precious metals from e-waste using heterotrophic organisms, base metals are recovered from waste materials, since they are available at high concentrations and form complexes with the metabolites, and thus reduce the mobilization of precious metals [36]. Cyanide-producing bacteria include *Chromobacterium violaceum*, *Bacillus megaterium*, and the *Pseudomonas* sp.; however, *Chromobacterium violaceum* is the most widely studied, followed by the *Pseudomonas* species (Table 3). Li et al. [25] reported a 70.6% bioleaching efficiency of gold from a pretreated waste PCB using *Chromobacterium violaceum* ATCC 12471. Chi et al. [70] studied the bioleaching of metals from waste mobile PCBs in the presence of *Chromobacterium violaceum*. The bioleaching experiment was conducted at 30 °C (150 rpm) at a pH range of 8.0–11.0 when 15 g/L pulp density was used. The recovery of gold and copper from the solid matrix was enhanced from 7.78% to 10.8%, and 4.9% to 11.4% as the pH increased from 8.0 to 11.0, and 8.0 to 10.0, respectively, in 8 d. In addition, indigenous *Pseudomonas balearica* SAE1 isolated from an e-waste recycling facility in India was employed for the bioleaching of precious metals from waste PCBs [20]. The results obtained showed recovery efficiencies of 68.5% (gold) and 33.8% (silver) at the optimum temperature of 30 °C, pH 9.0, pulp density 10 g/L, and glycine concentration 5 g/L. Marra et al. [71] recorded a recovery efficiency of 48% (gold) from e-waste shredding dust in the presence of *Pseudomonas putida* WSC361 at the optimized conditions of pH 7.2-8.4; glycine concentration 10 g/L; pulp density 10 g/L; 150 rpm, and 3 h. Mutated *Pseudomonas fluorescens* CICC21620 was able to leach 54% gold from waste PCB under the optimized conditions of pH 9.0, bacterial density 3.33% (*v*/*v*), pulp density 0.33% (*w*/*v*), glycine 0.133 mol/L, and a glycine–methionine ratio of 1:10 [21]. Motaghed et al. [72] investigated the extraction of platinum and rhenium from a spent refinery catalyst using *Bacillus megaterium* PTCC 1656. Maximum recoveries of 15.7% (platinum) and 98% (rhenium) were recorded at an optimum glycine concentration and pulp density of 12.8 g/L and 4% (*w*/*v*), respectively.

Table 3. Efficiencies of some heterotrophic bacteria (pure culture) in the bioleaching of metals from e-waste.

Bacteria	Metal Source	Recovery Efficiency (%)	Bioleaching Conditions	Reference
Pseudomonas chlororaphilis	Waste PCBs	8.2% (Au); 12.1% (Ag); 52.3% (Cu)	pH 7.0; 25 °C; glycine 4.4 g/L; +methionine 2 g/L; 72 h; 60 rpm	[73]
Chromobacterium violaceum	Electronic scrap materials	30% (Au)	Pulp density 0.5%; 30 °C; 170 rpm; 8 d	[74]
Pseudomonas putida	Waste PCBs	44% (Au)	10 g/L glycine; pulp density 1%; pH 7.3–8.6; 30 °C; 2 d	[36]
Chromobacterium violaceum	Waste mobile phone PCBs	24.6% (Cu); 11.31% (Au)	H_2O_2 0.004% (*v*/*v*); pH 8.0–11.0; 150 rpm; 30 °C; pulp density 15 g/L; 8 d	[70]
Bacillus foraminis	AMOLED display of smartphones	100% (Ag); 56.8% (Mo); 41.4% (Cu)	Incubation time 12 d; 160 rpm; 40 °C	[75]
Pseudomonas biofilm	Waste computer PCBs	14.7% (Ag)	Pulp density 2%; pH 7.0; 25 °C; 7 d	[76]
Bacillus megaterium	Computer PCBs	63.8% (Au)	Pulp density 2 g/L; pH 10.0	[55]

Table 3. *Cont.*

Bacteria	Metal Source	Recovery Efficiency (%)	Bioleaching Conditions	Reference
Pseudomonas fluorescens	Waste PCBs	54% (Au)	pH 9.0; bacterial density 3.33% (*v*/*v*); pulp density 0.33%; glycine 0.133; glycine: methionine ratio 1:10	[21]
Chromobacterium violaceum	Waste PCBs	70.6% and 52.4% (Au)	$MgSO_4 \cdot 7H_2O$ 4×10^{-3} mol/L; NaCl 1.7×10^{-1} mol/L; particle size 200 mesh; 7 d	[25]
Bacillus megaterium PTCC 1656	Spent catalyst	15.7% (Pt); 98% (Re)	Glycine 12.8 g/L; pulp density 4%; 30 °C; 170 rpm; 7 d	[72]
Pseudomonas balearica SAE1	Waste PCBs	68.5% (Au); 33.8% (Ag)	pH 9.0; pulp density 10 g/L; 30 °C; glycine 5 g/L	[20]
Chromobacterium violaceum	Mobile PCBs	11% (Au)	pH 11.0; pulp density 15 g/L; glycine 5 g/L; $MgSO_4$ 0.5 g/L; 8 d; 150 rpm	[77]
Chromobacterium violaceum MTCC 2656	SIM card waste	13.79% (Cu); 0.44% (Au); 2.55% (Ag)	pH 9.0; glycine 5 g/L; pulp density 10 g/L; 150 rpm; 30 °C; 7 d	[78]
Pseudomonas plecoglossicida	PCB	68.5% (Au)	pH 7.3; glycine 5 g/L; 150 rpm; 3 d	[79]
Pseudomonas putida	PCB	44% (Au)	pH 7.3–8.6; pulp density 5 g/L; glycine 10 g/L; 150 rpm; 2 d	[36]
Pseudomonas chlororaphilis	PCBs	8.2% (Au)		

The cocktail of cyanogenic bacteria enhances bioleaching efficiency. This is notable in a study conducted by Pradhan and Kumar [80] for the bioleaching of metals from personal computer e-waste, using a mixture of *Chromobacterium violaceum* and *Pseudomonas fluorescens* when the culture medium (pH 7.2) was incubated at 30 °C (150 rpm). Recovery efficiencies of 83% (copper), 8% (silver), 13% (iron), 73% (gold), and 49% (zinc) were achieved. Zhou et al. [81] assessed the efficacy of two cyanide-producing bacterial strains (*Pseudomonas putida* and *Bacillus megaterium*) that were co-cultured for the mobilization of precious metals from waste PCBs. Maximum gold mobilization of 83.59% was recorded at pH 10.0, pulp density 5 g/L, and leaching time of 34 h. Gold bioleaching from e-waste by *Chromobacterium violaceum* and *Pseudomonas fluorescens* at pulp densities of 2 and 4% (*w*/*v*) resulted in a recovery efficiency of 8% [82].

3.2.2. Bioleaching by Heterotrophic Fungi

This group of fungi secrete huge amounts of organic acids (such as carboxylic acid, gluconic acid, citric acid, and lactic acid), which induce the mobilization of metals from e-waste [83]. This is achieved through the reaction of organic acids with metals through acidolysis, redoxolysis, bioaccumulation, chelate, and complex formation mechanisms [84,85]. In addition, metal solubilization by fungi may also be due to the reduction in highly oxidized metal compounds in the presence of certain biodegradative extracellular enzymes [12]. The ability of fungi to adapt and tolerate environmental stress (e.g., metal pollution) makes them a better candidate in mineral bioleaching. Fungal genera such as Penicillium, Aspergillus, Trichoderma, Candida, Saccharomyces, and Phanerochaete are well documented for the solubilization of metals from e-waste due to the possession of bioconversion reactions in their metabolic machinery [6,32,85] (Table 4). Cui et al. [86] recorded 100% recovery efficiency of Indium from waste LCD panels when *Aspergillus niger* was inoculated into an optimized growth medium (pH 7.0) at 30 °C (200 rpm) in the presence of 1% (*w*/*v*) indium tin oxide for 15 d. Arshadi et al. [48] investigated the bioleaching of metals from PCBs in the presence of *Penicillium simplicissimum*. The results obtained showed the recovery of 90% (copper) and 89% (nickel) at pH 7.0 and spore concentration of 3.3×10^7 (copper) and pH 2.0, 30 °C, 150 rpm and spore concentration of 10^6 (nickel), respectively. Liu et al. [87] reported a 60.96% leaching rate of copper from waste PCBs by *Phanerochaete chrysosporium* at pH 5.0, pulp density of 2%, and 30 °C (150 rpm) for 14 d. In another study by Bahaloo-Horeh et al. [88], *Aspergillus niger* was employed for the

bioleaching of metals from spent lithium-ion mobile phone batteries. Maximum leaching capacities of 100% (lithium), 72% (manganese), 45% (nickel), 38% (cobalt), 94% (copper), and 62% (aluminum) were recorded at 30 °C for 30 d. Patel and Lakshmi [89] investigated the ability of *Aspergillus fumigatus* A2DS in the mobilization of metals from mobile phone PCBs. Maximum leaching efficiencies were obtained at the respective optimum conditions: pulp density of 0.5% (42.37% nickel and 62% copper), inoculum volume 1% (32.29% nickel and 58% copper), pH 6.0 (32% nickel and 58.7% copper), and incubation temperature of 40 °C (27.07% nickel and 61.8% copper). Netpae and Suckley [90] evaluated the efficacy of *Rhizopus oligosporus* and *Aspergillus niger* in the bioleaching of metals from PCB scrap collected from an e-waste recycling outlet. The best leaching efficiency was recorded by *Aspergillus niger* (30.63% lead and 46.92% copper) when compared to *Rhizopus oligosporus* (19.61% lead and 8.53% copper). A highly efficient recovery of 98% for gold (III) ion from an aqua regia leachate of a central processing unit was achieved by *Saccharomyces cerevisiae* at 5.0×10^{14} cells/m^3 within 10 min [91].

Table 4. Efficiencies of some heterotrophic fungi (pure culture) in the bioleaching of metals from e-waste.

Fungi	Metal Source	Recovery Efficiency (%)	Bioleaching Conditions	Reference
Aspergillus niger	Waste PCBs	100% (Zn); 80.39% (Ni); 85.88% (Cu)	Pulp density (0.5–20 g/L); 120 rpm; ambient temperature; 30 d	[92]
Trichoderma viride	Computer PCBs	1% (Pd); 10% (Au)	pH 5.0; 30 d; 1 g PCB	[93]
Penicillium simplicissimum	Cell phone PCBs	90% (Cu); 89% (Ni)	Cu: pH 7.0; 3.3×10^7 spores; sugarNi: pH 2.0; 10^6 spores; molasses	[48]
Phanerochaete chrysosporium	Waste PCBs	60.96% (Cu)	pH 5.0; pulp density 2%; 30 °C; 150 rpm; 14 d	[87]
Aspergillus niger	Spent lithium-ion mobile phone batteries	100% (Li); 72% (Mn); 45% (Ni); 38% (Co); 94% (Cu); 62% (Al)	30 °C; 30 d	[88]
Candida orthopsilosis	Cell phone PCBs	1% (Cu)	pH 4.4; 0.5 g PCB; 35 d	[93]
Aspergillus tubingensis	Computer PCBs	34% (Cu); 54% (Zn); 8% (Ni)	pH 5.0; pulp density 0.25%–1%; 33 d	[94]
Aspergillus fumigatus A2DS	Mobile phone PCB	42.37%, 32.29%, 27.07% (Ni); 62%, 58%, 61.8% (Cu)	Pulp density 0.5%; inoculum volume 1% (*v*/*v*); pH 6.0; 40 °C	[89]
Saccharomyces cerevisiae	PCB scrap	98% (Au)	5.0×10^{14} cells; 10 min	[91]
Trichoderma atroviride	Computer PCB	1% (Pd); 13% (Au)	pH 5.0; 1 g PCB; 30 d	[93]

A consortium of heterotrophic fungi enhances organic acid secretion for the mass mobilization of metals from e-waste [85]. Madrigal-Arias et al. [95] used a cocktail of *Aspergillus niger* MXPE6 and *Aspergillus niger* MX7 for the recovery of metals from cellphone PCBs. A maximum leaching efficacy of 87% (gold) was observed at a leaching time of 14 d. The biorecovery of metals from Ni–Cd batteries using a consortium of *Aspergillus niger*, *Aspergillus tubingensis*, *Aspergillus versicolor*, *Aspergillus japonicus*, *Aspergillus fumigatus*, and *Aspergillus flavipes* was reported [96]. Peak leaching efficiencies of 80% (nickel), 80% (cadmium), 80% (zinc), and 90% (cobalt) were obtained at a pulp density of 1 g/L, pH 5.0, 27 °C, 150 rpm, and particle size < 200 µm. Brandl et al. [97] recovered 65% (copper), >95% (zinc), >95% (lead), and >95% (nickel) from e-waste in the presence of *Aspergillus niger* and *Penicillium simplicissimum* at a pulp density of 1%–10% (*w*/*v*), pH 6.0, 150 rpm, and 30 °C. Similarly, a cocktail of *Aspergillus niger* and *Aspergillus tubingensis* has been employed for the recovery of 52% (cobalt), 95% (lithium), 95% (manganese), 77% (aluminum), and 72% (nickel) from cellphone batteries at a pulp density of 10 g/L, 30 °C, and 140 rpm [98].

4. Mechanisms of the Bioleaching of Metals from E-Waste

The bio-extraction of metals from solid waste materials differs among a broad range of microorganisms. However, it is governed by the following mechanisms of action in most organisms, including bacteria and fungi:

4.1. Acidolysis

Acidolysis occurs when the oxygen atoms present in a metal oxide are protonated, thereby resulting in the mobilization of metals from a solid matrix. The oxygen atoms interact with water molecules to facilitate the recovery of metals from its source into a bioleaching medium [6,99]. Organic acids such as gluconic acid, formic acid, pyruvic acid, etc., aid the protonation of oxygen atoms. In fungi, organic acid is essential for the maintenance of a low pH required for an enhanced bioleaching process [85]. In addition, acid reduces the availability of anions to cations in a metallic compound reaction, thus enhancing the solubility of metal ions from waste materials. This mechanism is rapid and prevalent among heterotrophic bacteria and fungi, and has been used for the extraction of metals such as lead, zinc, nickel, and copper [100]. For instance, Amiri et al. [101] reported the significance of organic acids from *Aspergillus niger* in the detachment of molybdenum, nickel, and aluminum from industrial waste. An increase in the secretion of itaconic acid and oxalic acid by *Aspergillus niveus* resulted in an enhanced recovery of zinc (75.7%), nickel (73.6%), and copper (80.3%) from waste PCB in 15 d [102]. In addition, Liao et al. [103] reported that ascorbic acid from *Acidithiobacillus caldus* and *Sulfobacillus thermosulfidooxidans* improved the bioleaching of copper (94%) and lithium (95%) from Li-ion batteries at a pulp density of 20 g/L.

4.2. Redoxolysis

Redoxolysis is an oxidation–reduction approach that permits the conversion of insoluble metals to corresponding soluble metallic form. This mechanism is common among chemolithotrophic microbes such as *Leptospirillum ferrooxidans*, *Acidithiobacillus ferrooxidans*, and *Acidithiobacillus thiooxidans*, among others [104]. During the bioconversion process, Fe^{3+} serves as an oxidizing agent and undergoes reduction to Fe^{2+}, followed by a subsequent oxidation to Fe^{3+} for metal mobilization. Various metals such as lead, zinc, nickel, copper, etc., are recovered from e-waste using this mechanism [100].

4.3. Complexolysis

Complexolysis is also known as a chelation mechanism. The target metals from a solid matrix form a complex with ligands, resulting in the formation of cyanides, organic acids, or siderophores, which then facilitate the mobilization of metals from waste materials. Complexolysis occurs upon acidolysis and is used for the extraction of metals such as silver, gold, iron, platinum, and palladium [100,105]. The secretion of iron-chelating compounds by microbes serves as a solubilizing agent for the recovery of Fe^{3+}. In addition, fungal organic acids produce protons and boost the complexing capacity during metal mobilization [85]. This mechanism is found among heterotrophic bacteria and fungi, including *Chromobacterium violaceum*, *Pseudomonas fluorescens*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Aspergillus niger*, etc. [12].

4.4. Biosorption

Biosorption is a metabolically independent accumulation of metals by microorganisms from the bioleaching medium [85]. It is an emerging physico-chemical adsorption technique that utilizes certain types of inactive, dead, or viable microbial biomass for the removal of metals from e-waste through complexation, chelation, coordination, and ion-exchange between the metals [12,39]. The microbial cell wall consists of many functional groups, including a carbonyl, phosphodiester, sulfonate, and phosphate group, that are needed for sequestering metals present in e-waste [106,107]. Biomass of several fungal, bacterial, and algal species has been used as a biosorbent for the recovery of heavy metals, precious metals, and rare earth metals from e-waste [12]. Microorganisms are considered better metal-absorbing agents due to their rapid growth under controlled conditions, adaptability toward environmental conditions, and large fungal hypha surface-area–volume ratio [108,109]. Di Piazza et al. [110] reported approximately 390 ppm lanthanum and 1520 ppm terbium following the biosorption of metals from waste electri-

cal and electronic equipment by *Penicillium expansum*. A cell-free culture supernatant of *Leptospirillum ferriphilum* and *Sulfobacillus thermosulfidooxidans* was used for the bioleaching of metals from PCBs (5 g/L). Maximum (100%) copper recovery efficiency was recorded in 2 h [1].

4.5. Bioaccumulation

It involves the uptake of metals from a bioleaching medium through the outer membranes of microorganisms [32]. In this microbe–metal interaction, soluble metals are mobilized intracellularly via the cell membrane, leading to the accumulation of metal ions in the cell vacuole [85]. The presence of certain functional moieties such as phosphate, amine, carboxyl, hydroxyl, etc., on the cell wall facilitates the solubilization of metals from a solid matrix. The metal ions serve as a cation exchanger and bind to the functional groups. Metal ion solubilization by fungal mycelium is accomplished through active bio-synthetic reactions and passive adsorption [85,99].

5. Strategies for the Biorecovery of Metals from E-Waste

The recovery of metals from e-waste in the presence of microorganisms is achieved with the strategies described below.

5.1. One-Step Bioleaching

In a one-step bioleaching technique, a freshly grown microbial culture is inoculated into a sterile bioleaching medium, along with the e-waste in shake flasks under aseptic conditions [2] (Figure 3). The bioleaching process is carried out at optimum bioprocess conditions suitable for the growth of the organisms, secretion of biolixiviants, and biorecovery of metals from waste materials. However, the toxicity of e-waste is a great challenge for the biorecovery process, as this affects the growth of the organisms, resulting in low yields of metabolites, with a consequential effect on metal recovery efficiency [9]. One-step bioleaching has been employed by various researchers for the mobilization of metals from e-waste [55,70]. For instance, Jujun et al. [73] investigated the one-step bioleaching efficacy of *Pseudomonas chlororaphilis* for the extraction of copper, gold, and silver from cellphone PCBs at optimum bioprocess conditions: glycine concentration 4.4 g/L, methionine 2 g/L, 25 °C, and pH 7.0. Leaching efficiencies of 52% (copper), 8% (gold), and 12% (silver) were obtained. Trivedi and Hait [111] employed single-step bioleaching for the recovery of 54% (copper) and 89% (zinc) from waste PCBs using *Aspergillus niger* at a pulp density of 2.5 g/L, pH 5.0–9.0, 30 °C, and 170 rpm. In addition, Arshadi et al. [112] recovered 72% (copper) and 3% (gold) by one-step bioleaching in the presence of *Bacillus megaterium* at optimized conditions of 10 g/L glycine concentration, pH 10.0, and pulp density of 0.8% (*w*/*v*). Garg et al. [113] achieved maximum (100%) copper recovery efficiency from cellphone PCBs within 6–8 d during a one-step bioleaching experiment in the presence of *Leptospirillum* sp. Khatri et al. [114] recorded 89% (copper) recovery efficiency within 10 d from a combination of cellphone and computer PCBs via one-step bioleaching in the presence of *Acidithiobacillus thiooxidans* at an optimum pulp density of 1% (*w*/*v*) and pH 1.0–1.6.

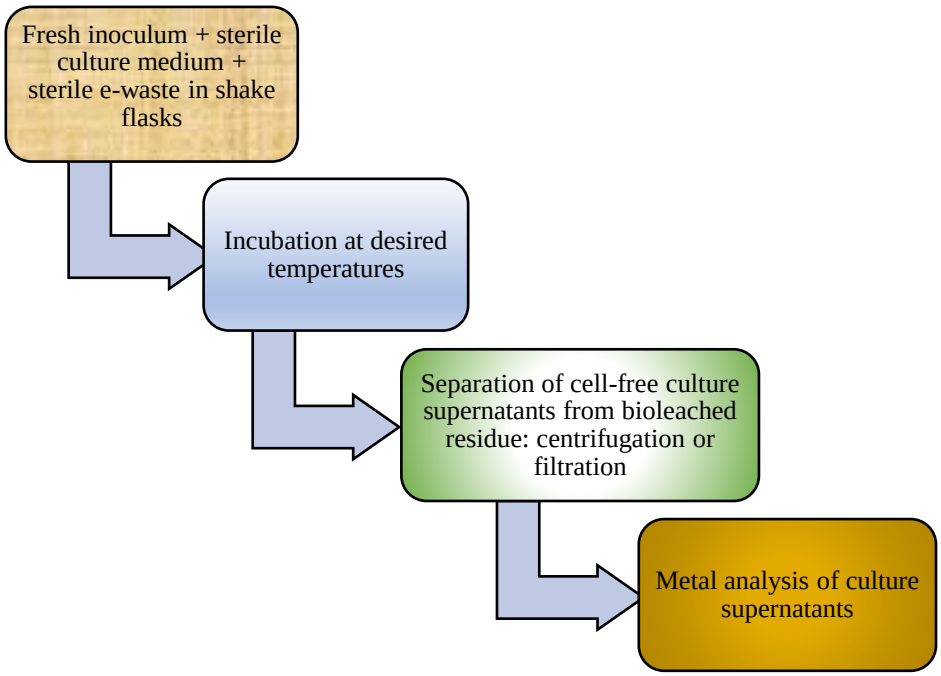

Figure 3. Schematic diagrams showing procedures for the one-step bioleaching of metals from e-waste.

5.2. Two-Step Bioleaching

In this approach, microbes are cultivated aseptically without e-waste, in shake flasks containing a fresh culture medium at optimum and appropriate bioprocess conditions until the organism attained maximum cell growth and biolixiviant production [36]. Thereafter, sterilized e-waste at a desired particle size and pulp density is added into the culture medium as a second step, followed by further incubation for a particular period [115] (Figure 4). This technique lessens the inhibitory effects of e-waste on microbial growth and metabolite secretion, thereby enhancing the metal leaching capacity of the organisms [116]. In addition, it is rapid and can be conducted at a high pulp density. The employability of two-step bioleaching as a preferred method for metal recovery from e-waste has been reported by several co-workers [25,117]. Mary and Meenambal [118] used a two-step bioleaching technique for the recovery of copper and lead from waste PCBs using *Penicillium chrysogenum* at pH 3.0, pulp density 1% (*w*/*v*), 25 °C, and 160 rpm. Maximum leaching efficiencies of 60% and 82%, respectively, were obtained. Işildar et al. [36] employed two-step bioleaching for the mobilization of 44% (gold) from discarded PCBs in the presence of *Pseudomonas putida* WCS361 at 1% pulp density, glycine concentration 10 g/L, pH 7.3–8.6, and 30 °C for 2 d. In addition, a metabolically engineered *Chromobacterium violaceum* pBAD strain was used for the recovery of 30% (gold) from electronic scrap materials using a two-step bioleaching approach at 0.5% pulp density, 30 °C, and170 rpm for 8 d [74]. Shah et al. [119] investigated two-step bioleaching for an enhanced recovery of metals from computer PCBs using *Leptospirillum ferriphilum*. Maximum recovery efficiencies of 85.26% (copper), 97.75% (zinc), and 93.22% (nickel) were recorded within an 8 d reaction time. The two-step bioleaching of copper from waste PCB resulted in 99%–100% recovery efficiency in the presence of *Leptosprillum ferriphillum* [59]. Becci et al. [120] investigated the potential of *Acidithiobacillus ferrooxidans* for the recovery of metals from computer PCBs using a two-step bioleaching approach at the optimum conditions of 30 °C, PCB concentration 5% (*w*/*v*), and Fe^{2+} 10 g/L. Maximum recovery efficiencies of 94% (copper) and 70% (zinc) were recorded with a leaching time of 9 d.

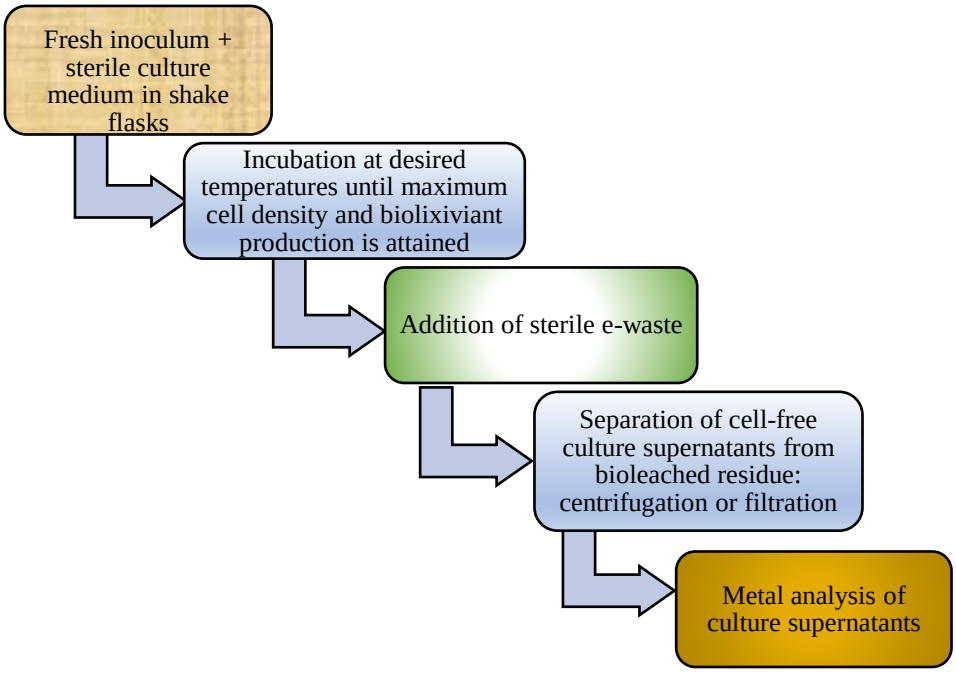

Figure 4. Schematic diagrams showing procedures for the two-step bioleaching of metals from e-waste.

5.3. Spent Medium Bioleaching

In this method, an organism is grown in a culture medium in shake flasks in the absence of e-waste, until it attains peak cell density and biolixiviant production. Thereafter, e-waste is added into a cell-free culture supernatant obtained via centrifugation or filtration of the cultured broth, and is subjected to further incubation [116] (Figure 5). The metabolites present in the supernatant facilitate the recovery of metals in e-waste. Unlike in single-step and two-step bioleaching, in this technique, the organisms are not in close contact with the e-waste. Spent medium bioleaching has been recognized as the most effective approach, as it allows for higher amounts of e-waste (i.e., higher pulp densities) at a higher pH, without any consideration of the toxic effect of waste materials on the microbes [121]. More so, it eliminates side reaction between the organisms and metabolites [2]. Natarajan and Ting [68] reported the efficacy of spent medium bioleaching in comparison to two-step bioleaching for the mobilization of gold (18%) from e-waste by *Chromobacterium violaceum* at 0.5% pulp density, 30 °C, and170 rpm for 8 d. A shift in the pH of the spent medium favored cyanide ion production, resulting in enhanced (30%) gold recovery. This was found to be greater than the 11.3% gold leaching efficiency obtained in the two-step bioleaching experiment. Similarly, Das et al. [122] confirmed the effectiveness of spent medium bioleaching for the mobilization of gold (30%) and copper (95.7%) from urban solid waste in comparison to two-step bioleaching at a pulp density of 0.5% and pH 10.0 by *Chromobacterium violaceum*. In addition, Wu et al. [1] employed *Leptospirillum ferriphilum* and *Sulfobacillus thermosulfidooxidans* culture supernatants as a preferred approach to eliminate the toxic effects of e-waste during metal bioleaching. The results obtained showed a maximum copper leaching capacity (93.4%) from PCBs (100 g/L) in 9 d. The spent medium bioleaching of rare earth elements (REEs) from waste materials (spent fluid catalytic cracking catalyst) was carried out using culture supernatants from *Gluconobacter oxydans*. Maximum leaching efficiency (49% of total REEs) was recorded, with preferential recovery of lanthanum over cerium. The leaching efficiency increased following an enhanced secretion of gluconic acid by the bacterial strain [121].

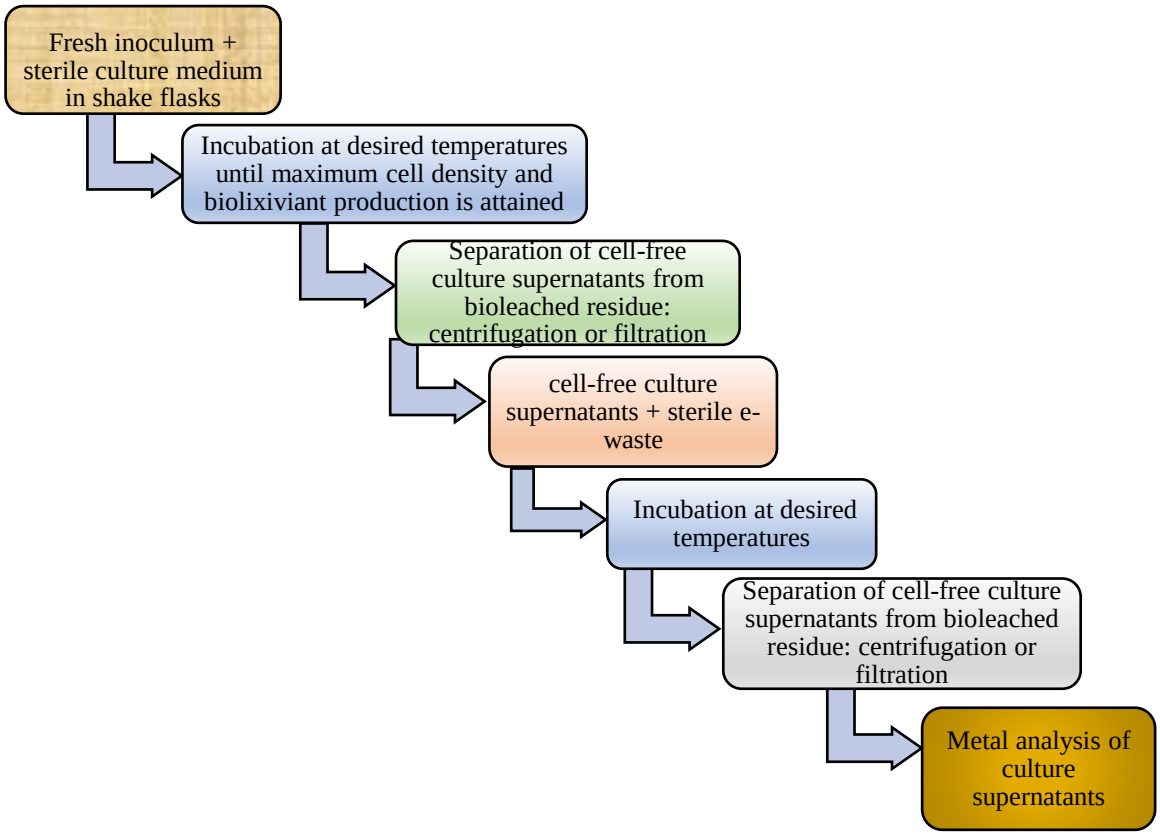

Figure 5. Schematic diagrams showing procedures for the spent medium bioleaching of metals from e-waste.

6. Influence of Bioprocess Parameters on the Bioleaching of Metals

The biorecovery of metals from e-waste is dependent on the physiology of the organisms and availability of suitable physico-chemical parameters at the optimum conditions required to support the growth of microorganisms and metabolite secretion [32] (Figure 6). Other factors that affect metal bioleaching include the effectiveness of the leaching methods: one-step, two-step, or spent medium bioleaching; and the mode of operation: heap, column, shake-flask, or bioreactor operation [6,123]. These parameters influenced bioleaching efficiency autonomously or as combined [124,125]. Some of the various factors that affect bioleaching are discussed below.

6.1. pH

The pH of the bioleaching medium is a crucial factor that influences the mobilization of metals from e-waste, since it affects the growth of microorganisms and biolixiviant production. However, a deviation in the optimum pH requirement of the organisms can have a detrimental effect on their growth, metabolic activities, and leaching efficiency [125]. The pH requirements differ among different groups of microbes. For instance, pH values in the range of 2.0–2.5 are optimal for iron- and sulfur-oxidizing bacteria, with an abundance of the proton concentration [83]. The optimal growth of cyanogenic organisms occurs at an alkaline pH [2]. Amiri et al. [126] reported different optimum pH values for organic acid secretion by *Aspergillus niger* during the biorecovery of metals from industrial waste. In addition, an increase from 8.0 to 11.0 (gold), and 8.0 to 10.0 (copper) in the pH of a yeast–peptone medium used for the cultivation of *Chromobacterium violaceum* enhanced gold and copper mobilization from 7.78% to 10.8%, and 4.9% to 11.4%, respectively, from e-waste [70]. Gold recovery from waste PCBs by *Pseudomonas balearica* SAE1 is enhanced (44% to 68.5%) following an adjustment in the pH of the bioleaching medium from 7.0 to 9.0 [20].

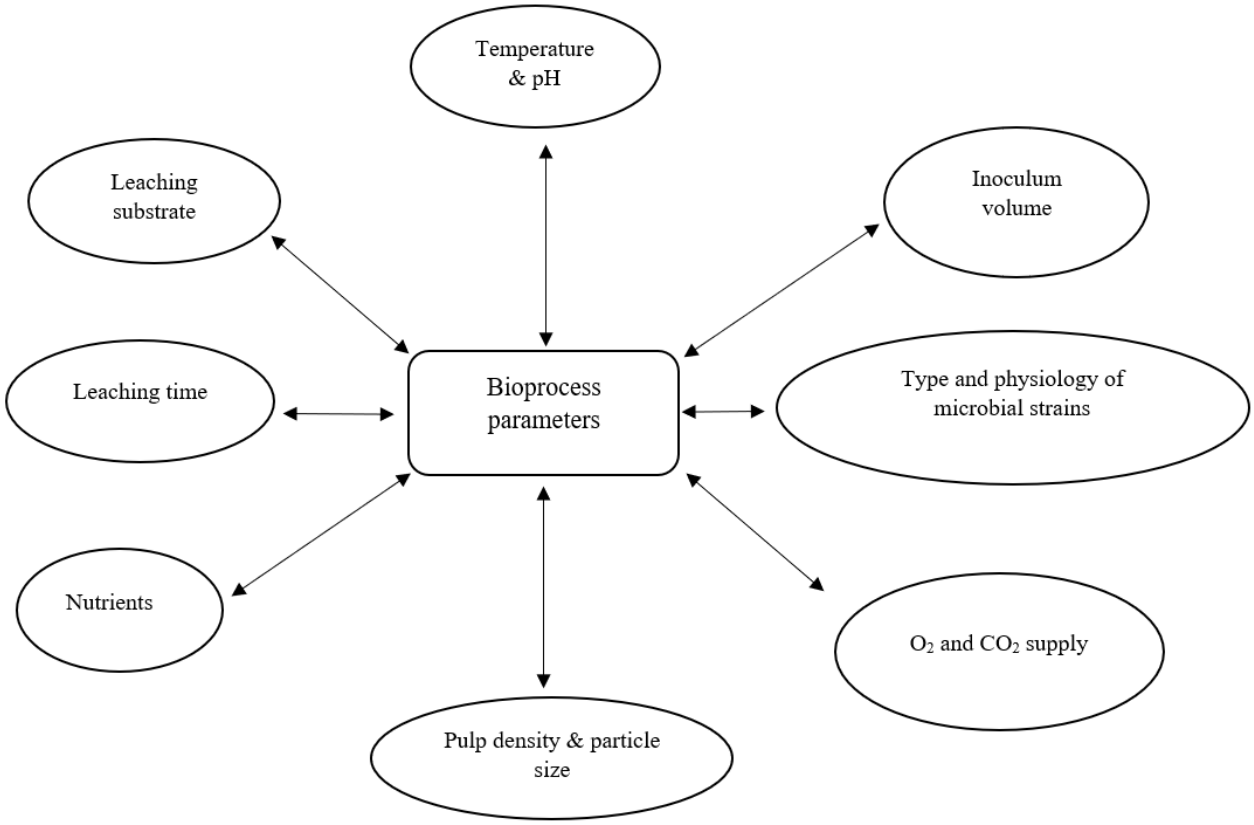

Figure 6. Some bioprocess parameters that affect the bioleaching of metals from e-waste.

6.2. Temperature

The efficiency of the bioleaching process is dependent on temperature, since each organism has a specific optimum temperature required for growth and metabolite production [2]. For instance, some acidophilic and cyanogenic bacteria and fungi have optimal temperatures in the range of 28–30 °C, 25–35 °C, and 25–30 °C, respectively [2,83,85]. However, thermophilic bacteria can be employed at elevated temperatures within the range of 50 to 80 °C for bioleaching [127,128]. Kumar et al. [20] investigated the influence of temperatures (25–40 °C) on the bio-extraction of metals from waste PCBs by *Pseudomonas balearica* SAE1. Maximum yields of 56.2% (gold) and 26.6% (silver) were recorded at 30 °C. *Bacillus megaterium* PTCC 1656 [72] and *Aspergillus niger* DDNSI [129] were employed for the bioleaching of platinum, rhenium, silver, copper, and iron from industrial waste, at 30 and 28 °C, respectively.

6.3. Pulp Density

Pulp density is also known as the liquid-to-solid ratio. It is the amount of a solid matrix (e-waste) present in a solution. A higher liquid-to-solid ratio signifies a low pulp density [125]. A higher pulp density contributes to the toxicity of the e-waste, affecting the growth and metabolic activity of organisms [130]. In other words, a higher pulp density limits the oxygen mass-transfer rate, inhibits microbial growth and metabolite production, and reduces the leaching efficiency [44,131]. Optimum pulp densities of 0.5%, 1%, and 4% have been reported for the biorecovery of precious metals from e-waste by *Chromobacterium violaceum*, *Roseovarius tolerans* DSM 11457 and *Bacillus megaterium*, respectively [10,68,72].

6.4. Culture Medium

The composition of the culture medium used for the cultivation of microorganisms determines the leaching efficiency, since it influences the metabolism of the organisms.

This medium consists of organic and inorganic nutrients, which are utilized by microbes for growth and metabolite production. A growth medium containing organic substances such as peptone, yeast extract, glycine, amino acids, etc., is used for the cultivation of cyanogenic organisms for the recovery of precious metals from e-waste [132]. A chemically defined medium consisting of ammonium sulphate, dipotassium hydrogen phosphate, magnesium sulphate, iron sulphate, elemental sulfur, etc., provides nutrients for the growth of chemolithotrophic organisms. These nutritive salts act as a cofactor for the enzyme-catalyzed biosynthetic pathways for the secretion of metabolites that aid metal mobilization from e-waste [2]. For instance, *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* utilized ferrous ion and elemental sulfur as energy sources, which are later converted to ferric ion and sulfuric acid, respectively, serving as lixiviants for the mobilization of base metals from e-waste [60].

6.5. Aeration

The supply of oxygen is essential for microbial growth and metabolic activity during metal bioleaching. Oxygen is provided to microbes (aerobes) via shaking or stirring using a shaker incubator, measured in revolutions per minute (rpm) [126]. An ideal shaking speed required for a bioleaching experiment is usually in the range of 130 to 170 rpm [24,48]. However, excess agitation renders the friction, abrasion, or disruption of microbial cells, resulting in a decline in metal biorecovery efficiency [133]. A limited oxygen supply can delay the oxidation of ferrous ion and elemental sulfur, leading to a reduction in bioleaching efficiency [134].

6.6. Type and Physiology of Microorganisms

Microorganisms (bacteria and fungi) or their metabolic products are utilized for the recovery of metals from e-waste. These organisms are classified, based on carbon and energy requirements, as chemolithotrophic and heterotrophic organisms. The former are further grouped as mesophiles, moderate thermophiles, and extreme thermophiles, depending on their temperature demands [6]. In addition, the microbes can be applied as pure cultures, mixed cultures, or a consortium for the bioleaching process. The leaching efficiency of these organisms is determined by their adaptation to the metallic environment and toxic nature of e-waste, optimal metal tolerance, and inoculum volume [133].

6.7. Leaching Substrate

The elemental and mineralogical composition of e-waste are crucial factors that influence bioleaching efficiency. This determines the type and amounts of metals present in the solid matrix [32]. The composition of e-waste is measured using analytical equipment such as inductively coupled plasma-mass spectrometer (ICP-MS), following acid digestion of the waste materials in aqua regia, consisting of concentrated nitric acid and hydrochloric acid [36]. In the biorecovery of precious metals from e-waste by cyanogenic organisms, pretreatment for the removal of copper is imperative, as this base metal can form a stable complex with cyanide, thereby reducing free cyanide yield and ultimately inhibiting the recovery of precious metals [68]. Such pretreatment is carried out with the aid of nitric acid, hydrogen peroxide, or *Acidithiobacillus ferrooxidans*.

In addition, the particle size of e-waste influences microbial growth and the biorecovery of metals from the solid matrix. It is the reduced particle size of e-waste and is usually measured in millimetres or micrometres. Bioleaching increases with decreasing particle size. Different particle sizes of e-waste, including <100 µm [74], 120 µm [129], 150 µm [20], <750 µm [34], <0.5 mm [124], and <22 mm [125] have been reported by several co-workers during the bioleaching process.

7. Techniques for the Large-Scale Bioleaching of Metals from E-Waste

7.1. Statistical Optimization of Bioprocess Parameters

The optimization of suitable bioprocess parameters that influence the growth and metabolism of microorganisms is vital for an improved bioleaching efficiency [72]. A conventional approach involving the change of one variable at a time, while keeping other parameters at a constant level, is generally employed to enhance metal yields [20]. However, this technique is laborious, costly, time-demanding, and is characterized by an inability to elucidate interactive effects among the tested variables [135]. As a result, statistical experimental designs are employed as a promising method for an enhanced recovery of metals from e-waste [25]. The significant parameters that affect metal biorecovery are chosen using the Placket–Burman design. Thereafter, optimal conditions and interactive effects of the variables are determined by an artificial neural network (ANN) or response surface methodology (RSM) [136]. Response surface methodology is commonly employed using the Box–Behnken design (BBD), Doehlert design (DD), or central composite design (CCD). For instance, Abdol Jani et al. [137] investigated the influence of the oxygen level, glycine concentration, and pulp density on the bio-extraction of gold from e-waste by *Chromobacterium violaceum* DSMZ 30191 using the BBD of RSM. A maximum leaching efficiency of 62.4% (gold) was recorded at optimal conditions of 0.56 mg/L (oxygen concentration), 2.49 mg/L (glycine concentration), and 1.95% (pulp density). The pulp density and glycine concentration significantly impacted on gold biorecovery. Similarly, Merli et al. [138] employed the CCD of RSM for the enhancement of cyanide production by *Pseudomonas aeruginosa* PA01-T. A maximum cyanide yield of 20 mg/L was generated at a pH 8.0 and glycine concentration of 1 g/L, resulting in the recovery of 90% (silver) and 20% (gold) from PCBs. Esmaeili et al. [139] assessed the influence of sucrose concentration, pulp density, and initial pH on the bioleaching of metals from computer PCBs in the presence of *Penicillium simplicissimum* using the CCD of RSM. The highest yields of 100% (copper), 98% (aluminum), and 70% (nickel) were recorded at the optimal conditions of 16 g/L pulp density, initial pH 6.0, and 60 g/L sucrose concentration. Pulp density was found to be the most significant variable affecting metal leaching efficiencies.

7.2. Bioreactor Bioleaching Experiments

The use of a bioreactor is vital for proper phase contact and accurately controlled process conditions for the large-scale bioleaching of metals from e-waste [140]. In addition, a bioreactor permits for synergy among microbial diversity involved in the metal bioleaching process at a lesser time [123]. Generally, bioreactors such as stirred tank bioreactor, column bioreactor, rotating drum bioreactor, fluidized bed bioreactor, etc., are employed for the bioleaching of metals. Minimol et al. [141] optimized the bioleaching of zinc from e-waste using *Alcaligenes aquatilis* in a fluidized bed bioreactor at the optimum conditions of a 5% e-waste load, 0.175 mm particle size, and 5% inoculum. A maximum zinc recovery of 13% was recorded. Three further sequential batch runs enhanced the zinc recovery to 38%. Maximum leaching capacities of 85.23% (zinc), 76.59% (copper), and 70.16% (aluminum) were reported during the bio-extraction of metals from waste PCBs using *Leptospirillum ferriphilum* and *Acidithiobacillus caldus* in a stirred tank bioreactor [142]. In addition, Ilyas et al. [143] applied a column bioreactor for the bioleaching of metals from electronic scrap using a mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* and *Thermoplasma acidophilum*. Maximum leaching efficiencies of 80% (zinc), 64% (aluminum), 86% (copper), and 74% (nickel) were obtained. A higher copper extraction of 85% at 50 °C in 8 d by *Sulfobacillus thermosulfidooxidans* was reported by Rodrigues et al. [144] using a rotating drum bioreactor. Hubau et al. [140] recorded peak recovery efficiencies of 96% (copper), 73% (nickel), 85% (zinc), and 93% (cobalt) during the bioleaching of metals from PCBs in a double-stage continuous bioreactor for 2 d in the presence of *Leptospirillum ferriphilum* and *Sulfobacillus benefaciens*. Vardanyan et al. [125] recorded approximately 87% (copper) and nearly 100% each of zinc and nickel during the bio-extraction of metals from waste PCBs by *Acidithiobacillus ferrooxidans 61* in a stirred tank bioreactor. In addition, a bubble

column bioreactor was used for the recovery of 97% (copper) and 72% (nickel) from mobile phone PCBs in the presence of *Penicillium simplicissimum* at a pulp density of 10 g/L [145].

7.3. Use of Genetically Engineered Microorganisms

The utilization of genetically modified organisms has been recognized as a promising and sustainable approach for the large-scale recovery of metals from a solid matrix [146]. Such modification in the genome of microbes can be achieved using techniques such as plasmid transfection, conjugation, protoplast fusion, mutation, etc., leading to an increase in the adaptation and resistance of the organisms to high metal concentrations, as well as an enhancement in metabolic activity of microbes at a particular optimal condition [147]. For instance, an improvement in arsenic bioleaching by *Acidithiobacillus ferrooxidans* TFBk using genetic engineering has been reported [148]. Natarajan and Ting [64] investigated the effect of mutagen (100 mm N-Nitroso–N-ethyl urea) on the bioleaching efficiency of *Chromobacterium violaceum* ATCC 12472 under alkaline conditions. The mutated bacterial strain recorded higher gold recoveries of 18%, 22.5%, and 19% at pH 9.0, 9.5, and 10.0, respectively, when compared to a wild strain. Mutation improved the bioleaching potential of the mutant under alkaline conditions by increasing the availability of hydrogen cyanide for gold mobilization from electronic scrap. Tay et al. [117] reported an increase in gold recovery by two genetically modified *Chromobacterium violaceum* strains: pBAD and pTAC. After 8 d of the bioleaching experiment, 30% and 25% of gold, respectively, was mobilized from electronic scrap material when compared to the 11% gold recovery from wild-type strain.

8. Challenges Affecting the Commercial Bioleaching of Metals from E-Waste

The bioleaching of metals from e-waste using microorganisms is efficient and promising. However, this technology is linked to some limitations, which makes its commercialization yet to be established. These include the following:

i Metabolite (e.g., cyanide, organic acids) production by microbial strains may be limited due to different optimal pH required by organisms for the growth and lixiviant secretion. In other words, dissimilar pH demands for microbial growth and metabolite production are challenging for a fruitful metal bioleaching experiment. Therefore, it is necessary to optimize the pH for metabolite production, without preventing the growth of the organisms. This can be achieved by statistical experimental designs (RSM or ANN), the use of a bioreactor, or the use of metabolically engineered organisms with a high pH tolerance.

ii Metal recovery from e-waste using microorganisms is low in comparison to chemical leaching, since bio-lixiviant concentrations are also relatively low. As a result, processing conditions and leaching parameters must be modified to enhance metal dissolution.

iii The toxicity of e-waste is a great challenge for the bioleaching process, as this affects the growth of organisms for metabolite production. Conventionally, high-throughput screening of microbial strains is carried out for the selection of organisms with a large tolerance to toxic metal ions. Furthermore, the organisms are allowed to reach a particular growth phase, in which maximal cell density and optimal biolixiviant production are attained before the addition of waste materials into the bioleaching medium. In addition, the toxicity of e-waste to microbes can be combated using autochthonous organisms that are native to waste materials. The exploration of indigenous organisms could be of great significant to metal bioleaching, since these microbes can be assumed to perform better in their native environments compared with exotic organisms.

iv The long duration of bioleaching is a crucial drawback that affects the large-scale (commercial) recovery of metals from e-waste.

v An efficient reclamation of metals from the bioleaching medium at a low cost is a great obstacle hindering the industrial recovery of metals from e-waste.

9. Conclusions and Recommendations for Future Perspectives

The global, fast growth of electronic and electrical industries, coupled with incessant demands for electrical and electronic equipment, has led to the indiscriminate disposal of e-waste, especially when the products have attained their service life, causing environmental and health hazards. Furthermore, e-waste is a complex material, consisting of metallic components in large quantities. Bioleaching is a promising and sustainable technology for the valorization of e-waste for the reclamation of value-added metals for the exploitation in various industrial applications. The efficiency of the microorganisms is determined by the presence of appropriate and optimum bioprocess conditions for the large-scale recovery of metals from e-waste.

There is a need to enhance the metal bioleaching efficacy through ground-breaking and sustainable technology for e-waste management. These innovative approaches for future studies include the following:

i The slow kinetics of metal recovery from e-waste can be ameliorated using suitable concentrations of metal ions (e.g., Ag^{+}, Cu^{++}, Hg^{++}, Co^{++}, Bi^{+++}, etc.) or non-metal ions (such as activated carbon and quartz) catalysts in the bioleaching medium as an efficient and cost-effective technique for the development of a successful large-scale bioleaching process. In addition, the cocktail use of these catalysts in the bioleaching of metals from e-waste should be encouraged, as this combination may induce greater catalysis, resulting in better microbe–mineral interactions with a consequential effect on improving metal yields.

ii Exploration of the leaching potential of unidentified or genetically modified microorganisms including thermophilic fungi, bacteria, and archaea under different bioprocess conditions can be a better alternative for enhanced metal recovery from e-waste. The genetic modification makes the engineered organisms highly efficient for metal recovery and rapidly adaptable to environmental changes when compared to wild-type strains. It involves enhancing the expression of genes that encode for biolixiviant production.

iii Further research should be carried out on the proper understanding of community distribution, synergistic relationships, and mechanisms of actions of mixed or a consortium of microbial strains for the bioleaching of metals from e-waste.

iv The utilization of non-conventional carbon sources, including agro-industrial wastes such as corncobs, rice bran, straw, mango-peels, etc., should be encouraged as a cost-effective and eco-friendly substrate for the cultivation of microbes for improved secretion of metabolite for metal recovery from e-waste.

v Due to the complexity of e-waste, which consists of a variety of metals, further studies should be geared toward application of hybrid technology for the efficient and enhanced extraction of metals from waste materials. This involves the integration of an assortment of leaching technologies (such as biological, chemical, and physical processes) for the effective recovery of metals from e-waste.

vi Prior to the bio-extraction process, the toxic level of e-waste can be reduced through the development of a novel physico-mechanical technique for the separation of hazardous components of the waste materials from non-hazardous elements.

vii Microbial cells can be immobilized on natural (e.g., cellulose, agar, alginate) or synthetic (such as polypropylene, polyvinyl, polyurethane) support materials for the efficient recovery of metals during the bioleaching process. In addition, the immobilization of microorganisms on a suitable carrier increases the stability of the immobilized cells over a broad range of temperatures and pH, and permits the reusability of the immobilized organisms with a decrease in operational costs.

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