recycling

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Review

Bioleaching of gold from printed circuit boards: potential sustainability of the thiosulphate

Zahra Ilkhani [1], Farid Aiouache [1,*]

1 Lancaster University, School of engineering, LA1 4YR, UK; z.ilkhani@lancaster.ac.uk
2 Lancaster University, School of engineering, LA1 4YR, UK; f.aiouache@lancaster.ac.uk
* Correspondence: f.aiouache@lancaster.ac.uk; Tel.: (+441524593526)

Abstract: The rapid consumption and disposal of electronic waste due to technological innovations and the changes in the living commodities are developing a significant environmental challenge. Among the components of these wastes, the spent printed circuit boards are particularly considered among the most valuables owing to their content of precious metals like gold first and potentially platinum available in less proportion. Effective methods as parts of gold recovery strategies by the industries and the policymakers are developed and envisioned from economic and environmental perspectives. Currently, the cyanidation dominates the global gold production from e-waste due to its selectivity for gold. The high toxicity of cyanide however poses serious environmental issues, leading the thiosulphate leaching to emerge as a non-toxic and promising alternative for gold extraction. Its industrial viability has been demonstrated by Barrick Gol Corporation at the Goldstrike site with the pretreatment of acidic or alkaline pressure oxidation. This review introduces bioleaching as a promising economic and environmentally friendly process for the gold extraction. The review explores the thiosulphate leaching of gold as an alternative to the conventional cyanidation with a particular interest in the bio-thiosulphate production by adapted microorganisms. The factors that affect the pretreatment, the reaction chemical mechanism and the design engineering are discussed. The consumption of thiosulphate was identified as one of the main challenges, restricting the reliability of the process. Various solutions for the reduction of its consumption and relevant process costs were discussed with a particular exam of the engineering aspect from the process design and the scalability to industrially relevant operating conditions by using bioreactors adapted to large pulp density loads of electrical waste.

Keywords: Bioleaching, Waste printed circuit boards, Gold, Thiosulphate, Metal recycling

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1. Introduction

Nowadays, the increasing reliance of modern lifestyle on electronic devices with relatively short replacement cycles has led to a substantial rise in the accumulation of electrical waste (e-waste) [1]. According to statistics by [2], 40 million tons of e-waste are produced annually, which makes up around 5% of the global solid waste. In 2019, Asia produced 46.6% of the world's total e-waste, with America ranking the second highest with

24.4% e-waste generation, followed by Europe (22.4%), Africa (5.4%), and Oceania (1.3%) [3]. [4] reported a production of 52.2 Mt global e-waste in 2021, which is expected to exceed 74.7 Mt by 2030 and 120 Mt by 2050. Despite this rise, only a proportion of 10–15% of generated e-waste is currently recycled, and the rest is stored or deposited in landfills often with reduced strategies for sustainable recovery of the metals they contain, leading to unprecedented contamination of the environment and risks of diseases such as cancer, kidney and heart malfunctions and brain swelling [5]. As a result, landfilling is considered the least option for e-waste destinations and there is an urgent need to recover metals from e-waste in order to mitigate the environmental contamination, promote the economic advantages as a secondary source of precious metals and save consumption of the natural ores [6].

Among e-waste, printed circuit boards (PCBs) contribute to approximately 3% of the total e-waste weight while noting that mobile phones and computer PCBs have been widely studied owing to their high disposal volumes and precious metal contents [7]. They are utilized as random-access memory motherboards and network interface cards to facilitate electrical and mechanical connections, and their compositions can vary depending on the technology, the year of fabrication, and the manufacturer brand but they are typically composed of a metallic portion (40%), non-metallic components such as plastics (30%) and ceramics (30%) [8]. They consist of a lamination layer of copper-clad fiberglass reinforced with epoxy resin material and other essential components such as capacitors, resistors, microchips and diodes. The hazardous components of PCBs include heavy metals (Pb, Hg, Cd, As, and Cr), polychlorinated biphenyls, polybrominated biphenyls and epoxy resins, which pose environmental challenges. Generally, PCBs contain about 10-20% Cu, 7% Fe, 5% Al, 3% Sn, 1-3% Ni, 1.5% Pb, 25% organic compounds, precious metals such as 200-3000 ppm Ag, 20-250 ppm Au, and 10-200 ppm Pt, which are used because of their favourable electrical conductivity, chemical stability, and resistance to oxidation, corrosion and acids [9]. The worth of Au and Ag in discarded PCBs in 2022 was estimated at $54,514.97/kg – $65,714.28/kg and $592.22/kg – $866.13/kg, respectively. Although their total weight is less than 1% in PCBs, they are still precious and financially important [10].

Every year, around 17 million PCBs are discarded, which is equivalent to the disposal of nearly 0.5 million tons of waste PCBs. In computer PCBs, the concentrations of Cu and Au reach 20–40 times and 25–250 times, respectively, more than natural ores and have the potential to be recovered from e-waste with less energy consumption and are seen as alternative sources to the natural ores [11]. As an example, 210 kg of Cu and 1.5 kg of Au can be recovered from one metric ton of waste PCBs while only around 5 g and 5.25 kg of Au and Cu can be recovered from relevant natural ores, respectively [3]. Thus, waste PCBs have drawn interest from industries and academia as a secondary metal resource to create efficient, sustainable, and economical metal recovery technologies [12]. Au is one of the first metals used by human civilization, even before 3400 BC, and has captivated attention for its wide-ranging applications (i.e., jewelry, high-tech industries, chemical processes, and medical applications) [13,14]. According to the U.S. Geological Survey, China leads the world Au production (420 tons), followed closely by Australia and Russia [15]. As a result, the growing demand for Au in different sectors and the depletion of natural resources make it essential to recover Au from alternative sources such as PCBs [14].

Pyrometallurgy and hydrometallurgy technologies are conventional methods that are still employed but face growing challenges due to issues linked to economic and environmental outlooks, including the emission of harmful gases, costs, energy requirements and

chemical contamination. Therefore, it is important to develop energy-efficient technologies, such as those that rely on bioprocessing technology operating at moderate conditions in terms of chemical and energy consumption [16-18]. Bioleaching as one of the bioprocess technologies relies on the ability of microorganisms to produce essential leaching agents such as organic acids (i.e., oxalic acid, citric acid, malic acid, gluconic acid, succinic acid and formic acid), amino acids (i.e., L-valine, glycine, DL-alanine, and L-histidine), biosurfactants such as Rhamnolipids (Rhls) produced from the bacterium *P. aeruginosa CVCM* to remove 11% Fe and 25% Zn at low concentration (0.4 mg/mL) and 19% Fe and 52% Zn at high concentration (1 mg/mL) [19], siderophore such as hydroxamate and catecholate mixed type pyoverdine PyoPpC-3B produced by the bacterium *P. putida PpF1* to selectively extract Zn and Mn from a primary and secondary mineral residue [20], chelating, and complexing agents and is becoming a promising technology for recycling e-waste because it is straightforward, requires less skilled labour, has reduced capital and operating costs and can be controlled at mild conditions. This process also reduces the costs of final disposal and residue treatment, creating an environmentally friendly waste stream while ensuring selective metal extraction [21]. Currently, about 15–25% of the world's copper production, 5% of Au production, and smaller proportions of Co, Ni, U, and Zn are produced by the bacteria-assisted leaching method [22]. For over a century, cyanide has been used as a cost effective and efficient agent for gold leaching. However, due to its high toxicity, thiosulfate has been proposed as a safer alternative. Past review papers have explored the principles of bioleaching including the methods and mechanisms, types of e-waste and microorganisms, and their role in the extraction of heavy and precious metals [23,24]. This review presents an update on the bioleaching methods for the extraction of Au from waste PCBs by revisiting the fundamental concepts and discussions involved in the most recent findings regarding the design methodologies, mechanisms, as well as the industrial perspectives and applications, contributing as a standing point to future directions. Thiosulphate-based leaching and associated challenges, the importance of engineering design and scale up in the bioleaching process, and its impact on cost reductions are discussed.

2. Pre-processing of PCBs for bioleaching

Typically, pre-processing is the initial step before recovering metals from waste PCBs. Dismantling various electronic components from PCBs is a crucial step in the recycling process. During the dismantling, priority is given to the removal of reusable or hazardous components such as batteries and cathode ray tubes in order to make the recovery of metals easier [10]. Selective removal of hazardous components from PCBs helps prevent toxic elements from entering the recycling process. Manual dismantling, oven heating and open burning are some common techniques for dismantling. However, some of these techniques are hazardous and might change the properties of waste PCBs and pose risks. For example, in the oven heating technique, certain toxic substances may be generated as a result of the high melting point of lead-free solders (270–280 °C). The development of semi-automatic and automatic machineries instead of manual dismantling methods is increasing the efficiency of the process and reducing the negative environmental impacts compared to oven heating, but the associated expenses remain high owing to the intricate geometries of PCBs of non-uniform structures, which highlights the application of manual dismantling.[25] estimated the costs for manual, mechanical and heating dismantling techniques, as illustrated in Table 1. Manual dismantling proved to be the most economical option when the quantity of waste PCBs was below 1,000 tons but as the quantity was increased, the mechanical dismantling and heating became more advantageous due to the high labour costs of manual dismantling. When the amount of waste PCBs exceeds 5,000

tons, the heating dismantling is considered the most cost-effective method [10,24]. A crushing stage is necessary to make the further handling of PCB waste easier. The size reduction follows and is done by cutting PCBs into small pieces (1-2 cm^2) by means of shredders or granulators. Further reduction of PCBs (5-10 mm) is achieved using ball mills, centrifugal mills, cutting mills and ring mills [26]. The next step that follows the pre-processing involves the recycling of waste materials in an economical and environmentally sustainable manner [24]. The production of fine dust during the crushing and grinding is a significant difficulty that can be challenging to control the process [27]. After removal of the hazardous components, different mineral processing unit operations such as shredding, crushing and grinding can be used to liberate metals from cladding materials such as resin, fiberglass and plastics. Various types of hammer crushers, rotary crushers, disc crushers, shredders, cutters equipped with a bottom sieve, are used for liberation. As the PCBs are made of reinforced resin, copper wires and glass fibers (multilayer), the conventional crushers may not achieve good liberation. In contrast, shredding or cutting, which works on the principle of shearing, is found to be more useful. Unlike mineral ores, PCBs do not have a particular size fraction for liberation; instead, different types of elements are liberated at different size fractions. Figure 1 outlines the pre-processing steps involved in preparing PCBs for bioleaching.

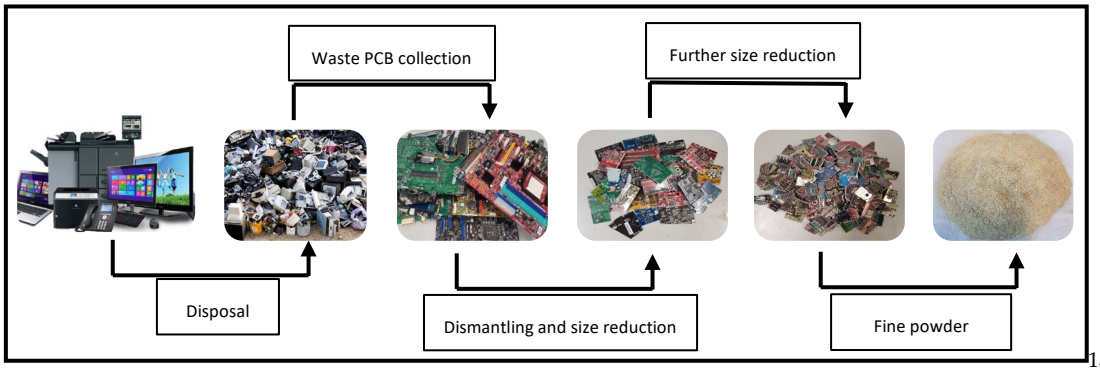

Figure 1. Pre-processing of the waste PCBs before the bioleaching.

Table 1. Cost of the dismantling methods [25].

Method	Cost ($) (below 1kt)	Cost ($) (5kt)	Cost ($) (10kt)
Manual	~500-1000	~350,000	~400,000
Mechanical	~50,000	~110,000	~350,000
Heating	~60,000	~90,000	~150,000

3. Bioleaching

3.1 Bacteria used for bioleaching and mechanism

The primary difference in how autotrophic and heterotrophic microorganisms leach metals lies in their energy sources and metabolic activities. Autotrophic bacteria, commonly known as sulphur-oxidizing and iron-oxidizing bacteria, which are capable of growing in acidic conditions, are the most prevalent [28]. During the bioleaching process, these microorganisms facilitate the oxidation of ferrous ions to ferric ions and elemental sulphur to sulphuric acid (as shown in Equations (1) and (2)). The biogenic ferric iron and sulphuric acid act as oxidizing agents (lixiviants) for the solubilisation of metals from e-wastes (Equation (3) and (4) [29]. *A. ferrooxidans* and *A. thiooxidans* are the most used

bacteria because they resist to the contamination by other microorganisms, tolerate the high concentrations of the heavy metals, the low nutrient requirement and can grow under extremely acidic pH conditions[5]. These bacteria are able to capture electrons from the substrates for their metabolic activities to release the heat and solubilise the metals without relying on external energy sources to facilitate the process [30]. The heterotrophic microorganisms, including fungi and bacteria, are extensively used in the bioleaching due to their ability to metabolize the organic compounds as energy sources and their adaptability to high pH levels and complex metal contents. The fungal species such as *Penicillium*, *Aspergillus*, *Trichoderma*, *Saccharomyces*, and *Phanerochaete* produce organic acids (i.e., citric acid, gluconic acid, oxalic acid, and formic acid) as a leaching agent, which facilitate the mobilization of metals from e-waste (Equation (5)). Additionally, these organic acids bind with metals to form stable complexes that significantly enhance the metal solubility in the leaching solution [31,32]. There are three main mechanisms that occur between microorganisms and e-waste during the bioleaching, namely the acidolysis (acid formation), the redoxolysis (microbial-driven oxidation and the reduction reactions), and the complexolysis (biogenic complexing agents). In the acidolysis, the oxygen atoms present in a metal oxide form interact with water molecules to facilitate the solubilisation of the metals into a leaching solution. During the the acidolysis, a number of sulphur-oxidizing autotrophs such as *A. thiooxidans*, *A. caldus*, *S. thermosulfidooxidans*, as well as heterotrophic bacteria species like *Bacillus*, *Pseudomonas*, and *Chromobacterium* and fungi including *Aspergillus* and *Penicillium* consume nutrients to produce a range of acids such as sulphuric, gluconic, acetic, malonic, oxalic, lactic, pyruvic, succinic and formic acids that facilitate the metal solubilization by maintaining low pH and reducing the anion availability. The acidolysis is a fast and effective method commonly used to extract metals like Zn, Ni and Cu from the waste [18,33]. In the redoxolysis mechanism, the metals are dissolved via the oxidation-reduction reactions. Through the electron transfer, the redoxolysis facilitates the supply of energy required for the microbial growth [2]. Iron plays the major role as an electron carrier. After the microbial oxidation of Fe^{2+}, Fe^{3+} acts as an oxidizing agent that is able to solubilise the metals such as Cu into Cu^{2+} and then can be chemically reduced to Fe^{2+} through the redox reactions. Subsequently, Fe^{2+} is re-oxidized to Fe^{3+} by the metabolic activity of the microorganisms [34]. Fe^{3+} ion is recognized to be an affordable oxidizing agent in the hydrometallurgical processes and has been proven to be the promising choice for the extraction of various metals from PCBs. The commercial-scale bioleaching commonly uses a combination of the redoxolysis by biogenic Fe^{3+} and the acidolysis by biogenic sulphuric acid for metal recovery of e-waste. In the complexolysis, the target metals interact with the ligands like cyanide, organic acids, or siderophores to form stable metal-ligand complexes. The complexolysis is used to leach the metals such as Au, Ag, Fe, Al and Pt by heterotrophic bacteria and fungi like *C. violaceum*, *P. fluorescens*, *B. megaterium*, *P. aeruginosa*, *A. niger*, etc. The common example of these metal-ligand complexes includes citric acid with Mg, oxalic acid with Al and Fe, and cyanide with Au, Ag and Pt [35].

$$S^0 + \frac{3}{2}O_2 + H_2O \rightarrow H_2SO_4 \qquad \Delta G° = -507\ kJ/mol \tag{1}$$

$$2Fe^{2+} + O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \qquad \Delta G° = -88.8\ kJ/mol \tag{2}$$

$$2Fe^{3+} + M \rightarrow M^{2+} + 2Fe^{2+} \tag{3}$$

$$H_2SO_2 + MS \rightarrow H_2O + MSO_4 \quad [M = Cu, Al, Mn, \dots] \tag{4}$$

$$M + H^+ + O_2 + H_2O \rightarrow M^{n^+} \tag{5}$$

3.2 Bioleaching methods

The bioleaching process is carried out through direct and indirect methods. The direct method is classified into the one-step and the two-step methods while the indirect method involves the spent medium [36]. The one-step method requires the addition of the samples of e-wastes samples and microorganisms directly into the sterile culture media at the same time, which allows the bacteria to grow in presence of waste. The intricate composition of e-waste and presence of toxic metals however may hinder microbial activity during the leaching process, decreasing the bioleaching effeciency. A study for instance has shown that in one-step bioleaching method, the growth of *Frankia spp.* decreased as the concentration of waste PCBs increased due to the lack of secondary metabolites and the decreased toxic effects of e-waste [37]. In the two-step method on the other hand, the microorganisms grow first without any addition of e-waste. In fact, microorganisms are cultivated in a culture media under adapted environmental conditions suitable for their activity until they reach the logarithmic growth stage [33]. The e-waste samples then are introduced into the microbial culture media. In contrast to the one-step method, the two-step method can notably mitigate the inhibition of microbial growth caused by e-waste and enhance the bioleaching efficiency. Since the microorganisms are still present in the two-step method, the toxicity of e-waste may partially affect the microbial growth and constrain the flexibility of the process compared to the spent medium method [34]. In the indirect leaching method, the microorganisms are cultivated until they reach the growth phase and produce their metabolites. After the metabolite production, the bacteria are filtered by centrifugation and removed from the culture medium to obtain a cell-free medium while the e-waste samples are added to the spent medium. This method eliminates the harmful effects of the e-waste on microorganisms and provides better control throughout the operation conditions [35]. Unlike the one-step and two-step methods, the spent medium method enables an independent optimisation of the biological and the chemical process such as a shortening the leaching process, increasing of temperature and rotation speed free of the shear limitation on the bacteria, which enhances the mass transfer rate and metal recovery [36]. For example, in a study by [32], fungal leaching of metals from the spent lithium-ion phone mobile batterie by *A. niger* was conducted under one-step, two-step and spent medium and the maximum leaching efficiency of Cu (100%), Li (95%), Mn (70%), Al (65%), Co (45%), and Ni (38%) was obtained at a pulp density of 1% in spent medium method. Another study reported that increasing the pulp density led to a decline in the bioleaching efficiency of Au to 11.3% by the bacterium *C. violaceum* at 0.5% pulp density when using the two-step method compared to 18% by the spent medium method [38].

3.3 Effective factors during the bioleaching process

Many studies have shown that factors such as pH, temperature, pulp density, microorganism type, nutrient and aeration affect the bioleaching efficieny. Maintaining an optimal pH in the culture medium is crucial for enhancing microbial activity and metal solubilization during bioleaching. The pH influences both the effectiveness of metal leaching and the stability of metal ions in solutions. The acidophilic bacteria perform best at a pH level 2.0-2.5. Although *A. ferrooxidans* can tolerate pH levels below 2.0 but higher pH levels (above 2.5) reduce the bioleaching efficiency due to the precipitation of the ferric iron as jarosite and the bacterial attachment to these compounds, which hinders their activity. Temperature plays a key role in the bioleaching, as different microorganisms

thrive at specific temperature ranges. The mesophilic acidophiles typically grow at temperature between 25–30 °C, while thermophiles are grown at 40–45 °C. Fungi generally grow at temperature within a range of 25–35 °C, with *A. niger* showing optimal performance at 25 °C [18,35]. The microorganisms involved in the bioleaching process are typically aerobic, meaning they require oxygen for their metabolism and growth. In the laboratory, the aeration is provided using shaker incubator, with an agitation speed typically maintained between 120 and 145 rpm for bacterial cultures. An excessive agitation however can cause physical stress on the bacteria and negatively affect their activity [23,39]. The effectiveness of microbial leaching is significantly influenced by the composition of the culture medium, as it directly affects the metabolic activities of microorganisms. This medium includes both organic and inorganic nutrients that support microbial growth. For the cultivated cyanogenic bacteria used in leaching valuable metals from e-waste, the nutrient-rich medium containing organic components like peptone, yeast extract, glycine, and amino acids is typically employed. The acidophilic bacteria however are grown in a chemically defined medium that supplies essential nutrients such as ammonium sulfate, dipotassium hydrogen phosphate, magnesium sulfate, iron sulfate, and elemental sulphur [40]. The pulp density, which is defined as the weight ratio of the solid material (i.e., e-waste) to liquid in a solution (i.e., leching agent), is a key factor affecting the effectiveness of the metal leaching. High pulp densities increase the toxicity, reduce the oxygen transfer, and inhibit the microbial growth, leading to a reduced leaching efficiency. Although some microorganisms, like acidophilic bacteria, tolerate heavy metals, their activity decreases at high pulp densities due to a limited presence of oxygen and increased medium viscosity. Several studies have identified 1% (w/v) as an optimal pulp density for e-wastes. For example, [41] found that the consortium of *C. violaceum*, *P. aeruginosa*, and *P. fluorescens* were able to leach 69.3% Au from waste PCBs at a pulp density of 1%, but the leaching rate decreased to 20.28% when the pulp density was increased to 10%. The pulp density is also one of the important parameters that affects the reactor design, the culture media consumption and the operating cost. When the pulp density is for instance increased from 1% to 10%, the volume of the liquid phase and the size of the reactor decrease notably by 90%, leading to a substantial decline in the operation cost [2]. According to [42], there was a significant drop in operating costs when the pulp density was increased from 1% to 5% with pH adjustment, which led to an annual profit of AUD $ 2749 per ton of PCBs. In spent medium bioleaching, the optimization of the operating parameters such as temperature, agitation speed, and pulp density are critical. Unlike the one-step and the two-step methods, the absence of live microbial culture allows for broader flexibility in the parameter selection. In this context, maximizing metal leaching efficiency becomes the primary focus, independent of conditions required to sustain the microbial viability. For example, high temperatures or agitation speeds may be used to accelerate reaction kinetics without concern for microbial tolerance, enabling greater adaptability and a potential for scale-up to industrial applications.

3.4 Progress in the culture growth

The toxic metals present in e-waste can negatively impact the microbial growth, metabolism, and survival during the leaching process. To maintain a sufficient and active microbial population in the solution, it is often necessary to use the microorganisms that are resistant or adapted to these toxic conditions. Therefore, before starting the bioleaching, the microorganisms should undergo a gradual adaptation process. This involves serial sub-culturing over time with a gradually increasing the pulp density of the waste material, allowing microbes to increase their tolerance to levels of the metal toxicity

[17,43]. For instance, [30] studied the bioleaching of base metals from PCBs using adapted *A. ferrooxidans*. The adaptation process involved stepwise increases in waste concentration from 1 to 15 g/L over 187 days in a flask, followed by further adaptation in a bubble column bioreactor with waste concentrations raised to 40 g/L over 44 days. Under the optimal conditions with a solid content of 20 g/L, this approach resulted in 54% of Cu, 75% of Ni and 55% of Fe recovery after 9 days. Although microorganisms have been widely used across various scientific and industrial fields, most research in the bioleaching focused on pure microbial cultures, which often lack in terms of adaptability, efficiency, and their ability to manage complex substrates. The mixed cultures, which consist of two or more microorganisms in a consortium of culture media offers a more effective approach for bioleaching compared to the pure culture. Species like *A. thiooxidans*, *A. ferrooxidans*, *A. caldus*, *L. ferrooxidans*, and *L. ferriphilum*, have been combined for the extraction of metals in bioleaching processes to form biofilms that generate microenvironments, enhancing leaching kinetics. Although, the precise mechanisms that illustrate the interaction within these communities remained weakely known, the growing prevalence of mixed cultures in the bioleaching is partly due to the presence of diverse metal ions in ores and soils, which cannot be effectively processed by pure cultures. The applications of mixed cultures of microorganisms in bioleaching demonstrated that these cultures can bioleach the concentrate more rapidly and extensively than pure cultures [44,45]. For example, in one study, the Cu leaching in a mixed culture of *A. ferrooxidans* and *A. thiooxidans* was 10% higher than in pure cultures [46].

The use of genetically modified organisms (GMOs) has emerged as a promising approach to improving the efficiency of the bioleaching process. Through genetic engineering, the microorganisms can be designed to tolerate and adapt to high metal concentrations, enhancing the metabolic activity under optimal conditions, and significantly reduce the time needed for metal extraction. Recent advancements in the genetic engineering, along with progress in DNA synthesis, sequencing, and integrated -omics technologies (such as genomics, proteomics, transcriptomics, and metabolomics), are providing new opportunities to develop high-performing engineered microorganisms suitable for enhanced leaching performance and resilience in harsh environments [40]. For example, the genetic engineering has been successfully used to enhance arsenic bioleaching by *A. ferrooxidans* TFBk [47]. In another study, Tay et al. observed an improved Au recovery using two genetically modified strains of *C. violaceum*, named pBAD and pTAC, with a leaching of 30% and 25% of Au, respectively, compared to only 11% leaching rate by the non-modified microorganism [48].

4. Precious metal leaching

4.1. Cyanide-based leaching

The recovery of precious metals like Au and Ag from PCBs has been given much attention as they are utilized in different industries such as electronic industries owing to their stability and conductivity [49]. [50] reported a global demand of Au in electronic industries of 254 tons in 2015, which highlights the importance of the recovery of Au through sustainable methods. Cyanidation was introduced by John Stewart MacArthur in the 1880s, and since then it has been the primary method for Au leaching due to its efficiency for the selective recovery of Au [51]. Cyanide refers to inorganic compounds containing a cyano group (C-N), which are highly toxic, particularly at temperatures above 25.6°C. The total reaction of precious metal dissolution by cyanide is represented

in Equation (6). Although cyanide has been the main leaching agent for Au extraction, it has some drawbacks such as absence of possible solutions for cyanide regeneration as well as the environmental concern owing to the cyanide leakage into groundwater and the overall management of hazards associated with its toxic nature [52]. According to statistics by [53], cyanide leakage from metallurgical plants caused some serious accidents. For instance, in 1995 in Guyana, a tailings dam at the Omai mine collapsed and discharged approximately 2.9 million m3 of cyanide-laced tailings, which contaminated the nearby Omai River. Another accident happened in 2000 in Baia Mare, Romania, when the collapse of Aurul S.A. tailings dam released a swage containing up to 100 tons of cyanide, which finally flowed into the Danube River. Gold leaching with a bio-cyanide solution has emerged as a cost-effective, simple, and efficient alternative to traditional cyanidation methods. Recent studies have shown that bacteria such as *C. violaceum*, *Pseudomonas* species, *E. coli*, and *B. megaterium* can produce biological cyanide. But still the high consumption of cyanide by Cu poses challenges. To address this issue, certain autotrophic bacteria have been employed to target the base metals and remove them selectively in the first step in order to facilitate the leaching of precious metals [35]. Several studies have reported high Au extraction rate after Cu removal. For instance, in the study by [54], 98.4% of Cu was extracted by *A. ferrivorans* and *A.thiooxidans* under acidic condition (pH 1-1.6) and room temperature after 7 days. In the second step, *P. putida* was employed, and 44% Au was solubilised under alkaline condition (pH 7.3-8.6) after 2 days. In another study, PCBs were pretreated with bio-oxidation using *A. ferrooxidans* and over 80% of Cu removal enhanced the Au/Cu ratio in the residual PCBs. Before the pretreatment, the Au leaching rate was only 20.8%, which doubled to 40.1% after the treatment [55]. Table 2 summarises some previous studies on the bioleaching of precious metals from e-waste by different microorganisms. The cyanide-producing microorganisms are the main source for the selective leaching of Au and Ag from waste PCBs.

$$4M + 8CN^- + O_2 + H_2O \rightarrow 4M(CN)_2^- + 4OH^- \quad [M: Ag, Au] \tag{6}$$

Table 2. overview of the previous studies on bioleaching of precious metals under different conditions.

Bacteria	Waste	Leaching (%)	Leaching condition	Leaching agent (mg/l)	Reference
P. balearica *SAE1*	PCBs	Au: 68.5 Ag: 33.8	Pulp density: 1.5%, 30℃, 7 d	Glycine: 5000	[56]
P. Chlororaphis	PCBs	Au: 8.2 Ag: 12.1	Pulp density: 1.6%, 25℃, 72 h	Cyanide: 8.71	[57]
C. violaceum	PCBs	Au: 11.3	Pulp density: 1.5%, 30℃, 8d	Cyanide: 68	[58]

C. violaceum	Landfill of e-waste	Au: 16	Pulp density: 0.5%,30℃, 8d	Cyanide: 5-15	[59]
p. biofilm	PCBs	Ag: 14.7	Pulp density: 2%, 25℃, 7 d	Cyanide: 5	[60]
F. casuarinae	PCBs	Au: 75	Pulp density: 0.2%, 28℃, 30 d	Biomass: 3620	[37]

4.2. Thiosulphate-based leaching

Thiosulphate ($S_2O_3^{2-}$), a sulphur oxyanion with a tetrahedral structure is considered among the most promising alternatives to cyanide that has numerous benefits compared to various cyanide and non-cyanide leaching agents, including low corrosivity, stability, rapid leaching kinetics, operability in a safe workplace and lower risk of environmental pollution [61]. Because of its efficiency, thiosulphate is recognized as a highly effective reagent for extracting Au from waste PCBs [51]. The first report on the use of thiosulphate in precious metal recovery dates back to 1905, when Au was extracted from ores by employing ammonia-thiosulphate leaching [62]. The overall reaction for the thiosulphate leaching of Au is represented by Equation (7). After the formation of the Au(I)-thiosulphate complex, a slightly acidic to highly alkaline condition is needed to prevent the decomposition of the complex and ensure its stability in solution [14]. Thiosulphate-based leaching requires an oxidant to facilitate the Au solubilisation. Cu^{2+} is one of the prevalent oxidants that accelerate Au dissolution by 17−20 times. The high redox potential of Cu^{2+}/Cu^{+} however results in a high thiosulphate consumption which is often moderated by presence of ammonia to form the cupric ammonia complex ($Cu(NH_3)_4^{2+}$) as a stable catalyst to weaken the interaction between Cu^{2+} and thiosulphate as well as to reduce copper hydroxide precipitation [63-65]. Additionally, ammonia has a non-negligible role in reducing the formation of the passivation layer on the Au surface and enhancing the leaching kinetics [66].

$$4Au + 8S_2O_3^{2-} + O_2 + 2H_2O \rightarrow 4Au(S_2O_3)_2^{3-} + 4OH^- \qquad \Delta G° = -97.9\ kJ/mol \qquad (7)$$

4.2.1. Thiosulphate leaching methods

4.2.1.1. Ammonia- based method

There are two main methods of thiosulphate leaching: non-ammonia and ammonia-based methods. The ammonia-based methods consist of copper ammonia, nickel ammonia and cobalt ammonia leaching. Ammonia accelerates the dissolution of Au, stabilises thiosulphate and maintains the pH level [64]. In an ammonia-based thiosulphate system, Cu^{2+} acts as a redox mediator to facilitate Au oxidation through both anodic and cathodic reactions [15]. In the anodic area (Equation (8)-(10)), NH_3 moves toward the Au surface and forms a complex with Au+ ($Au(NH_3)_2^{+}$). NH_3 is then replaced by $S_2O_3^{2-}$ and a more stable complex is formed ($Au(S_2O_3)_2^{3-}$). Although Au can form different complexes with ammonia and thiosulphate, $Au(S_2O_3)_2^{3-}$ is the most stable complex in ammonia-based

system. In the cathodic area, $Cu(NH_3)_4^{2+}$ is reduced to $Cu(S_2O_3)_3^{5-}$ and rapidly oxidized back to $Cu(NH_3)_4^{2+}$ by dissolved oxygen (Equation (11)-(12)). The values of associated Gibbs free energy offer an overview of the level of spontaneous reactions to occur with reference to the chemical equilibrium. While most values support the forward path, the spontaneous solubilisation of the metal gold for instance (equation 8) is not favoured. In one study, around 98% of the Au was solubilised from 1% (w/v) PCBs by using ammonium thiosulphate as leaching agent [55]. In another study, 1M ammonium thiosulphate was utilized to extract around 91% Au from 1% (w/v) PCBs after 24 h [67]. [68] compared thiosulphate and thiourea for Au dissolution and reported approximately 70% of Au from PCBs with different concentrations of ammonium thiosulphate (0.08 to 0.12 M) at 20 ℃ and pH of 10.5 while only 40% dissolved Au with thiourea. Table 3 summarises some key information on the ammonia-based thiosulphate leaching of Au reported in previous studies, noting the boundary conditions of typical operations (i.e., thiosulphate 0.1-1 M, ammonia 0.1-4 M and $Cu^{2+} < 0.1$ M, with leaching rates ranging from 15% to 99%). Despite the substantial number of lab-scale experiments and advances in understanding the chemical mechanisms and fundamental principles, the development toward an ammonia-based method has been slow, partly due to the high volatility of ammonia and the environmental issues related to the leakage of ammonia during storage or transportation [69]. Table 4 summarizes the advantages and disadvantages of employing ammonia-based system in thiosulphate leaching.

$$Au \rightarrow Au^+ + e^- \quad \Delta G° = 163.2\ kJ/mol \tag{8}$$

$$Au^+ + 2NH_3 \rightarrow Au(NH_3)_2^+ \qquad \Delta G° = -74.1\ kJ/mol \tag{9}$$

$$Au(NH_3)_2^+ + 2S_2O_3^{2-} \rightarrow 2NH_3 + Au(S_2O_3)_2^{3-} \qquad \Delta G° = -74.9\ kJ/mol \tag{10}$$

$$Cu(NH_3)_4^{2+} + 3S_2O_3^{2-} + e^- \rightarrow Cu(S_2O_3)_3^{5-} + 4NH_3 \quad \Delta G° = -21.9\ kJ/mol \tag{11}$$

$$2Cu(S_2O_3)_3^{5-} + 16NH_3 + O_2 + 2H_2O \rightarrow 4Cu(NH_3)_4^{2+} + 4OH^- + 12S_2O_3^{2-} \quad \Delta G° = -67.1\ kJ/mol \tag{12}$$

Table 3. Ammonia-based thiosulphate leaching of Au under typical operating conditions.

Agent (M)	Condition	Au (%)	Waste	References
$S_2O_3^{2-}$ 0.072, NH_3 0.266, Cu^{2+} 0.01	20-25 ℃, 400 rpm, 5 min	~ 50%	Printed circuit boards	[63]
$S_2O_3^{2-}$ 0.2, NH_3 0.2, Cu^{2+} 0.0015	Pulp 30%, 30 ℃, aeration (0.2l/min), 6 h	89%	Pressure oxidized sulphide gold concentrate	[70]
$S_2O_3^{2-}$ 0.2, NH_3 0.4, Cu^{2+} 0.01-0.02	30 ℃, 300 rpm, 20%, 24 h, pH 11.5	30%	Gold ore	[71]

$S_2O_3^{2-}$ 0.08-0.12, NH_3 0.1-0.2, Cu^{2+} 0.015 M	20 ℃, pH 10.5, 2 h	up to 70%	Waste PCBs	[68]
$S_2O_3^{2-}$ 0.3, Cu^{2+} 0.05	pH 10, pulp 10%, 15 h	94%	Gold ore	[72]
$S_2O_3^{2-}$ 1, NH_3 1, Cu^{2+} 0.01	Pulp 10%, 24 h	99%	Waste PCBs	[73]
$S_2O_3^{2-}$ 0.2, NH_3 1, Co^{2+} 0.03	50 ℃, pH 10	70%	Gold ore	[13]
$S_2O_3^{2-}$ 0.3, NH_3 1, Cu^{2+} 0.03	25 ℃, pH 10	72.7%	Gold ore	[74]
$S_2O_3^{2-}$ 0.5, NH_3 1, Cu^{2+} 0.04	70 ℃, pH 6, 1 h	99%	Silver-bearing ore	[75]
$S_2O_3^{2-}$ 0.8, NH_3 4, Cu^{2+} 0.05	25 ℃, pH 10.2, 48h	90%	Gold-copper sulphide concentrate	[76]
$S_2O_3^{2-}$ 0.1, NH_3 0.2, Cu^{2+} 0.015-0.03	25 ℃, pH 9-11	15%	PCB	[77]

Table 4. The advantages and disadvantages of using ammonia in thiosulphate leaching system [66,69].

Advantages	Disadvantages
Prevents passivation film on Au surface and accelerates dissolution rate	Volatile reagent
Hinders the dissolution of undesirable minerals including silicates, carbonates and iron oxides	Easy escape from vessel and environmental pollution
Forms stable complexes with Cu and reduces the reactivity of Cu with thiosulphate	Toxic to human and aquatic animals
	Difficult transport and store

4.2.1.2. Non-ammonia method

Many studies have employed thiosulphate leaching by eliminating ammonia. Alternative non-ammonia methods in thiosulphate leaching include copper thiosulphate, oxygen thiosulphate, ferric Ethylenediaminetetraacetic acid (EDTA) thiosulphate, copper EDTA thiosulphate and ferric oxalate thiosulphate to mitigate the issues associated with ammonia. [66] employed a copper-citrate-thiosulphate method for Au leaching from a refractory carbonaceous gold concentrate and the findings demonstrated that substituting citrate with ammonia had a similar extraction capability but reduced the thiosulphate consumption from 0.045 M to 0.025 M. [78] observed that the copper ion–ethanediamine–thiosulphate was more effective than the copper ion–ammonia–thiosulphate leaching of Au from gold ore. The use of cetyltrimethyl ammonium bromide (CTAB) was found to increase the extraction rate to 94.3% and to decrease the thiosulphate consumption to 1.12 kg/t in a leaching system containing 0.1 M sodium thiosulphate, 0.06 M ethanediamine,

0.005 M Cu^{2+} and 1.5 kg/t of CTAB. In this process, CTAB broke down into [CTA^{+}], attracted the negatively charged particles of [$Au(S_2O_3)_2^{3-}$]·nH_2O and [CTA^{+}][$AuBr2^{-}$]·nH_2O, formed ion pairs that facilitated the stabilization of [Au(S2O3)2^{3-}]. Overall, ethylenediamine can form a more stable complex with Cu^{2+} than ammonia. The cupric-Ethylenediamine complex is considered an interesting agent in reducing the catalytic oxidation ability of Cu^{2+} over the cupric/cuprous redox equilibrium potential, which in turn lowers the consumption of thiosulphate. In some literature, the effect of external factors on the leaching rate was assessed. For example, in one experiment, ultrasound was applied in a cobalt ammonia thiosulphate leaching system (0.2 M $S_2O_3^{2-}$, 0.03 M Co^{2+}, 1 M NH_3, 50 ℃, and 750 W ultrasonic power). The results showed that the leaching rate was 8 times faster and the extraction yield with ultrasound reached 89%, which was found to be 25% higher than the one without ultrasound. Temperature, ultrasonic power and the reduction in activation energy from 22.65 kJ/mol to 13.86 kJ/mol were the main reason for the high leaching rate [13].

4.2.2. Challenges with the thiosulphate-based leaching

The primary challenge associated with the use of thiosulphate is the high cost of reagent consumption and its impacts on the generated sulphate, tetrathionate, and trithionate [63]. Addressing this challenge requires implementation of strategies, including the control of the key factors of operations such as temperature, flow rate and the mixing speed, oxygen level, Eh, pH, and the concentration of Cu^{2+} and ammonia. Depending on pH and Eh, unstable compounds such as tetrathionate, sulphite, and trithionate may form, thereby controlling the Eh and pH is essential to minimize thiosulphate loss [79]. Using inorganic additives like phosphate, sulphate, and chloride and organic additives such as EDTA, humic acid (HA), polyamine, CDTA, Ethylenediamine (EDA), Triethanolamine (TEA), ammonium alcohol polyvinyl phosphate, citric acid, carboxymethyl cellulose (CMC), and amino acids can reduce thiosulphate consumption as they have the potential to compete with thiosulphate anions and form complexes with Cu^{2+} that decrease the interaction between Cu^{2+} and thiosulphate [80]. For example, in the copper-ammonia-thiosulfate leaching system, 1 L of leach solution was applied to 400 g of sulphide ore, with an initial gold concentration of 4.3 mg/kg. The addition of a low concentration of EDTA (2.0 mM) enhanced the efficiency of Au leaching to 100% and decreased the consumption of ammonium thiosulphate from 9.63 kg/t to 3.85 kg/t after 24 h [81]. [65] employed TEA as an additive for Au leaching in a thiosulphate-copper-ammonia system. The results demonstrated that the dissolution rate increased by 50% while thiosulphate consumption was reduced by around 10%. In a study conducted by [82], ethydiaminedhephen acetic (EDDHA) was employed as a non-toxic, environmentally friendly, and cost-effective organic additive to form a stable complex with Cu^{2+} owing to numerous coordination sites of carboxylic acid and amine functional groups in it and led to a stable Cu-EDDHA complex ion and the dissolution of 82.84% Au, which was 56.02% higher than the cyanide leaching. Moreover, EDDHA reduced the thiosulphate consumption from 103.19 kg/t to 10.54 kg/t. [83] investigated the effect of different additives on the Au oxidation and revealed that potassium ethyl xanthate (KEX), imidazole, sulphinic acid, sodium di-ethyl dithiocarbamate, pyridine, thioglycolic acid and mercaptobenzothiazole completely passivated the Au surface while a small amount (5 mM) of thiourea and thioacetamide improved the oxidation of Au. Another study found that the Au dissolutions in thiosulphate-oxygen-copper medium was only 2% over 24 h and increased to 95% in presence of activated carbon as an additive [79]. In some experiments, Cu^{2+} was substituted by Co as oxidizing agent. According to [84], in ammoniacal thiosulphate solutions, Co(III)-NH_3 complexes were able to extract

around 80% of Au, while they reduced thiosulphate consumption by 44.2% thiosulphate. Table 5 summarises the effect of different additives on Au leaching and the consumption of thiosulphate. As seen, adding additives increased the dissolution of Au approximate by 20% and decreased the thiosulphate consumption by 30%.

Table 5. Effect of additives on Au dissolution and thiosulphate consumption.

Additive (mM)	Au dissolution (%)	Thiosulphate consumption (%)	Reference
Humic acid (80)	Before: 72.6 After: 81.4	Before: 42.4 After: 13.2	[85]
EDTA (2.0)	Before: ~ 80 After: 100	Before: 9.63 After: 3.85	[7]
TEA (12)	Increased by 50	Decreased by 10	[65]
EDDHA (450)	Before: 56.02 After: 82.84	Before: 10.31 After: 1.05	[82]
CTAB (60)	Before: 50 After: 94.3	Before: 1.3 After: 0.11	[78]
EDA (10)	Before: 60.3 After: 80.3	Before: 1.13 After: 0.414	[86]

4.2.3. Bio-thiosulphate produced by microorganisms

Over the past two decades, efforts have been made to minimise the breakdown of the thiosulphate during thiosulphate leaching of Au by using different types of additives and controlling the parameters affecting the process. It was discovered that producing thiosulphate by means of microorganisms (bio-thiosulphate) offers a potential pathway toward the development of a feasible, innovative and sustainable metal recovery technology [51,87]. The benefits of using biogenic thiosulphate for Au leaching include, for instance, fewer toxic substances, less energy requirements associated with intermediate chemical productions, and scalability combined with e-waste recycling operations. Currently, an industrial thiosulphate leaching process is employed at Nevada Gold Mines that involves thermal treatment of sulphur and calcium hydroxide at 90 °C followed by oxidation of substances under pressure at 550 kPa. Therefore, developing a biogenic process in moderate conditions (e.g. ambient temperature and atmospheric pressure) can be recognized as an innovative alternation to reduce the CAPEX and the OPEX costs [88]. The two most common species that produce thiosulphate are cyanobacteria and proteobacteria. Table 6 summarises the ability of various microorganisms to produce thiosulphate under different environmental and substrate conditions [89]. The bacterium *Microcoleus chtonoplastes* produces thiosulphate (695 mg/L)

using light, sulphide and hydrogen and consumes carbonate as a carbon source at 22 °C and a pH around 8 [90]. *Methylophaga sulfidovorans* consumes methanol or Dimethyl Sulphide (DMS) as substrates to produce thiosulphate, as shown in Equation (13), and grows at pH above 7.5 and temperature of 22–30 °C. In the thiosulphate production by the bacterium *Thiobacillus thioparus*, sulphide, thiocyanate, elemental sulphur (S0), DMS and polythionates can act as electron donors. Around 18.5% of thiosulphate production by this bacterium results from sulphide oxidation, which occurs under oxygen-limiting conditions and pH 7 [91]. *Streptomyces fradiae* produced up to 485 mg/L of $Na_2S_2O_3$ as a main byproduct in mineral media at pH 7.5, which remained stable without degradation during 20 days of incubation [92]. [93] discovered that the addition of 1 mM sulphide to the media containing recombinant *E. coli* resulted in the production of 0.45 mM sulphite, 0.2 mM thiosulphate, and 0.1 mM sulphur. *Sulphurimonas sp.* was observed to consume sulphide to produce 1mM thiosulphate after intermediate production of elemental sulphur [91]. In a mixed culture with a neutral pH and temperature range of 20–32 °C, *T. oxidans* and *T. neapolitanus* produced sulphate and thiosulphate through sulphur oxidation. Sulphate was produced as a main product under atmospheric conditions and at pH 8.0 but during oxygen-limited conditions especially when O_2/S_2 consumed ratio was around 0.5, thiosulphate formation (35 mg/L) occurred as a result of increased sulphide-oxygen ratio [94].

$$(CH_3)_2S + 4O_2 \rightarrow 2CO_2 + H^+ + 0.5S_2O_3^{2-} + 2.5H_2O \quad \Delta G° = -1606.5\ kJ/mol \tag{13}$$

Biogenic thiosulphate as an intermediate metabolite is extremely unstable under acidic conditions but can be stabilised through the adjustment of pH and presence of inhibitors [51]. For example, biogenic thiosulphate (500 mg/l) obtained from *A. thiooxidans* was able to leach 65% Au from 0.5% w/v of PCBs in presence of 3.25 mg/L sodium azide (NaN_3) as an inhibitor and 1M ammonia at pH 6–7 after 36 h [95]. [96] investigated the thiosulphate leaching of Au from ore by the bacterium *Methylophaga sulfidovorans* using sodium sulphide as the substrate. The bacterium was able to produce thiosulphate in a rotating sealed flask of high salinity medium at pH 7−8 and temperature of 30 °C. A maximum of 61.9% of Au was leached in 24 h under conditions of 10% pulp density, air flow rate of 0.1 L/min, and 50 °C. The bio-thiosulphate approach was found to be a viable substitute of the chemical thiosulphate opening the door to further investigation of other thiosulphate producing microorganisms from inexpensive substrates. This is considered a promising area in bioprocess engineering for metal recovery in an efficient and environmentally-friendly way.

Table 6. Illustration of thiosulphate production by microorganisms[89].

Microorganism	Substrate	Thiosulphate production condition	Produced thiosulphate (mg/l)
Microcoleus chtonoplastes	Sulphide	pH 8, temperature 22°C, Anoxic photooxidation	695
Methylophaga sulfidovorans	Ethanethiol, hydrogen sulphide	pH 7.5, temperature 22-30°C,	672
Streptomyces fradiae	L-Cystine	pH 7.5, temperature 22°C, 20 days	485

Pseudomonas sp. C27	Sulphide, acetate	pH 7-8, temperature 30˚C, coupled with nitrogen fixation process	451
Calothrix sp. HI 41	Sulphide, hydrogen sulphide	temperature 25°C, Anoxic photooxidation, 10 days	392
Oscillatoria sp. BO 32	Sulphide	temperature 25°C, Anoxic photooxidation	146
Sulfurimonas sp. CVO	Sulphur, sulphide	Temperature 30°C,	112
Anabaena cylindrica	Sulphide	Temperature 25°C	73
E. coli	Sulphide	Temperature 37°C,	45
T. neapolitanus	Sulphide	pH 5-9, temperature 30°C, limited oxygen	35
D. desulfuricans	Sulphide	pH 1-6, Temperature 85°C	10

5.Engineering perspective and scaling up

The phase of scaling up operation of the laboratory scale has been an important challenge in the bioprocessing to achieve representative microbiological and technological enhancements and bring it as close as possible to the industrial applications [22]. Currently, the industrial-scale metal extraction is performed using methods such as the dump bioleaching, the heap bioleaching, and the tank bioleaching. Among these, the dump leaching is the oldest technique used in the industry, primarily for extraction of Cu, Ni, Zn, Au and U from ores. In this method, a lixiviant generated by bacteria is sprayed onto the ores piled in the dump and the leachate solution is collected in ditches at the bottom of the dump, recovered and then directed to an oxidation basin, where the bacteria are regenerated for reuse in the process. This technique generally has low metal recovery efficiency and requires several days to complete the process. Moreover, there is a risk of leachate seeping into the nearby water sources with a potential risk of water pollution. Appropriate safety measures must therefore be undertaken to control the potential runoff [97]. Heap bioleaching is another method suitable for low-grade ores that are not economically viable for leaching through grinding and smelting and is particularly effective for instance for treating complex sulfide ores that have a porous structure[98,99]. Heap bioleaching involves stacking ores on an impermeable surface and applying leaching agents such as acidified solutions for Cu or cyanide solutions for Au over the top of the heap. The leaching occurs through microbial activity and mineral oxidation within the heap and metal-rich solution is collected at the base and directed to a pond to recover metals [100]. Despite its technical maturity and widespread industrial application, it has drawbacks such as yielding a low leaching rates within a long processing time. Extending

the leaching time increases the demand for manpower and material resources, which restricts further development of this technology [98]. Table 7 provides a summary of commercial-scale bioleaching operations for the recovery of Au and Cu from refractory gold concentrates, copper ores, and sulphidic ores across various global mining sites. The table shows details about the mine location, operator, year of operation commencement, production amount, and the type of bioleaching technology employed.

Table 7. Commercial scale bioleaching operation for refractory gold concentrate, copper ores or sulphid ores [101,102].

Mine	Location	Operator	Year started	Production	Type
Obuasi	Ghana	Anglo Gold Ashanti	1994	Au: 200,000 ounces	stirred-tank
Laizhou (BioGold)	China	Sino Gold Mining	2001	Au: 75,000 ounces	stirred-tank
Olimpiada	Russia	Polyus Gold	2001	Au: 965,000 ounces	stirred-tank
Suzdal	Kazakhstan	Nordgold	2005	Au: 90,000 ounces	stirred-tank
Kokpatas	Uzbekistan	Navoi Mining and Metallurgical Combinat	2009	Au: 432,000 ounces	stirred-tank
Fosterville	Australia	Kirkland Lake Gold	2005	Au: 150,000 ounces	stirred-tank
Iranian Babak Copper Company	Iran	Iranian Babak Copper Company	2020	Cu: 50,000 t/y	Heap
Zaldivar	Chile	Barrick Gold Corporation	1995	Cu: 150,000 t/y	Heap
Whim Creek and Mons Cupri	Australia	Straits Resources	2006	Cu: 17,000 t/y	Heap
Jinchuan Copper	China	Zijin Mining Group Ltd	2006	Cu: 10,000 t/y	Heap

To enhance ore leaching efficiency, the process is initially performed in laboratory flasks prior to expanding to larger bioreactors. These bioreactors play a key role in process scale-up and are commonly operated in either batch or continuous modes. The development of an adapted bioreactor is the first step to move from an experimental scale to industrially relevant operating conditions. Bubble column reactors (BCRs) and continuous stirred tank reactors (CSTRs) have been the widely used bioreactors in the bioleaching process.One of the advantages of these bioreactors compared to others is the ability to control the concentration of product and substrate during the process and to maintain the environmental operating conditions [22,30]. [103] investigated the extraction of heavy

metals from a petroleum spent catalyst using *A. thiooxidans* in a bubble column reactor and results showed 87% % Mo, 37% Ni and 15% Al extraction with a pulp density of 0.9%, particle size of 60.7 μm, and aeration rate of 209 ml/min after 7 days. In another study, [30] employed adapted *A. ferrooxidans* in a bubble column and leached approximately 54% Cu, 75% Ni and 55% Fe with 2% waste PCBs in 9 days. Figure. 2 rillustrates a schematic diagram of two types of reactors employed in the bioleaching process. As seen in Figure. 2a, the stirring in a BCR is conducted by air bubbling and the aeration happens via air injection through a sparger, operating at high airflow. Water cooling thermostat is fitted to maintain the temperature. Figure. 2b shows the continuous bioleaching in a 50 L CSTR. The mixture of sediment slurry and deionized water was pumped to the reactor filled with sulphur-oxidising microorganisms and supplied with aerator [104]. Figure. 2c represents a combination of BCR and STR in the bioleaching of metals by acidophilic consortium. The continuous cultivation experiment of microorganisms carried out in the BCR and bioleaching in a batch and continuous mode was done in STR [6,105]. The CSTRs are more advantageous in comparison with the BCRs because of the presence of high mass transfer rates, homogeneous mixing and better control over parameters such as pH, temperature and aeration [22] A typical design of CSTRs involves a circuit of continuously flowing aerated tanks set up in series, parallel or a combination of both, equipped with agitated impellers that keep the grounded minerals suspended in the solution and ensure an effective transfer of oxygen, which is necessary for both the dissolution of the metals as well as the growth of aerobic microorganisms. In CSTRs, the feed is added to the first tank and overflows from tank to tank in co-current flow with the microorganisms. When microorganisms are injected at the start-up of an operation, a batch culture is maintained until microorganisms reach a certain stage close to the middle of the logarithmic phase, at which point fresh substrate is supplied continuously from the feed tank to the reactor. The continuous flow of the substrate through the tanks is provided to ensure the optimal growth of microorganisms for a high metal dissolution rate at the steady-state phase[106]. Since its introduction in 1986, the CSTR related technologies have undergone significant advancement such as high temperature performance in the bioleaching. During the first decade of this century, the Beaconsfield plant in Australia and the Laizhou plant in China, utilizing Mintek BacTech technology, have conducted bioleaching operations at moderate to high temperature, ranging between 45-55 ℃. The BioCop™ process, pioneered by the BHP Billiton biotechnology group based in Johannesburg in South Africa, employed thermophilic bacteria thriving at temperature between 70-80 ℃ to leach chalcopyrite [107].

In a study conducted by [97], bioleaching with thermophilic bacteria, *L. ferriphilum* and *A. caldus* was employed at a high pulp density of 8% PCBs using a 3 L stirred tanks reactor, resulting in a recovery of 85.23% Zn, 76.59% Cu, and 70.16% Al in 7 days. [108] employed a 12 L aerated rotating-drum reactor operated at 50 ℃ for the bioleaching of 76% Cu with a pulp density of 2.5% by thermophilic bacterium *S. thermosulfidooxidans* and Fe^{2+} concentration of 5 g/l in 8 days. In another study, [109] achieved a 99% leaching rate of Cu within 72 h using a 3 L CSTR containing 5% of low-grade PCBs and mixed acidophilic culture, supplemented with 7.8 g/l of Fe^{2+}. However, the large scale bioleaching has not been sufficiently investigated owing to factors such as reaction conditions, the complexity of the mechanism and the weakly efficient mass transfer rates [110]. More comprehensive investigation is crucial as the findings are found not linearly scalable when the size of equipment is increased. While controlling conditions for a complete reaction at the laboratory scale is straightforward and flexible, maintaining microbial populations along with the aeration rate is challenging at the industrial scale, which affects the steady state operations at long time of streams [24].

In the recent years, several projects in Europe, such as BIORECOVER, RAWMINA, RUBICON, and BIOCriticalMetals, have employed the bioleaching for the recovery of various metals from e-wastes. Among the industrial contributors, the UK-based IT lifecycle services provider N2S is applying bioleaching for the extraction of precious metals from the printed circuit boards and aligning with the UK's environmental and cybersecurity regulations [111]. In Estonia, BiotaTec (formerly BiotaP), which was initially focusing on environmental monitoring using metagenomics, has since expandedtheir activities to development of microorganism-driven bioleaching and biomining solutions for the recovery of metals from low-grade ores and various waste streams. Another notable company is Ekolive, a Slovak start-up which offers an EU/ETV-certified eco-innovative bioleaching method (InnoBioTech®) for processing minerals and waste using heterotrophic bacteria. Their large-scale pilot project established in Slovenia in 2019 demonstrated the market maturity of this approach for industrial mineral recovery from mining waste [112].

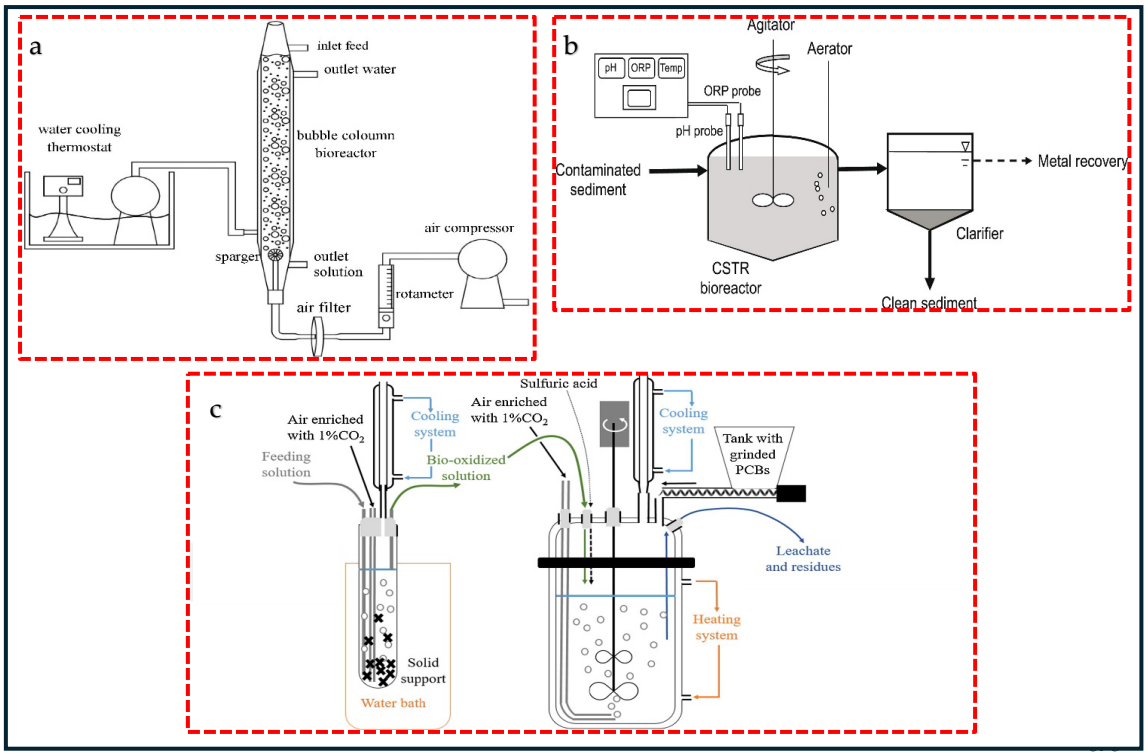

Figure 2. A schematic diagram of two types of reactors in the bioleaching. Copyright © 2020, Elsevier (a), Copyright © 2021, Elsevier (b), Copyright © 2022, Elsevier (c) [6,30,104]

6. Process economics

6.1. Cost estimation

Estimating both capital expenditure (CAPEX) and operational expenditure (OPEX) is essential to assess the economic feasibility of the bioleaching process and give insight into the opportunities to improve, optimise and further research if needed. CAPEX includes the cost of equipment, installation, construction, piping, electrical systems, service facilities, and land and OPEX covers ongoing costs like electricity, water, materials, labour, and maintenance [113]. Estimating capital costs help identify the key factors that affect the economic. For instance, while lower pulp densities often yield higher metal leaching rates, they are associated with higher capital costs. In contrast,

higher pulp densities may reduce leaching efficiency but result in lower CAPEX, making the process more economically viable. In one study, leaching of base metals from PCBs at 1% pulp density led to high operating costs and an annual loss of AUD 1,007 per tonne of PCBs. However, increasing the pulp density to 5% significantly lowered the costs, resulting in an annual profit of AUD 2,749 per tonne. Although the raw material costs can change over time, the process would be profitable. Even with a 40% increase in raw material costs, the study showed an annual profit of AUD 2,463 per tonne of PCBs was still achievable [114]. Labor costs for pre-processing of e-waste such as collecting, dismantling, and sorting are the highest operating expenses, making it difficult to expand e-waste recycling in regions such as Australia, New Zealand, and other parts of Oceania. A techno-economic study assessed the use of biooxidation and cyanidation to extract gold from refractory ore. Over a 25-year mine life, processing 1,200 tonnes of ore daily, the project required a $220 million investment and had an operating costs of $58.27 million annally. With an expected annual revenue of $78.48 million and a net present value (NPV) of $34.4 million at a 7% interest rate, the process was found to be economically viable [115]. Another study compared two bioleaching technologies including an aerated bioreactor and an aerated and stirred bioreactor for Cu recovery from goethite using *A. ferrooxidans*. The aerated and stirred bioreactor proved to be financially viable at a pulp density of 10%, achieving a NPV of $1.275 billion and an internal rate of return (IRR) of 65% over 20 years. With a CAPEX of $119.8 million and annual OPEX of $5.9 million, the plant is expected to become profitable within one year [39].

6.2. Cost minimisation strategies

In the bioleaching process, the initial investment required for setting up a reactor plant may seem to be high, but the ongoing operational expenses can be reduced through the optimization of process parameters and the utilization of cost-effective substances. For example, the use of commercial iron, sulphur and specific additives contributes to the overall cost [28], but can be reduced by using low-grade pyrite as an alternative source [116]. In the case of heterotrophic bacteria, glucose is the main nutrient carbon source for bacterial growth and accounts for approximately 44% of the total initial costs. Using alternative affordable substrates such as molas is an option to reduce the cost of the process [24]. [117] reported that using potato wastewater reduced the cost by 17% compared to the glucose and replacing corn stover with potato wastewater reduced the costs by 22% in comparison with potato wastewater. The formation of iron hydroxides and jarosite precipitations during bioleaching, which leads to a decrease in diffusion rates of oxidants to the surface of PCBs and results in non-economic losses in metal yields, can be avoided by maintaining low pH and using effective sulphur agents that oxidize sulphur to sulphuric acid. The costs of the pre-processing of e-waste (dismantling, separation and grinding) and operation (labour, raw materials and utilities) can render the bioleaching technique economically impractical without careful management of the design and the process automation. Cost reduction in the grinding step can be achieved by maintaining a particle size that does not significantly impact both the mass transfer rate and the bioleaching yield [28,110]. For example, a study reported that using PCB chips instead of powdered PCBs resulted in high metal leaching efficiency and significantly lowered operational costs, as it avoided the energy-intensive milling process and reduced the risk of metal loss during pre-processing [118].

7. Conclusions

Metal recycling from printed circuit boards (PCBs) as the major component of e-waste is a beneficial option to protect the environment, conserve natural resources and meet the needs for metals particularly the valuable ones that are critical for the present industries. The bioleaching process as a green technology for metal extraction is typically conducted by direct (one-step and two-step) and indirect (spent medium) methods and relevant chemical mechanisms fundamentally include acidolysis, complexolysis, and redoxolysis. The latter is the primary mechanism associated with the iron-oxidizing bacteria. For over a century, Au extraction has been relying on the cyanidation process, but the environmental concerns are limiting its expansion and offering the thiosulphate-based process as a safe alternative for Au recovery. The high thiosulphate consumption remains however one of the main challenges associated with this method and various strategies such as the use of additives, control of operation conditions and employing bio-thiosulphate are the options being considered. Upscaling bioleaching to an industrially feasible process at cost-effective operations remains a challenge but the selection of a suitable microorganism and adapted design of the bioreactor are contributing as close as possible to the success of the process feasibility from economic and environmental perspectives. These studies indicate that the biological extraction of metals from PCBs presents a promising viable alternative to the physico-chemical methods and improvement in handling large pulp density of PCB within the bioreactors is necessary for a practical achievement of a cost-effective and competitively upscaled process.

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