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Review

Biohydrometallurgical Recovery of Metals from Waste Electronic Equipment: Current Status and Proposed Process

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Abstract: Electronic waste (e-waste) is an emerging health and environmental burden due to the toxic substances present within e-wastes. To address this burden, e-wastes contain various base, rare earth and noble metals, which can be recovered from these substances, thus serving as secondary sources of metals. Pyrometallurgical and hydrometallurgical processes have been developed to extract metals from e-waste. However, these techniques are energy-intensive and produce secondary wastes, which will add to the operating costs of the process. However, the biohydrometallurgical approach has been deemed as an eco-friendly, cost-effective, and environmentally friendly process that does not produce large quantities of secondary waste. However, research has focused chiefly on one-stage bioprocesses to recover the metals of interest and majorly on base metals recovery. Hence, this review proposes a two-stage bio-hydrometallurgical process where the first stage will consist of acidophilic iron and sulphur oxidising organisms to extract base metals, followed by the second stage which will consist of cyanide-producing organisms for the solubilisation of rare earth and precious metals. The solid waste residue that is produced from the system can be used in the synthesis of silica nanomaterials, which can be utilised for various applications.

Keywords: bioleaching; waste printed circuit board; acidophilic bacteria; cyanogenic bacteria

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1. Introduction

The world is dependent on various metals, which have numerous functions in society. With the increased focus on the 4th industrial revolution (4IR), which relies on metals for technology development and/or advancement, there is a need to find alternative eco-friendly, cost-effective, and sustainable technologies to recover metals from secondary metal sources, since the primary resources are being exhausted [1]. Electronic waste (e-waste) has been deemed as a secondary metal source, since it contains low concentrations of base, critical rare earth, and precious metals. This characterisation is informed by the mineralogy of electronic waste [2]. The rapid exponential growth in e-waste is due to industrial development and the extensive utilisation of electrical and electronic equipment (EEE), which has resulted in the generation of enormous amounts of e-waste worldwide when these electronic products reach the end of their lifecycle [3,4]. E-waste consists of various substances such as toxic metals and metalloids (copper, iron, chromium, lead, nickel, mercury, zinc, etc.), chlorofluorocarbons, polybrominated diphenyl ether, and brominated flame retardants that pose severe threats to the environment and public health when improperly disposed of [5]. E-waste is mainly disposed of in landfills or through incineration, which results in the significant release of hazardous materials into the environment [5]. To date, there are no eco-friendly standards and regulatory mechanisms, especially in developing countries, that have been developed for the safe disposal of e-waste [4]. Recycling e-waste using economic, sustainable, and eco-friendly technologies can serve as a secondary source for metals, especially for critical metals of high value. The recovery of metals from e-waste can minimise environmental and public health deterioration through exposure to heavy hazardous metals such as mercury, lead, and cadmium [6,7]. The quantity of e-waste recycled globally remains small, and South Africa only recycles 11% of their generated e-waste annually, with a significant percentage of valuable metals contained in complex materials such as Printed Circuit Boards (PCBs), being exported for subsequent processing [8].

The annual production of e-waste was estimated to be 20–30 million tons by the United Nations Environment Programme (UNEP) and 4000 tons per hour globally, and the production is growing exponentially [3,9–11]. It was reported that 44.7 million tons of e-waste were generated in 2016, and it is estimated that by 2021, e-waste generated will have risen to 52.2 million tons annually and will continue to grow exponentially at a growth rate of 3% to 4% annually [12]. Figure 1 represents e-waste generated from 2011 to 2020, and this graph demonstrates the increasing e-waste generation worldwide.

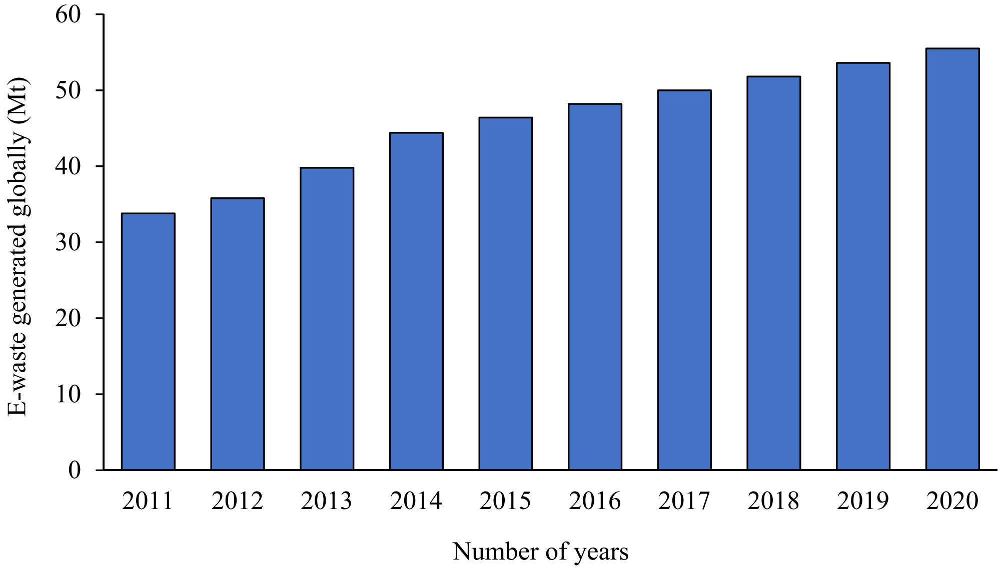

Figure 1. Electronic waste generated globally in the past decade [13].

Computer waste seems more beneficial amongst other e-waste categories, allowing economic and environmental benefits for recovering/recycling metals. It was projected that by 2020, the e-waste from computers would have risen by 500% in India and by 300–400% in South Africa [10]. The PCBs are the core component of many EEE, which are used for conventional functioning in small and large electronic devices, and they contain various metals and several hazardous pollutants that cannot be disposed of in landfills or incinerated. The disposal of e-waste in landfills or via incineration are the methods majorly adopted for e-waste management and this results in the release of toxic gases such as greenhouse gases and dioxins to the atmosphere, even at low temperatures, and the contamination of soil and groundwater by heavy metals (mercury, lead, and cadmium) that have leached from the e-wastes [5,7]. These pollutants can eventually accumulate and be absorbed by plants, directly affecting the lives that feed on such resources. Therefore, e-waste management research has intensified over the years to mitigate the impacts of e-waste on the environment and recover valuable resources [4]. PCBs are the primary and/or major carriers of valuable and hazardous metals due to their high composition of precious and other commercial metals. PCBs majorly constitute approximately 40% metals, 30% plastics, and 30% ceramics [11,14], where the major metal constituents include copper (19.9%), aluminium (7.06%), nickel (5.35%), iron (3.56%), tin (2.03%), lead (1.01%), and precious metals such as silver and gold (0.2%) [10].

The traditional approaches of extracting metals from PCBs include mechanical, hydrometallurgical, and pyro-metallurgical processes [15,16], which are processes which have been extensively explored. These processes are considered unsustainable due to their cost intensiveness, the excessive pollutants they generate, and high energy consumption, amongst others [4]. Bio-metallurgical processes utilise microorganisms to extract metals from the solid PCBs into soluble metals in an aqueous solution by generating lixiviants, which are responsible for metal extraction [17]. The use of such organisms is promising, since this process is eco-friendly, can be operated at an ambient temperatures and pressure, is cost-effective and generates minimal secondary wastes [4,5,18]. However, this process is hampered by commercialisation prospects, since there are still insufficient scientific data (optimum conditions) for up-scaling the process and the synchronisation of the acidic and alkaline bio-metallurgical processes.

2. Definition and Classification of Electronic Waste

There are several definitions of e-waste, but the simplest definition that is widely used is: e-waste is any broken/unwanted electrical and electronic equipment (EEE) that has reached the end of its lifecycle or economic life span [4,19]. The European Union (EU) has defined e-waste as any appliance that requires an electric power supply and has reached the end of its life cycle. E-waste has been grouped into 10 primary categories by the European Commission (2012/19/EU), shown in Table 1 and classified per product type and legislative relevancy category. The 10 categories are further broken down into 58 sub-categories, with approximately 920 products in which all disposed devices are included [19,20]. Printed circuit boards (PCBs) are the core part of most electronic appliances and electric equipment: a component which controls the functionality of most equipment and with a Cu-clad laminate basic structure consisting of glass-reinforced epoxy resin and various metallic materials, including precious metals [4,11]. PCBs can be classified as single-sided, double/multi-layered sided depending on the structure and alignment, and material present in the PCBs can be categorised into three groups: metals, ceramics, and organic materials. PCBs have much higher concentrations of precious metals such as Ag, Pd, Au, and Pt than their respective primary resources, making PCBs an attractive secondary resource for recovery metals [11]. Nevertheless, PCBs pose a great danger to the environment if disposed in landfills or through incineration, since they contain various hazardous metals such as Cr, Hg, Cd, etc., and rare earth metals such as Ta, Ga, etc., including flame-retardants (Br) coupled with high concentrations of precious metals. Therefore, the extraction of metals from PCBs is important for both economic and environmental perspectives due to the high concentration of precious metals (Au and Pd) they possess compared to natural ores and the danger they pose to the environment and the human health [11,21].

Table 1. Waste Electrical and Electronics Equipment categories [20].

WEEE Classification	Description
1 Large household appliances	Washing machines, refrigerators, dryers, air conditioners, radio sets, electric shavers, toasters, microwaves, dishwasher
2 Small household appliances	vacuum cleaners, microwaves, ventilation equipment, toasters, electric kettles, electric shavers, scales, calculators, radio sets, video cameras
3 Information technology and communication (ICT) equipment	PCs, laptops, mobile phones, fax machines, printers, telephones and photocopiers
4 Consumer electronics	Televisions, VCR/DVD/CD players, hi-Fi sets, radios, train sets, coin slot machines, parking ticket equipment
5 Lighting	Fluorescent lamps, high-intensity discharge lamps, and LED lamps
6 Electrical and electronics tools	Drills, electric saws, sewing machines, lawnmowers, large stationary tools, machines
7 Toys	Electrical and electronic toys
8 Leisure and sports equipment	
9 Medical devices	Surveillance and control equipment, medical instruments and equipment
10 Automatic dispensers	

3. E-Waste Management and Legislations

E-waste is poorly managed worldwide, as it is either disposed in landfills or transported to developed countries through legal or unregistered routes that create environmental problems due to the high presence of hazardous substances, regardless of the legislation in place in various countries [20,22]. Different nations and associations have adopted various regulations such as the Waste Shipment Regulation (WSR) to control e-waste, which was implemented in 1993 and amended in 2007. The EU passed the e-waste directive for changing item designs and expanding the reuse or recycling of disposed e-waste in 2003 and implemented restrictions on the utilisation of certain unsafe substances [23]. The Packaging Directive for managing essential e-waste was adopted into UK law through two separate directives: Packaging (Essential Requirements) Regulations 2003, which stated that packaging material should be recyclable and recoverable, and dangerous substances such as heavy metals should be controlled [24], and the Producer Responsibility obligations (Packaging Waste) Regulation 2007, which obligated all UK companies handling more than 50 tons of packaging annually to reduce their packaging [25]. India placed a legal liability on producers for reducing and recycling e-waste under the e-waste (management and handling) Rule 2011, further restricting the use of hazardous substances [26,27]. In 1992, the Basal Convention was drafted under the United Nation Environment program to manage and direct the transboundary stream of hazardous e-waste and their transfer. In South Africa, laws on the regulation of e-waste were included in the National Environmental Management Act, 1998 and the National Environmental Management: Waste Act, 2008 [28]. The National Environmental Management: Waste Act, 2008 (Act No. 59 of 2008) regulates waste management to protect public health and the environment by providing reasonable measures to mitigate pollution and ecological degradation [29,30]. Since many substances contained in e-wastes are hazardous, e-waste is considered a hazardous waste and these are listed in Annexure 1 of The Basel Convention for the Control of Transboundary Movement of Hazardous Waste; South Africa has been part of this Convention since 1992. Hazardous Substance act No: 15 of 1973 regulates the management of hazardous waste. South Africa's e-waste Association (eWASA) seeks to enforce producers, importers and distributors to return old or end-of-lifecycle EEEs for repair and reuse, thus bolstering e-waste recycling [31,32]. Over the past decade, China has adopted a variety of regulations, environmental laws, standards, technical guidance, and norms in relation to e-waste management, and to date, China still faces challenges to implement and the enforce this legislation. Despite the policies and legislation adopted by the Chinese and Japanese governments, the illegal dumping, exporting, and importing of e-waste continues [25,29,33].

4. Pre-Treatment of E-Waste

The pre-treatment process of e-waste can be carried out physically with mechanical techniques that involve dismantling, which requires human labour and is the first crucial step for all types of e-waste recycling. Dismantling is the pre-treatment process, usually carried out with a hammer, screwdrivers, and conveyer beds for dissembling the components into different categories for their recycling. The shredding of materials mechanically is the second step in the pre-treatment process of e-waste, where e-waste is placed in crushers and grinders and can either be processed further through an electrical magnetic separator to separate non-metallic components. Finally, the pre-treated e-waste is further processed for metal recovery in various processes such as hydrometallurgical, pyro-metallurgical, bio-metallurgical, and electrometallurgical [17].

5. Metallurgical Technologies to Treat E-Waste

E-waste has been traditionally treated with metallurgical technologies such as mechanical separations, pyro-metallurgical, and hydrometallurgical methods. The technologies such as the pyro-metallurgical process to treat e-waste has been studied and linked with environmental challenges such as the generation of large quantities of harmful by-products, the formation of brominated and chlorinated di-benzo furans, and dioxins from halogens present in the plastic part of e-waste during the burning process [34]. The pyrometallurgical process requires high-energy to treat e-waste and is inefficient in the recovery of precious metals. Hydrometallurgical technology has also been explored, mostly involving chemical reagents such as strong acids (sulphuric acid, nitric acid, hydrochloric acid) and complex chemical reagents such as cyanide and thiosulfate to leach base, rare earth, and precious metals. Hydrometallurgical methods have also been associated with environmental deterioration due to the generation of large secondary toxic waste streams that require pre-treatment before disposal [19,35].

5.1. Pyro-Metallurgical Processes

The pyrometallurgical processes involve smelting, combustion, incineration, and pyrolysis in a furnace or blast furnace. Pyrolysis is carried out at elevated temperatures of up to 900 °C (or even higher in some instances) to treat e-waste, which generates 70% metal-rich residue, 23% oil, and 5% gases [19,36]. These processes are associated with high-energy consumption, are expensive and generate toxic compounds, including dioxins and furans. This is because e-wastes, especially PCBs, contain halogenated flame-retardants [35].

5.2. Hydrometallurgical Process

The hydrometallurgical process primarily involves the use of chemical reagents, where strong acids are used to leach base metals while chemical reagents such as cyanide, halide, thiourea, and thiosulfate are used to leach out precious metals from e-waste [35,36]. These processes require large quantities of chemical reagents and produce a variety of by-products and high volumes of effluent waste. It is noteworthy to mention that the recovery of precious metals from ores and e-waste is preferred via cyanide leaching due to its potential to yield high recovery and economic cost. However, this method has some disadvantages, such as increasing the additional work of treating the effluent before disposal, and cyanide is one of the controlled chemicals due to its toxicity [37].

5.3. Biohydrometallurgical Processes to Treat E-Waste

Biometallurgical processes are mostly referred to as bioleaching. This is a process that has received significant attention as a promising sustainable technology to recover metals from e-waste due to its advantages such as low operational cost, low energy consumption, reduced chemical reagents usage, and easy manageability of secondary waste effluents [3–6]. However, this process is slow to leach metals; hence, it has not been fully industrialised for ores with high metal content and e-waste [5,38]. Table 2 shows the metallurgical process used to leach metals from e-waste, including their advantages and disadvantages.

5.3.1. Bioleaching Technology

Bioleaching of metals from e-waste is divided into two processes: (1) the One-step and (2) the Two-step bioleaching process.

One-Step Bioleaching Process

In one-step bioleaching, the microbial inoculum sourced from the exponential growth phase is added to a suitable bioleaching medium and the e-waste. The process produces ferric iron (Fe^{3+}) through the oxidation of ferrous iron (Fe^{2+}) and protons, which slowly solubilises the embedded metals within the e-waste. The one-step bioleaching process can only be operated at low pulp density ranging between 1 and 10% (w/v) due to the presence of the toxic substances in e-waste that inhibit microbial growth [4]. Hence, the direct culturing of microorganisms in the presence of e-waste is not advisable due to the presence of toxic substances in e-waste that inhibits microbial growth, which in-turn lowers the availability of the extractant, leading to low metal extraction efficiencies [41]

Two-Step Bioleaching Process

In the first step of the two-step bioleaching process, microorganisms are cultured in the absence of e-waste in their respective culture medium under their optimum conditions. When the cells have reached their maximum cell growth and lixiviant production (Fe^{3+} and protons), the e-waste is then added to the medium as a second step [4]. This process reduces the inhibition of microbial growth caused by the toxic substances available in the e-waste. The two-step bioleaching process has been found to be more attractive as it results in rapid and high metal extractions, and it can be carried out at high pulp density, unlike the one-step bioleaching process. A large quantity of Fe^{3+}/protons is readily available to react with the added e-waste and dissolution metals at the fastest possible rate [42].

Table 2. Comparison of applied metallurgical processes [17,18,39,40].

Process Description	Advantages	Disadvantages
	Pyro-metallurgy	
The process involves the use of high temperature liquid phases such as fused salts (LiCl and KCl) and fused metals (Cd, Bi, Al)	The energy obtained in this process can be utilised in upstream or downstream processes and requires minimal chemical consumption.	High energy consumption due to high operational temperatures within the process. The process generates toxic compounds such as dioxins and furans since e-waste contains halogenated flame-retardants. Emits strong gas emissions such as CO_2, and CO is used as reducing agent.
	Hydrometallurgy	
The process involves the use of chemical reagents such as H_2SO_4 and HNO_3 solutions to extract metals from e-waste	It saves time as it has a short process time and high efficiency on leaching metals	Expensive sulphur conversion technology. Generates a high volume of effluents which may pollute local water sources. High concentrations of metals in ores or e-waste are required. Uses a large quantity of chemical reagents. High operational cost.
	Bio-hydrometallurgy	
The process uses microorganisms such as bacteria and fungi to exact metals from e-waste and ores instead of chemical solutions	Environmentally friendly. Low operational cost. Less use of chemical reagents. Low energy consumption. Simple to maintain.	Bio-reactions not easily controlled. The technology is still under development for industrial use. Long operational period and time-consuming.

5.3.2. Bioleaching of Base Metals from E-Waste

The well-known acidophilic microorganisms *Acidithiobacillius ferroxidans, Leptospririllum ferrooxidans, Acidithiobacillus thiooxidans* and others play a vital role in the extraction of metals from e-waste. These are characterised by (1) survival or operation at low pH values (<2), (2) high metal concentration tolerance, and (3) they assist in the generation of lixiviants for the solubilisation of metals from the solid phase into the aqueous phase [6,43]. The metals embedded within the e-waste are in their insoluble form, whereas in sulphide minerals, they are present in the form of metal sulphides, where these iron and sulphur-oxidising organisms derive their energy from. Microorganisms in the bioleaching system use pyrite/sulphur as substrates to continually produce Fe^{3+} and protons as lixiviants. Therefore, in the bioleaching system of e-waste, it is necessary to add ferrous iron (Fe^{2+}) and a source of sulphur, since such substrates are not available within the e-waste [6]. The acidophilic microorganisms oxidise Fe^{2+} to produce Fe^{3+}, and sulphur oxidation to produce protons which are responsible for the conversion of insoluble metals (Cu^0, Zn^0, and Ni^0) to their respective water-soluble metals (Cu^{2+}, Zn^{2+}, and Ni^{2+}) as represented from Equations (1)–(6) [4,34,44].

$$4\mathrm{Fe}^{2+}+\mathrm{O}_2 \underset{\text{Bacteria}}{\overset{\text{Fe}-\text{oxidizer}}{\rightarrow}} 4\mathrm{Fe}^{3+}+\mathrm{H}_2\mathrm{O} \qquad \left[\Delta\mathrm{G}^0 = -39.7\ \mathrm{kJ\ mol}^{-1}\right] \tag{1}$$

$$2\mathrm{Fe}^{3+}+\mathrm{Cu}^0 \rightarrow 2\mathrm{Fe}^{2+}+\mathrm{Cu}^{2+} \qquad \left[\Delta\mathrm{G}^0 = -347.1\ \mathrm{kJ\ mol}^{-1}\right] \tag{2}$$

$$2\mathrm{Fe}^{3+} + \mathrm{Zn}^0 \rightarrow 2\mathrm{Fe}^{2+} + \ \mathrm{Zn}^{2+} \qquad \left[\Delta\mathrm{G}^0 = -1235.9\ \mathrm{kJ\ mol}^{-1}\right] \tag{3}$$

$$2\mathrm{Fe}^{3+} + \mathrm{Ni}^0 \rightarrow 2\mathrm{Fe}^{2+} + \ \mathrm{Ni}^{2+} \qquad \left[\Delta\mathrm{G}^0 = -822.6\ \mathrm{kJ\ mol}^{-1}\right] \tag{4}$$

$$\mathrm{S}^0 + 105\mathrm{O}_2 + \mathrm{H}_2\mathrm{O} \overset{\text{microbes}}{\rightarrow} \mathrm{SO}_4{}^{2-} + 2\mathrm{H}^+ \qquad \left[\Delta\mathrm{G}^0 = -774.5\ \mathrm{kJ\ mol}^{-1}\right] \tag{5}$$

$$2\mathrm{Cu}^0 + 2\mathrm{H}^+ \rightarrow \mathrm{Cu}^{2+} + 2\mathrm{H}_2\mathrm{O} \tag{6}$$

Copper, Zinc, and Nickel dissolution from e-waste occurs through the action of Fe^{3+} in acidic conditions, maintained by the oxidation of sulphur by the microorganisms (see Equations (2)–(5)). All these chemical reactions occur at an ambient temperature and atmospheric pressure [4]. Biohydrometallurgical studies on recovering metals from e-waste have been widely reported with the use of mesophilic and chemolithotrophic bacteria such as *Acidithiobacillus ferroxidans* and *Acidithiobacillus thioxidans*, and acidophilic, moderately thermophilic microorganisms such as *Sulfobacillus thermosulfidooxidance* and *Thermoplasma acidophilum*, including cyanogenic microorganisms such as *Chromobacterium violaceum* and *Pseudomonas fluorescens* [34,38].

Işıldar et al. [45] studied copper bioleaching in a two-step process using e-waste from desktop computers, laptops, and mobile phones. The bioleaching of copper was carried out in 300 mL Erlenmeyer flasks by pure *Acidithiobacillus ferroxidans, Acidithiobacillus thiooxidans,* and a mixture of *Acidithiobacillus ferroxidans* and *Acidithiobacillus thiooxidans*. These were all incubated at 30 °C for 7 days with an incubator shaker speed set at 150 rpm. The mixture of *Acidithiobacillus ferroxidans* and *Acidithiobacillus thiooxidans* achieved the highest copper bioleaching efficiency of 98% compared to the reactor tanks with pure *Acidithiobacillus ferroxidans* and *Acidithiobacillus thiooxidans,* which achieved 94% and 89%, respectively at the pulp density of 10 g/L [46]. Benzal et al. [42] investigated the recovery of copper from mobile phone WPBCs using a two-step bioleaching process in a batch 500 mL Baffled Erlenmeyer flask with *Acidithiobacillus ferroxidans*, incubated at 30 °C with an incubator shaker speed set at 130 rpm for 2 days. The study achieved 95–100% copper recovery at a pulp density of 7.5 g/L in 48 h [42]. Benzal et al. [42] investigated the recovery of copper in a two-step bioleaching process, extracting copper from non-pulverised PCBs, which were in the form of plates using *Leptospirillum ferriphillum* with the cell density of 2×10^7 incubated in a 5 L bubble reactor aerated with air at 4.5 L/min. It was reported that more than 99% of ferrous sulphate was oxidised. The study achieved 99% copper recovery at the end of the process, corresponding to 168 ± 1.3 g of copper recovered [47].

A study by Harikrushnan et al. [6] investigated the bioleaching g of copper (Cu), Nickel (Ni), and Zinc (Zn) from computer e-waste using an integrated method that combines both biohydrometallurgical and hydrometallurgical processes where *Acidithiobacillus ferooxidans* microorganism, and 2 M HNO_3 was used. *A. ferooxidans* was incubated at 30 °C with the incubator shaker speed set at 100 rpm for 10 days, and the culture pH was maintained between 2.5–3. It was discovered that the hybrid treatment could achieve nearly 100% metals recovery for all selected metals (Cu, Zn, and Ni) at the pulp density of 10 g/L and noticed that as they increase the pulp density, the metal recovery efficiency decreased. The authors achieved the metals recovery efficiency of 85% for Cu, 98% for Ni and Zn and approximately 60% of Cu and 97–98% for Ni and Zn at the pulp density of 50 g/L and 100 g/L, respectively [6]. de Andrade et al. [48] studied the bioleaching of copper from mobile phone e-waste using *A. ferrooxidans* incubated at 30 °C with an incubator shaker speed set at 185 rpm. The study demonstrated that magnetic separation improved copper concentration in the non-magnetic (NM) fraction (1950 g/L) compared to one without magnetic separation (MIX) fraction (1435 g/L). It was observed that the presence of microorganisms in the culture medium increased the copper extraction from 6854 to 7387 mg/L and from

6309 to 6606 mg/L for both NM and MIX fraction, respectively, which meant that the presence of microorganisms in the culture medium increased copper extraction by 8 and 5%, respectively [48].

A study by Shah et al. [47] investigated the extraction of Cu, Ni, and Zn from computer e-waste with a two-step bioleaching process using ferrous sulphate as a substrate. The authors evaluated the factors affecting metal extraction percentage to achieve the optimum process conditions. The authors used 250 mL Erlenmeyer flasks to grow *Leptospirillum ferriphilum* microorganism with an incubator shaker speed set at 150 rpm at 32 ± 2 °C. The authors achieved 85.25%, 96.75%, and 92.33% metal extraction efficiency of Cu, Zn, and Ni, respectively, in the two-step bioleaching process without process optimisation and the experiments were run for 8 days at 10 g/L pulp density. The authors further investigated the non-pre-treated and pre-treated e-waste powder with acidified distilled water, and they noticed that the metal extraction percentage increased by 3.80–7.79%, corresponding to 94.08% Cu, 99.80% Zn, and 97.99% Ni, respectively. The authors evaluated the effects of pH (1.5, 18, 2.0, 2.5, and 3) at 10 g/L pulp density, and they noticed that medium with the pH of 2.0 achieved maximum metal extraction efficiency of 88%, 99.8%, and 97.13% of Cu, Zn, and Ni, respectively, compared to the pH of 1.5 and 1.8. They reported a significant marginal difference in the metal extraction percentage between the pH of 1.8 (79.50% Cu, 85.20% Zn, 89% Ni) and the pH of 2.0 and concluded that the pH of 2 was the optimum condition at a pulp density of 10 g/L. The authors also evaluated the impact of particle size on metals extraction percentage. They noticed that metals extraction percentage increases with the decrease in particle size from 3360 to 74 µm, and the metal extraction percentage achieved by Cu and Zn were 71.25% and 79.56%, respectively, at 3360 µm particle size and 97.35%, and 99.80%, respectively, at 74 µm particle size and noticed that a further decrease in particle size to 54 µm decreases the extraction percentage. These could be due to particle–particle collision, which may lead to severe attrition and disrupt the cells or decrease air diffusion and the development of thick slurry caused by small particle sizes [47].

Pradhan and Kumar [41] studied the bioleaching of metals from personal computer e-waste with both one and two-step bioleaching processes using cyanogenic microorganisms (*Chromobacterium violaceum, Pseudomonas aeruginosa and Pseudomonas fluorescens*) at a pH of 7.2. They were incubated at 30 °C with an incubator shaker speed at 150 rpm. The researchers also studied the impact of pulp density of 10 and 20% *w*/*v* and discovered that the bioleaching rate depends on the pulp densities. The authors reported that *C. violaceum* and the mixture (*C. violaceun* and *P. aeruginosa*) achieved maximum bio-leachability efficiency of more than 79, 69, 46, 9, and 7% (*C. violaceum)* and 83, 73, 49, 13, and 8% (*C. violaceun* and *P. aeruginosa*) of Cu, Au, Zn, Fe, and Ag, respectively [41]. The mentioned studies are a demonstration that the two-step bioleaching process is more effective compared to the one stage process.

5.3.3. Bioleaching of Precious Metals from E-waste

Different cyanogenic bacterial strains such as *Chromobacterium violeaceum, Pseudomonas fluorescens, Pseudomonas aeruginosa, Bacillus megaterium* have been exploited to extract precious metals and metalloids. These cyanogenic bacteria can detoxify and degrade the excess cyanide to β-cyanoalanine by β-cyanoalanine synthase during the late stationary and/or death growth phase, which makes the bio-cyanidation process attractive due to significant environmental and health risk mitigation, thus making the bio-cyanidation process eco-friendly [6,48,49]. Cyanogenic bacteria are effectively used to recover precious metals and metalloids such as Au, Ag, Pt, Pd, Ti and Mo from e-waste through a process referred to as alkaline bioleaching or the heterotrophic bioleaching process [40]. The cyanogenic bacteria are all capable of producing hydrocyanic acid (HCN)/cyanide ion (CN^-) as their secondary metabolite at their late stationary phase during the decarboxylation of glycine, which serves as a lixiviant for the dissolution of the solid metals [39,50]. Gold cyanidation is an electrochemical process that consists of anodic and cathodic reactions, where gold is dissolved in alkaline cyanide solution to form a gold cyanide complex, as summarised by Elsner's equations (see Equations (7)–(9)) [36,37,51]. This cyanide can be produced by the mentioned organisms and the extraction process can be carried out as demonstrated by Elsner's equation [50,52,53].

$$4\mathrm{Au} \;+\; \mathrm{S8CN}^- \rightarrow 4\mathrm{Au(CN)}_2{}^- + 4\mathrm{e}^- \qquad \left[\Delta \mathrm{G}^0 = -433\mathrm{kJmol}^{-1}\right] \quad (7)$$

$$\mathrm{O}_2 + 2\mathrm{H}_2\mathrm{O} + 4\mathrm{e}^- \;\rightarrow\; 4\mathrm{OH}^- \quad (8)$$

$$4\mathrm{Au} + 8\mathrm{CN}^- {+}\mathrm{O}_2 + 2\mathrm{H}_2\mathrm{O} \;\rightarrow\; 4\mathrm{Au(CN)}_2{}^- + 4\mathrm{OH}^- \quad (9)$$

It is essential to extract base metals from e-waste before the recovery of precious metals since they are present in high quantities and can quickly form cyanide complexes at higher concentrations than precious metals, thus lowering the recovery of precious metals [46,48,54]. Li et al. [36] investigated the bio-cyanidation of gold using a two-step bioleaching process, where *P. fluorescens* was used to recover gold from mobile phone e-waste powder in a 250 mL stirred airlifted tank reactor incubated at 30 °C with an incubator shaker speed at 150 rpm. The authors observed a 54% bioleaching efficiency of gold when they added glycine and methionine in the growth medium. In addition, these authors [36] also studied the effect of pulp density (0.33% *w*/*v*, 0.67% *w*/*v*, 1% *w*/*v*, 1.5% *w*/*v* and 1.67 *w*/*v*) on the bioleaching efficiency of gold and achieved 42% bioleaching efficiency of gold with a pulp density of 0.33% *w*/*v* and also noticed that when the pulp density increased, the bioleaching efficiency decreased [36]. Işıldar et al. [46] used *Pseudomonas fluorescens* and *Pseudomonas putida* to extract gold from desktop computers, laptops, and mobile phone e-waste in 300 mL stirred tank bioreactors incubated at 30 °C and achieved the highest gold recovery of 44%, using *P. putida* at 0.5% *w*/*v* pulp density [46].

Natarajan et al. [49] studied the bio-cyanidation of gold from electronic strap material (ESM) powder with a particle size of less than 100 µm in a two-step bioleaching process using *Chromobacterium violeaceum* and engineered strains and achieved the highest gold recovery efficiency of 30% with engineered strains at a pulp density of 0.5% *w*/*v*, compared to 11% achieved by *C. violeaceum*. The study also demonstrated that the increase in pulp density decreased gold recovery efficiency. This was due to the increased toxicity levels of the metals [49]. Marappa et al. [55] studied bio-cyanidation of gold and other precious metals from PCBs powder in a one and two-step bioleaching process using two *Frankia* bacterial strains (*Frankia casuarinae* and *Frankia sp.*). They observed that *Frankia casuarinae* achieved the highest gold bioleaching efficiency of 75% compared to *Frankia sp.* In contrast, *Frankia sp.* achieved the highest copper recovery efficiency of 94% compared to *Frankia casuarinae* [55]. Arshadi and Mousavi [56] investigated the biocyanidation of gold and copper simultaneously from computer e-waste using the central composite design of response surface methodology (CCD-RSM) method to achieve maximum metal bioleaching efficiency and the optimum conditions. The authors evaluated four factors that affect bioleaching activity using *Bacillus megaterium* in a 250 mL stirred tank reactor and achieved maximum gold extraction efficiency of 36.8% at an initial pH of 10 and 2.5% *w*/*v* pulp density and glycine concentration of 0.5 g/L. The authors pre-treated the e-waste with *A. ferrooxidans* to bioleach copper as a first step, while the second step involved the extraction of gold by cyanide-producing *Bacillus megaterium* at an extraction efficiency of 63.8% [56]. Pourhossein et al. [54] investigated the bioleaching of precious metals from spent light diode lamps (LED) using *A. ferrooxidans* as the first process to pre-treat LED e-waste to extract base metals and thereafter, used *Bacillus megaterium* to bioleach precious metals from the residue that was generated in the first process. In this study, *A. ferrooxidans* bioleached 80% Cu, 94% Ni, 93% Sn, 68% Al, 51% Pb, 46% Cr and 35% Fe, whereas *Bacillus megaterium* produced a maximum cyanide concentration of 15 g/L and achieved a high dissolution efficiency of 93% Au, 91% Ag, 98% Ni, 87 Cu, and 84% Ga after 4 days. The authors also noticed that there was a low dissolution of precious metals when untreated LED powder was used [54]. These studies demonstrate the efficacy of using the two-step process for the bioleaching of base and precious metals [57,58], which is proposed in the subsequent section as the only viable extraction process of base and precious metals from e-waste. These studies are summarised in Table 3.

Table 3. The bioleaching of metals from e-waste by different biological approach.

E-Waste Material	Parameter Investigated	Microorganisms Used	Reactor Type	Bioleached Metals	Noteworthy Findings	References
PCBs (particle size less 100 µm)	Pulp density (1 to 15 g/L)	*Acidithiobacillus ferrooxidans*	Stirred tank (130 rpm and 30 °C)	Ni and Cu (99%) at day 11 and 98% at day 14	Noted that *A. ferrooxidans* adopted at high pulp density	[59]
PCBs (particle size less 150 µm)	Pulp density (10, 50, 100 g/L), Glycine (2.5, 5, 7.5, 10 g/L), Temperature (25, 30, 35, 40), and pH (7, 8, 9)	*Pseudomonas balearica*	Stirred tank (150 rpm)	Au and Ag (68.5% and 33.8%)	Further increase in pulp density (100g/L) decreased the bioleaching efficiency and the optimum conditions were 10 g/L pulp density, 5 g/L glycine, pH 9, and 30 °C	[60]
PCBs (personal computer) (37 to 149 µm particle size)	E-waste concentration (1.5, and 10% v/w)	*Chromobacterium violaceum*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*	Stirred tank (150 rpm and 30 °C)	Cu, Au, Zn, Fe and Ag,	Discovered higher bioleaching efficiency of 73. 17% with mixture of *P. aeruginosa and C. violaceum* and 69.3 with *C. violaceum* alone and its seems to have higher tolerance in metal toxicity as metal concentration increased from 1 to 10% v/w	[41]
PCBs (computer)	35 g/L pulp density, pH controlled at 1.8	*Acidithiobacillus ferrooxidans* (10% v/v) and mixed of bacteria from AMD	Stirred tank (30 °C, 170 rpm)	Cu (92%)	Cu recovered at high pulp density with significant bioleaching efficiency	[61]
PCBs	0,5 g pre-treated PCBs powder, pH (7, 8, 9), 10 g/L mixture glycine and methionine (2, 2.5, 5, 10 g/L)	*Pseudomonas fluorescens*	Bubble tank (30 °C, 150 rpm, and air at 100 mL/min)	Au (54%)	Discovered that the pH of 9 achieved the highest Gold bioleaching efficiency compared to pH of 7 and 8, respectively, and the Gold bioleaching efficiency decreased when the glycine substrate is 20 g/L	[62]
PCBs	Ground PCB concentrations of 1, 5, 10 and 20% (w/v) and pyrite as source of lixiviant	*Acidithiobacillus caldus*, *Leptospirillum ferriphilum*, *Sulfobacillus benefaciens and Ferroplasma acidiphilum*	Stirred tank (150 rpm and 37 °C)	Cu, Cr, Ni, Sn, Zn	Discovered that the pulp density of 5% and above have a significant negative impact on bioleaching efficiency	[63]

6. Proposed Process for Future Work

To date, the majority of research has focused on the extraction of base metals from e-waste in a one-stage process. Recent studies have focused on the bioleaching of both base and precious metals in a one-stage and two stage process. However, there have been no studies that have focused on the optimisation of cyanide production from cyanogenic microorganisms. Even though a couple of studies have indicated that a glycine concentration that is above 10 g/L inhibits microbial growth [61], this has not been tested with various cyanide producing organisms, therefore, it can only be limited to the study that was undertaken by the mentioned authors. In addition, no studies have been conducted to evaluate different parameters such as pH, temperature, pulp density, bacterial growth, particle size, and agitation speed in the bioleaching of precious metals using cyanogenic microorganisms. Therefore, these parameters need to be taken into consideration when assessing the efficacy of the organisms that produce cyanide for extraction purposes. Most studies have focused on the utilisation of *Bacillus* sp. to produce cyanide, therefore there is a need to explore various organisms such as *Pseudomonas* sp. and the co-cultures of *Bacillus* sp. and *Pseudomonas* sp. for maximum cyanide production. This will in turn result in higher extraction efficiencies of rare earth and precious metals.

Furthermore, the majority of acidophilic metal extraction studies using microorganisms have been carried out in batch processes, and for future studies, continuous processes for the bioleaching of base and precious metals from e-waste should be undertaken, where the first stage will consist of an acidophilic microbial community which will be responsible for the extraction of base metals, while the second stage will consist of cyanogenic organisms for the extraction of precious and rare earth metals, as proposed in Figure 2 However, before metals can be extracted from the PCBs, there would need to be a separation process that is undertaken, since PCBs contain numerous components. Wang Zhao [64] demonstrated the physical-mechanical separation processes that take place prior to the metal extraction process. Figure 2 proposes a process where the acidophilic iron and sulphur oxidising mixed culture (stage 1) is utilised for the extraction of base metals from pulverised PCBs using pyrite as a substrate to produce the lixiviant and as an energy source for the organisms. Bryan et al. [60] observed that the addition of pyrite in the bioleaching of PCBs resulted in improved microbial activity, which in turn supported the extraction of metals by producing the lixiviant. After the extraction period, the base metals can be recovered and the resultant residue will proceed to a different stage where the precious and rare earth metals would be extracted. The second stage involves the utilisation of *Bacillus* sp. and *Pseudomonas* sp. co-cultures to produce cyanide, which would be transferred to the third stage where the residue that was produced in the first stage would be mixed with the produced cyanide to extract precious and rare earth metals. After the extraction process, the metals of interest can be recovered using existing processes (i.e., electrowinning).

However, the challenge lies with the resultant residue, which contains a high content of silica. The silica content has been shown to vary depending on the particle size from 7.26 to 18.85 wt%, with a particle size of +1 to −0.09 mm [63] This residue also poses an environmental burden, therefore processes that make use of the resultant residue must be considered when extracting metals from PCBs. One of the materials that could be synthesised from the residue are silica materials, due to the silica content of the residue. Silica materials such as mesoporous materials and nanoparticles can be synthesised from the residue, depending on the intended application. In a review article by Díaz de Greñu, de los Reyes [65], the authors described in detail how these nanomaterials can be synthesised from various silica sources, including their applications, which range from environmental catalysis, adsorption and drug delivery. For example, in their medical application, MCM-41, which is a silica nanomaterial, has been shown to be cytotoxic against various cell lines such as human neuroblastoma (SK-N-SH) cells [64] and Chinese hamster lung fibroblasts (V79 cells), with respect to amorphous silica nanomaterials [66]. The vast application of silica nanomaterials makes them an ideal material that can be synthesised from the residue of PCBs.

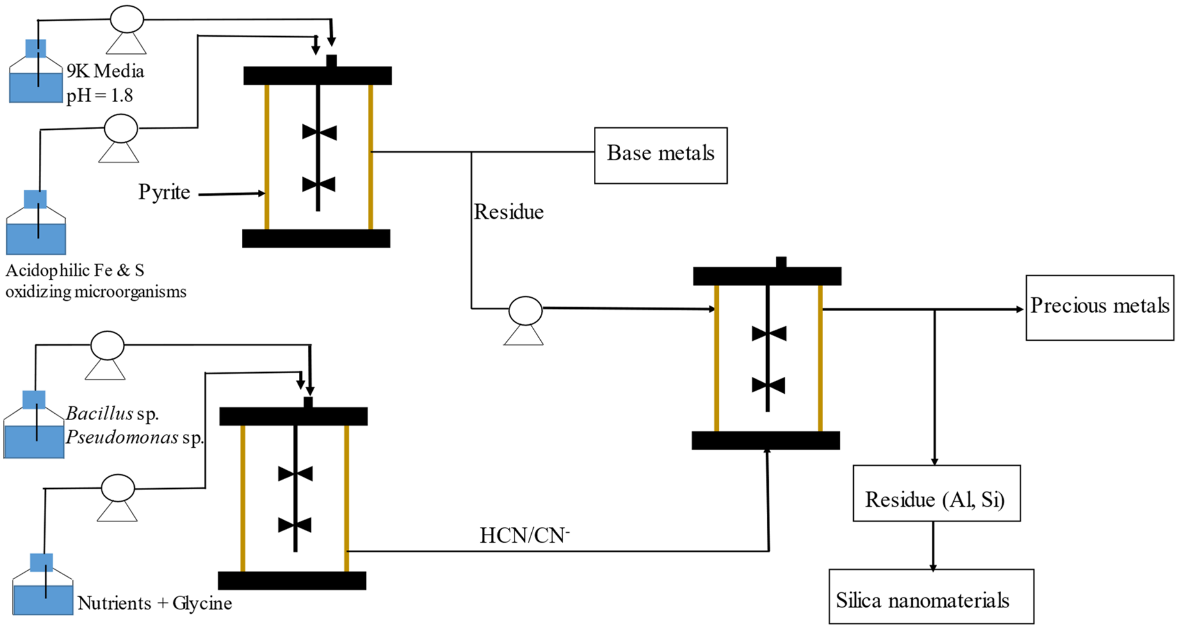

Figure 2. Proposed metal extraction process from electronic waste.

Another challenge associated with the proposed process is how cyanide would be managed. Since cyanide is a toxic compound, the residual cyanide including its complexes needs to be remediated after the extraction process. This can be achieved by utilising the cyanide producing organisms *Bacillus* sp. [67,68] and *Pseudomonas* sp. [69,70] for the bioremediation of cyanide. These organisms have been previously shown to be effective cyanide degraders and, hence, they can be utilised in a separate process where the cyanide-containing wastewater would be remediated.

7. Conclusions

The emerging challenges that facilities face for the recycling/recovery of metals from e-waste are technology challenges. Traditional technologies such as hydrometallurgical and pyro-metallurgical processes have been used for a decade to recover metals from e-waste. However, such technologies pose a high environmental risk; hence, these technologies are considered not sustainable due to the high operation cost, production of greenhouse gases, and the production of secondary waste. New technologies that are sustainable, cost-effective, and environmentally friendly, such as the bio-hydrometallurgical process, must be investigated effectively and adequately to recover metals from e-waste, especially the recovery of precious metals using cyanogenic microorganisms. The recovery of metals from e-waste using acidophilic microorganisms has been adequately investigated, and the process is well understood, while the two-step bioleaching of precious metals from e-waste using cyanogenic microorganisms still requires intensive research to be well understood. In light of this, this review proposes an integrated approach to the extraction of metals (base, rare earth and precious) from PCBs, where the first stage will consist of iron and sulphur oxidising microorganisms, which will facilitate the extraction of base metals, while the second stage will consist of cyanogenic organisms, where cyanide will be produced by predominantly *Bacillus* sp. and *Pseudomonas* sp. and this cyanide will be mixed with the residue from the first stage so that rare earth and precious metals can be extracted. It has been proposed that the resultant residue after the extraction process, which is high in silica content, should be utilised for the synthesis of silica nanomaterials, which could applied in various fields such as environmental engineering and in the medical field.

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