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**Bioleaching of platinum group metals from  
electronic waste**

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# **Bioleaching of platinum group metals from electronic waste**

## **Abstract:**

The rapid economic development together with the short life span of electronic products lead to the generation of ever more electronic waste (e-waste). In this context, the researchers from all over the world have paid much attention to the study of metals and metalloids present in the composition of e-waste. In the present thesis methods for disposing of e-waste are introduced, including physical, mechanical, and biological technologies. Considering advantages in energy savings and eco-friendliness, biological approaches appear to be the most effective ones. This thesis evaluated the worldwide generation of e-waste and the implementation of multiple technologies in the recovery of e-waste, specifically, the different applications of the biological method which utilizes microorganisms to extract the platinum group metals. The mechanisms as well as influencing factors for the biological method are discussed, some examples of the method are also introduced. Finally, the conclusions on the implementation of the biological treatment method for e-waste and the prospects for its further use are presented.

## **Keywords:**

bioleaching, printed circuit board (PCB), platinum group metals (PGM), copper, silver, gold, platinum, e-waste

## **CERCS:**

B230 Microbiology, bacteriology, virology, mycology

T490 Biotechnology

## **Plaatinarühma metallide bioleostamine elektroonikajäätmetest**

### **Lühikokkuvõte:**

Kiire majandusareng koos elektroonikaseadmete lühikese elueaga tekitab üha enam elektroonikaromusid (e-jäätmeid). Sellega seoses on kogu maailma teadlased pööranud palju tähelepanu e-jäätmete koostises olevate metallide ja metalloidide uurimisele. Käesolevas lõputöös tutvustatakse e-jäätmete kõrvaldamise meetodeid, sealhulgas füüsikalisi, mehaanilisi ja bioloogilisi tehnoloogiaid. Arvestades energiasäästu ja keskkonnasõbralikkuse eeliseid, näivad kõige tõhusamad olevat bioloogilised lähenemisviisid. Selles lõputöös hinnati e-jäätmete ülemaailmset teket ning mitmete tehnoloogiate rakendamist e-jäätmete taaskasutamisel, täpsemalt bioloogilise meetodi erinevaid rakendusi, milles plaatina rühma metallide eraldamiseks kasutatakse mikroorganisme. Käsitletakse bioloogilise meetodi mehhanisme ja mõjutegureid, toodud on ka mõned meetodi näited. Lõpuks tutvustatakse järeldusi e-jäätmete bioloogilise töötlusmeetodi rakendamise kohta ja selle edasise kasutamise väljavaateid.

### **Võtmesõnad:**

Bioleostumine, trükkplaadid, plaatinarühma metallid, vask, hõbe, kuld, plaatina, e-jäätmed

### **CERCS:**

B230 Mikrobioloogia, bakterioloogia, viroloogia, mükoloogia

T490 Biotehnoloogia

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## **TERMS, ABBREVIATIONS AND NOTATIONS**

BFR	brominated flame retardants
DTPA	diethylenetriaminepenta acetate
DO	dissolve oxygen
EDTA	ethylene diaminetetraimid
EPS	extracellular polymeric substances
ENIG	electroless nickel immersion gold
FR	flame retardent
HALS	hot air levelling solder
IC	integrated circuit
MS	metal sulfide
PBDE	polybrominated diphenyl ethers
PCB	printed circuit board
PBDD	polybrominated dibenzodioxins
PBDF	polybrominated dibenzofurans
PGM	platinum group metal
RPM	rotations per minute
SAC	spent automotive catalysts
WEEE	waste electrical and electronic equipment

## INTRODUCTION

The rapid development in technology, the continuous and unexpected growth of consumer demand for electrical and electronic equipment (EEE) especially combined with their shorter life spans has greatly increased e-waste generation around the world. E-waste is created up to three times faster than other waste streams in the modern world. The printed circuit board (PCB) is the central component of e-waste, and the rapid rise in the amount of waste PCBs with the growth in e-waste production is unavoidable (Hadi et al., 2015; Widmer et al., 2005). At the same time the PCBs represent only approximately 3–6% of the overall e-waste weight (Das et al., 2009). The composition of waste PCBs is heterogeneous, typically consisting of approximately 40% of metallic components and 60% of non-metallic (plastic, ceramic, etc) components, although their composition varies depending on their specific class (Duan et al., 2011). Amongst these metallic constituents, non-ferrous metals such as Cu, Pb, Zn, Ni, and Sn and precious metals such as Au, Ag, Pd, and Pt are significant (Huang et al., 2009; J. Li et al., 2007). The e-waste toxicity is linked to the presence of both metallic substances such as As, Hg, Cd, Cr, Pb and non-metallic substances such as brominated flame retardants (BFRs) if they exceed the allowable concentrations (Hagelüken, 2006a). Depending on the nature of such compounds in higher concentrations, e-waste is usually considered as toxic waste which can cause serious threats to human as well as environmental safety unless adequately handled in a coordinated way. Due to the abundant precious metal content with the simultaneous presence of toxic substances, the recycling of e-waste is a significant issue not only from the viewpoint of disposal of waste for the benefit of the environment but also from the viewpoint of recovery of metals for economic development.

Various traditional metallurgical approaches have been developed for the recovery of metals from e-waste, e.g. pyrometallurgical processes that use heat treatment such as roasting, smelting, and hydrometallurgical processes based on acid/alkali wash chemical leaching (Cui & Zhang, 2008). However, both pyrometallurgical and hydrometallurgical methods for e-waste processing have also seen to be energy-intensive, expensive and correlated with secondary emissions such as release of poisonous gases, metal dust, furans and release of large volume lixivants (Cui & Zhang, 2008; Dalrymple et al., 2007; Ilyas et al., 2010). Biotechnology implementation into the hydrometallurgical method by adding biocatalysts (e.g., microorganisms and enzymes) has been developed into an alternative metallurgical method commonly known as biohydrometallurgy or bioleaching for e-waste recycling (J. Wang et al., 2009; Xin et al., 2009). Bioleaching includes transforming metallic substances

into water-soluble states utilizing a number of micro-organisms, particularly chemolithoautotrophs, which are using CO<sub>2</sub> as a source of carbon and inorganic compounds such as Fe<sup>2+</sup> and reduced sulfur as a source of energy (H. Brandl et al., 2001; Ilyas et al., 2007). Traditional methods of recovery of metals such as solvent extraction, precipitation, adsorption, ion exchange, and electrowinning can also be implemented by leaching. It is claimed that bioleaching provides numerous advantages over conventional metallurgical techniques, along with its simple nature, lower energy requirement and operating costs, decreased skilled labor demands, highly efficient detoxification of industrial effluent, reduced secondary pollution and environmental friendliness (Huang et al., 2009; X. Wang et al., 2013).

The overview of this thesis provides a detailed insight into the different processes involved in the processing of e-waste for metal recovery with a strong emphasis on bioleaching, discusses limitations in research and points out important areas where more work may be useful. The paper starts with the e-waste production situation in the world and a description of elements and substances used in e-waste. It then moves on to review the current status of e-waste management in terms of bioleaching techniques for the extraction of metals from e-waste together with their realistic evaluation from the viewpoint of economic and environmental feasibility. In addition, a description of the bioleaching process is provided with various biotic and abiotic influences influencing the mechanism for extracting metals from e-waste. A concluding part illustrates important research observations and provides recommendations for a pre-defined e-waste.

# 1 LITERATURE REVIEW

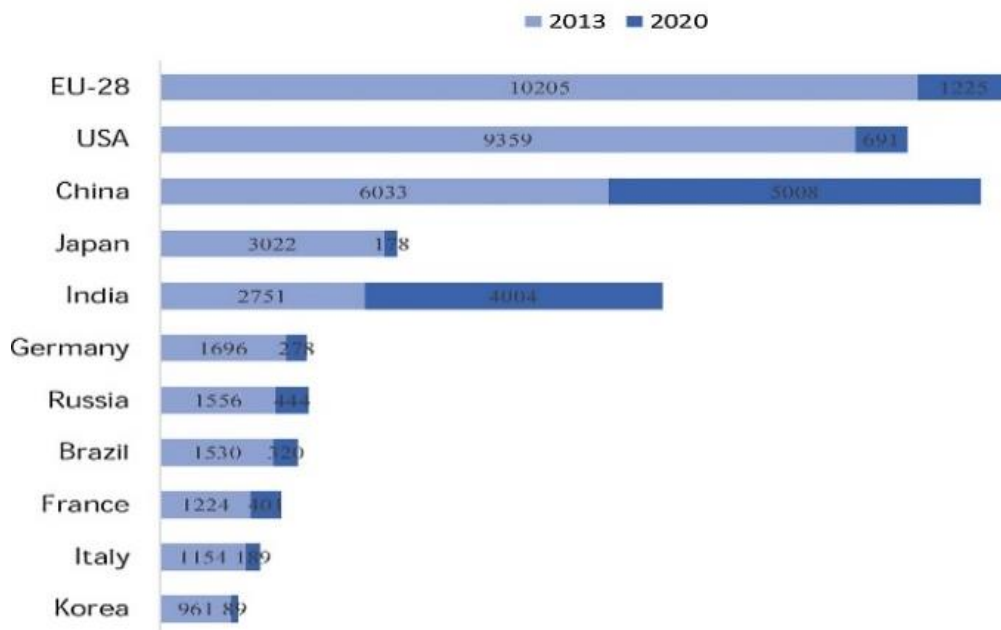
## 1.1 Global waste electrical and electronic equipment (WEEE) management

Electrical waste relates to discarded electrical and electronic products which is no more used by consumers at the end of their economic life span. It is generally simplified as e-waste and known as WEEE.

WEEE includes a wide range of discarded devices and is categorized by product type and legislative relevancy. WEEE is grouped into 10 major categories according to the WEEE Directive by the European Commission (2012/19/EU), i.e. (i) large household appliances, (ii) small household appliances, (iii) information technology and communication (ITC) equipment, (iv) consumer electronics, (v) lighting, (vi) electrical and electronic tools, (vii) toys, (viii) leisure and sports equipment, (ix) medical devices, and (x) automatic dispensers (Dominguez-Benetton et al., 2018).

The natural minerals, energy and chemicals used for the production and after usage of the electronic material are disposed of at the end of its working life thus WEEE is a rapidly growing global environmental problem. A considerable number of WEEE generated is land-filled or shipped to developing countries where it poses a significant hazard to the environment and local communities (Creamer et al., 2006). The hazards are associated with the presence of heavy metals, brominated flame retardants (BFRs), polybrominated diphenyl ethers (PBDEs), dioxins and other potentially harmful substances either contained in or formed during waste processing (Hadi et al., 2015). In addition, personal computer components contain about 50% of hazardous arsenic, hexavalent chromium and mercury.

The quantification of WEEE is essential for sustainable development but it is so challenging due to the lack of reliable data on the proper constituents and amounts as well as because of the sensitivity of the related issues. In developing countries this task is cumbersome as the data quality and informal waste management systems are poorly documented. In fact, there is a prevalence of a lack of quantitative comprehension of the quantities involved in the transboundary WEEE movement. A summary of the total WEEE generation in 2013 and its 2020 projection is shown in Figure 1 (Işıldar et al., 2019). According to the forecasted economic growth, a significant rise in WEEE generation is also anticipated in developing countries (Yamane et al., 2011). As a result, areas with high populations and fast economic development are projected to become major producers of WEEE in the future years.



**Figure 1: Annual generation of electronic waste in 2013 and future projection in 2020(Işıldar et al., 2019).**

Proper e-waste management is necessary for all countries because e-waste pollutes the groundwater, acidifies the soil after burning, generates toxic fumes and gases, accumulates the fastest in municipal disposal areas and releases harmful byproducts into the air. The most favored to least favored choice hierarchy pyramid has been offered for proper management of e-waste (Figure 2). For effective handling of e-pollution, minimization of material usage, reuse and prevention as the most preferred choices are on top of the pyramid of the e-waste hierarchy. Burning and disposal by landfill is the least preferred option of the e-waste.



**Figure 2: The EU waste hierarchy(European Commission, 2009)**

### 1.1.1 Bangladesh: E-waste situation and practice

Bangladesh's digitalization is the key motivating force behind rising electronic and e-waste use over the last decade. Bangladesh introduced its National Environmental Strategy in 1992, in 1995 the Environmental Protection Act and the Medical Waste Management Rules in 2008. There are actually no laws directly covering e-waste. However, the Bangladesh government has made the preparations for 'Electrical and Electronic Waste (Management and Handling) Laws' in 2011 a top priority. Moreover, the government has already planned a Regional 3R (Reducing, Reusing and Recycling) plan integrating some areas of e-waste management. There are various categories of e-waste available in Bangladesh including items from ship-breaking yards, television sets, cell phones, dental waste and household electrical appliances (Figure 3). Owing to their unintentional disposal activity, the high volume of e-waste per annum is environmentally harmful. In 2005-2012, the production of e-waste in Bangladesh increased by 2,81-5,181 million tons, according to the Environment and Social Development Organization. The average growth rate of electronic waste worldwide is about 5%, where 48% in Bangladesh (Herat & Agamuthu, 2012). Bangladesh had 10.3 million television sets in operation at the end of 2008, with about 6 million television sets being discarded as e-waste each year. Bangladesh had 58 million mobile phone subscribers as of May 2010. In Bangladesh, about 25 million cell phones are projected to be lost annually. Every year 68% of e-waste is produced in Bangladesh from medical and dental electronics (Herat & Agamuthu, 2012).



**Figure 3: E-waste in Bangladesh(Buffalo, 2020)**

Initiatives for e-waste management and environmental conservation should be taken by the Bangladesh government. Bangladesh does not have any regulations on e-waste management and particularly e-waste management policies. For the proper management of e-waste, several measures should be taken by the Government of Bangladesh. Overall, there is a need for a significant encouragement for researchers to pursue a safe alternative for the recovery of precious metals from e-waste.

## 1.2 Assembly framework of printed circuit board (PCB)

All PCBs consist essentially of three basic components (Kaya, 2016):

- i) non-conductive substrate or laminate.
- ii) Conducting Cu-substrate printed on or inside the laminate.
- iii) Substrate-mounted modules (chips (Ga, In, Ti, Si, Ge, As, Sb, Se and Te), connectors (Au, Ag), capacitors (Ta, Al), etc.)

PCBs may be classified as single-sided, double-sided, or multilayered, depending on the structure and alignment. Single and double-sided PCBs have a conductive coating on one or both sides of the laminate and plated holes interconnect the sides. The thickness of the PCB boards can range from 0.2 to 7.0 mm. The PCBs have a Cu thickness of between 17.5 and 175  $\mu\text{m}$ . The minimum diameter of a drill hole is 0.2 mm. Solder types are water-soluble leaded or non-leaded solder paste. Surface finishing is usually HALS(hot air levelling solder), chemical Sn, chemical Au, ENIG(electroless nickel immersion gold), immersion Au / Ag or Au plating type.

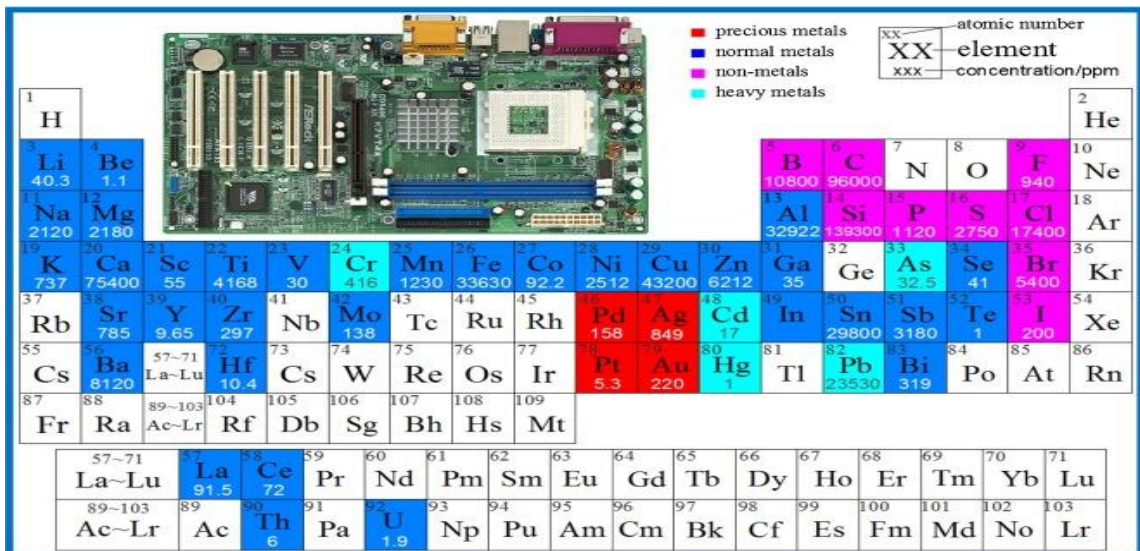
PCBs comprise approximately one-third of metallic materials such as Cu and Fe, approximately one-fourth of organic resin materials including elements such as C and H, and approximately one-third of glass products used as resins reinforcing fiber (Hino et al., 2009). Of metals Cu, which is used in the circuit board composition has the highest content, followed by Sn, Fe and Pb, which are used in the soldering and in the lead frames. Due to their high conductivity and chemical stability Au, Ag and Pd are found in IC(integrated circuit) as contact materials or as plating layers (Hino et al., 2009; Jung & Bartel, 1999).

The substances present in PCBs may be divided into three groups: organic materials, metals, and ceramic materials. According to the previous studies, the material composition of PCBs is shown in Table 1 (Duan et al., 2011) and Figure 4.

**Table-1: PCB material composition (Duan et al., 2011)**

Material	Element	Content (%) mass	Overall mass(Approx.)
Metals	Cu	6-27	40%
	Fe	1.2-8.0	
	Al	2.0-7.2	
	Sn	1.0-5.6	
	Pb	1.0-4.2	

	Ni	0.3-5.4	
	Zn	0.2-2.2	
	Sb	0.1-0.4	
	Au(ppm)	250-2050	
	Ag(ppm)	110-4500	
	Pd(ppm)	50-4000	
	Pt(ppm)	5-30	
	Co(ppm)	1-4000	
Ceramics	SiO <sub>2</sub>	15-30	30%
	Al <sub>2</sub> O <sub>3</sub>	6.0-9.4	
	Alkali-earthoxides	6.0	
	Titanates-micas	3.0	
Plastics	PE	10-16	30%
	PP	4.8	
	PS	4.8	
	Epoxy	4.8	
	PVC	2.4	
	PTPE	2.4	
	Nylon	0.9	

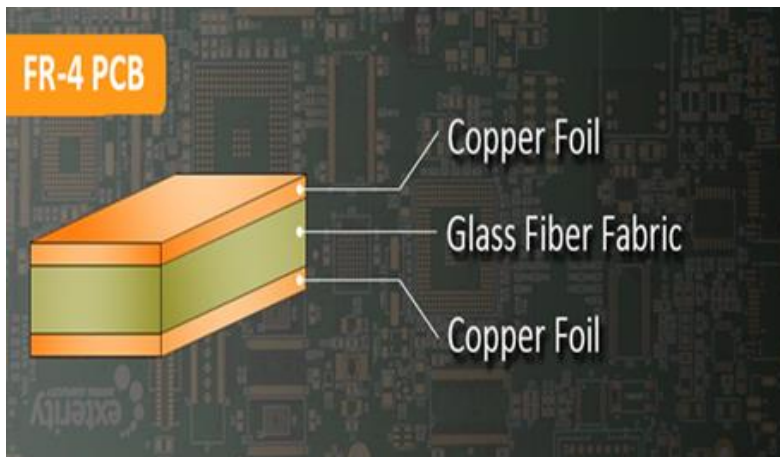


**Figure 4: Metals contained in PCBs (Lu & Xu, 2016)**

### 1.2.1 Characterization of waste PCBs

Base metals (i.e. Cu) present mainly in PCBs are used because of their high conductivity (Veit et al., 2005). Two types of PCBs are widely found in PCs and cell telephones. i) FR-4 and ii) FR-2. The type FR-4 (FR = Flame Retardent) is made of an epoxy resin multilayer, fiberglass coated with a layer of Cu. It is the substance most widely used for PCBs. FR4 uses



8 layers of fiberglass material. The FR-2 type contains a single layer of fiberglass or cellulose paper and a phenolic coated Cu layer (Hall & Williams, 2007; Murugan et al., 2008). Both resins are thermosetting (i.e., they can not be remelted or reformed).



**Figure-5: Most widely used FR-4 PCB(Assembly, 2019)**

The type FR-4 is used in small electronics, such as cell phones, and the type FR-2 is used in television sets and home appliances, such as PCs (LaDou, 2006). There are three kinds of PCBs according to their value, i) low (contain less than 100 ppm Au), ii) medium (contain 100-400 ppm Au) and iii) high (contain more than 400 ppm Au) (Hagelüken, 2006b). The epoxy resins FR-4 are green in color and have a high value, whereas the phenolic resins FR-2 are colored yellow/brown and have a low value (Table 2).

**Table 2: PCB types and properties (Kaya, 2016)**

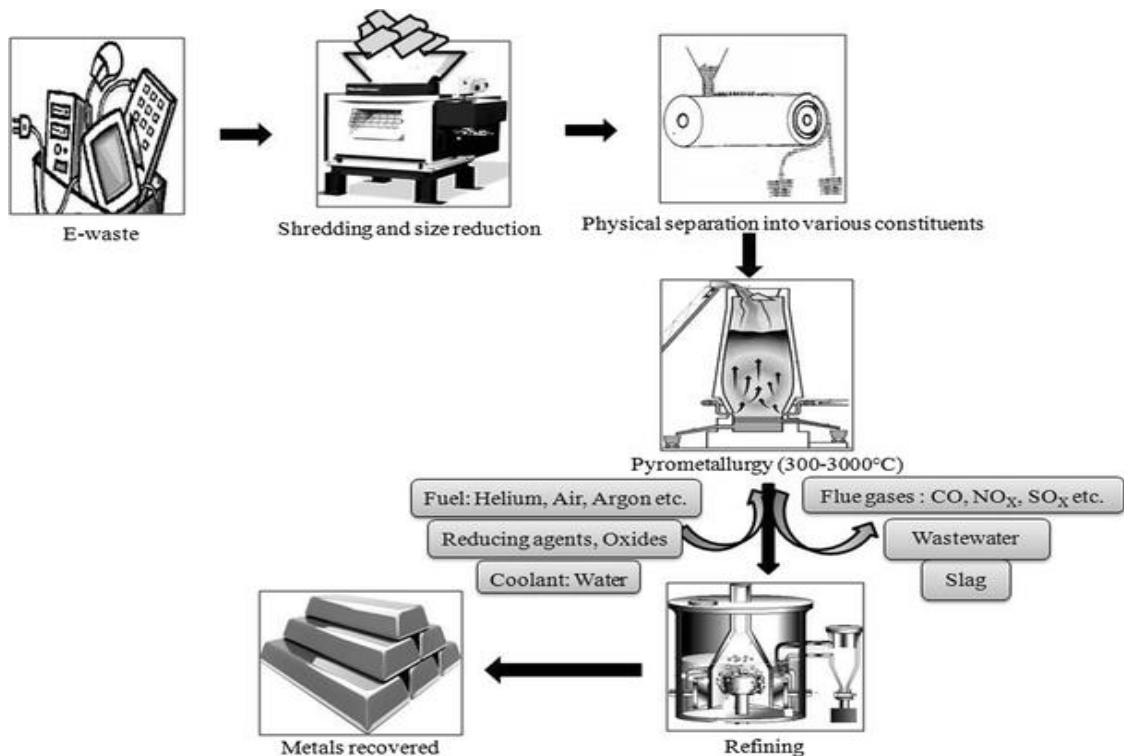
Type	color	layer	value
FR-2 (reinforcement) 	Yellow/brown	Single layer, Phenolic cellulose paper	Low value EEE (TV, home electronics)
FR-4 (reinforcement) 	Green	Multilayer, Epoxy glass fiber	High value EEE (PC, phones)

## 1.3 Fundamental processes of e-waste recycling

### 1.3.1 Pyrometallurgical process

The pyrometallurgical cycle includes thermal treatment including incineration, smelting, scrubbing, sintering, fusion and gas reactions in a regulated high-temperature setting to extract non-ferrous as well as precious metals from e-waste (Lee et al., 2007). The heating process is performed in highly specialized incinerators, blast furnace or plasma arc furnace, in which metallic oxides interact with reducing agents such as carbon monoxide (CO) releasing coke or charcoal. Here CO<sub>2</sub> can not be used as a reducing agent since it is much more stable than CO thermodynamically (Quora, 2016). Slag is the non-metallic component of the e-waste called gangue after heating with flux materials like charcoal, coke, lime, and borax. Slag is a liquid substance that is lighter than the metal component that flows over it which contains metallic residues that can easily be separated (Cui & Zhang, 2008; Dalrymple et al., 2007). Generalized steps covering the recovery of pyrometallurgical metals from e-waste are shown in Fig-6. Metals that are found in pure form in e-waste are quickly extracted by smelter melting (Reck & Graedel, 2012). The stability of the alloys coupled with their poor separation behavior during melting, however, limits the recovery of the pure metals and makes pyrometallurgical processes energy-intensive (Nakajima et al., 2010). It has also been documented that e-waste emits many dangerous gases and compounds, such as polybrominated dibenzodioxins (PBDD), polybrominated dibenzofurans (PBDF), phenol, naphthalene, biphenyl, anthracenic or phenanthrenic acids and many others, which lead to extreme secondary pollution, including dibenzofuran, dibenzop-dioxin, tribromobenzene and many more (Barontini & Cozzani, 2006; Ebert & Bahadir, 2003; Ni et al., 2012). It has also been mentioned that the informal manufacturing of metallic components in e-waste at open burning areas releases significant amounts of toxic heavy metals such as Hg, Zn, and polychlorinated dibenzodioxins and dibenzofurans contributing to long-term environmental and health problems.(Cui & Zhang, 2008; Kahhat & Williams, 2009; Tsydenova & Bengtsson, 2011). A new, ultrasonically aided pyrometallurgical metal recovery procedure with reduced waste emissions and high recovery performance has been developed. The technique recovered Cu and Fe with an efficiency of 95.2–97.5% and 97.1–98.5%, respectively (Xie et al., 2009). (Zhan & Xu, 2008) have integrated vacuum technology into the metallurgical method to remove Cu from e-waste with a purity of 99 % and gold recovery with 99.9% purity from e-waste using an altered refining process, ensuring that the electronic scrap reacts with chlorine at the temperature of 300–700°C. Afterward the impurities were

washed out with a combination of hydrochloric acid, ammonium hydroxide, and nitric acid (Cui & Zhang, 2008).



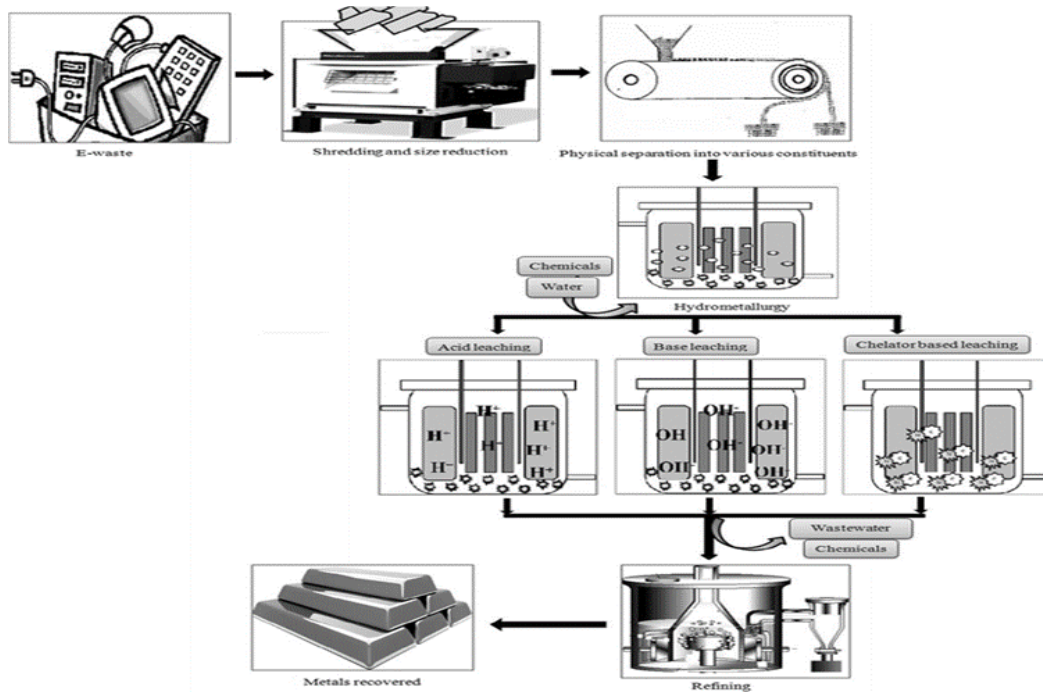
**Figure-6: Schematic description of phases involved in e-waste recovery of pyrometallurgical metal(Priya & Hait, 2017)**

### 1.3.2 Hydrometallurgical process

Major obstacles associated with pyrometallurgical processes include possible release of toxic metals in gaseous form, especially lower melting point metals such as Cu, Cd, and Pb. On the other hand, major investments and running costs are required for hydrometallurgical processes to realize it as a viable solution to WEEE metal treatment and recovery. Leaching is the initial phase in the recovery of metals in traditional hydrometallurgical processes using suitable chemical lixiviants that lead to the dissolution of metal fractions of e-wastes (Cui & Zhang, 2008; Das et al., 2009; Silvas et al., 2015). Hydrometallurgical lixiviants or leaching reagents typically contain acid or alkaline solutions that in liquid phase solubilize metals from solid matrix. Since the metals in WEEE are sometimes embedded in a polymer or ceramic matrix, a mechanical scraping method (figure-7) might be required to remove embedded metallic particles to enhance extraction (Cui & Forssberg, 2003; Cui & Zhang, 2008; Zhang & Forssberg, 1997). Crushed electronic scraps are processed with sulfuric acid, nitric acid, aqua regia, thiourea, thiosulfates, cyanides, and halides, for the purpose of leaching metals from e-waste (Choubey et al., 2015; Huang et al., 2009; J. Li et al., 2007;

Pant et al., 2012). Overall, oxidative reactions are typically involved in the successful degradation of the base and precious metals from e-waste. HCl, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub> / H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub>, and NaClO are the popular inorganic and oxidant acid leaching agents (Huang et al., 2009). Cyanide, thiourea, thiosulfate and halides are widely used to recycle precious metals from e-waste (Tuncuk et al., 2012). Cyanide leaching is very efficient and economical for gold and can remove 1–3 g/t of gold but it also has an environmental toxicity issue (Tuncuk et al., 2012). Thiosulfate and thiourea both have a high rate of leaching efficiency and were introduced as an alternative to cyanidation (Tuncuk et al., 2012). Thiosulfate forms complex with metals such as Au and Ag in the presence of ammonia and Cu ion, speeds up metal dissolution. The thiosulfate compound has poor chemical stability and is therefore rapidly disintegrated into metal sulfide (Helmut Brandl et al., 2008). Acid leaching is typically performed as a first step leaching to recover other unprecious base metals like Cu (Tuncuk et al., 2012). Chemical leaching was also tested for metal extraction utilizing different chemical ligands such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepenta acetate (DTPA), and other chelators such as oxalate and citric acid (Pant et al., 2012). After leaching of metals by an appropriate lixiviant, the leach solution is purified using techniques such as electro-refining, adsorption and solvent extraction to concentrate and recover specific pure metals (Cui & Zhang, 2008).

While the technological viability of the hydrometallurgical method has been developed, the wastewater produced during the recovery process is extremely hazardous, difficult to recycle and greatly restricts the recovery process (Norgate et al., 2007). Researchers also suggested many upgrades and modifications in the hydrometallurgical method that are already in use. In order to recover metals (Rath et al., 2012) has used thermal plasma coupled with acid leaching. This system provides low effluent production, fast slag metal isolation and quick processing time. Another method suggested a mixture of thermal and acid treatment for quicker and more effective dissolution and recovery of gold (Chmielewski et al., 1997). (H. Yang et al., 2011) has investigated the sustainable method of Cu recovery from shredded waste particle PCBs with sulfuric acid and hydrogen peroxide solutions at room temperature. Hydrometallurgical treatment of e-waste nevertheless brings questions regarding the generation of significant amounts of hazardous, strongly acidic, alkaline or flammable reagents with the production of voluminous effluents, threats of exposure to acid fumes, liquid acid, along with cleaning solvents that could present a serious danger of persistent exposure of worker as well as a threat of environmental pollution (Helmut Brandl et al., 2008; Cui & Zhang, 2008; Huang et al., 2009).



**Figure 7: Schematical description by hydrometallurgy of metal production from e-waste(Priya & Hait, 2017)**

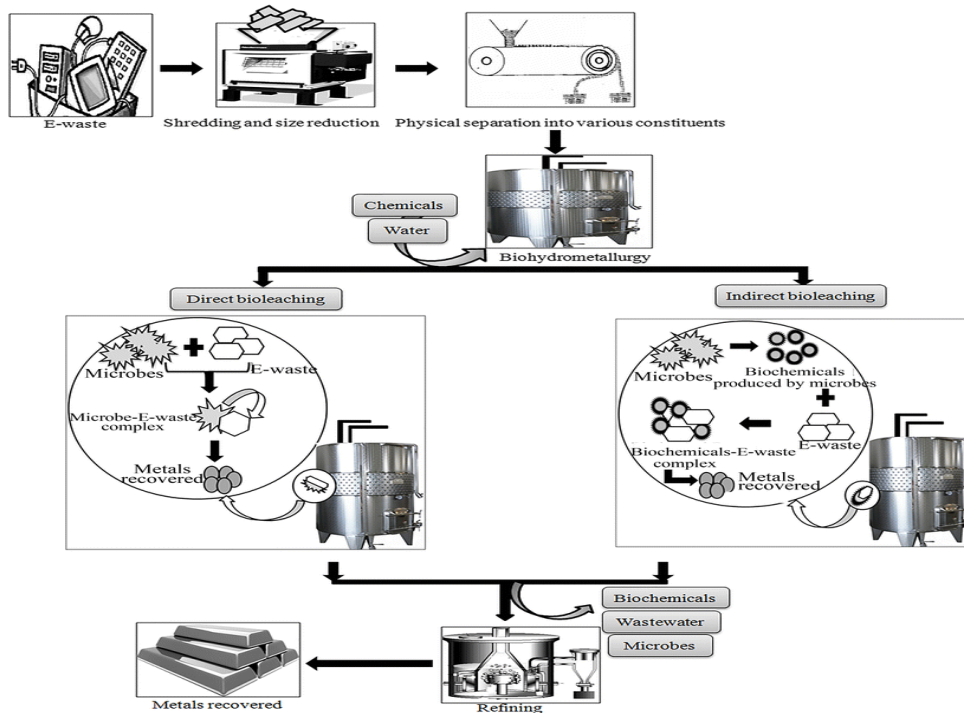
### 1.3.3 Biohydrometallurgy process or bioleaching

Biometallurgy or biohydrometallurgy is also known as bioleaching is a revolutionary technique that has built up an environmentally-friendly, energy-conserving way for the recovery of metals. It utilizes the leaching capability of so-called bioleaching microorganisms to turn metals into their soluble extractable forms (figure-8), which are then retrieved from the solution (J. A. Brierley & Brierley, 2001; Krebs et al., 1997). In recent decades, microorganisms primarily responsible for mineral leaching have been detected and described in natural ecosystems (Beolchini et al., 2012; Bosecker, 1997c; Ivanus et al., n.d.; Krebs et al., 1997). These microorganisms are generally iron (Fe) and sulfur-oxidizing bacteria that can oxidize metals from solid substrates by direct mechanism or indirect mechanism by oxidizing ferrous iron (Fe<sup>2+</sup>) to ferric state (Fe<sup>3+</sup>) producing a strong oxidizing agent, or by oxidizing elemental sulfur and its compounds to produce the required leaching reagents such as sulfuric acid and sulfur-oxidizing reagents (Beolchini et al., 2012; Bosecker, 1997c; Krebs et al., 1997; Sand et al., 2001). Chemolithoautotrophic bacteria such as *Thiobacillus ferrooxidans*, *T. thiooxidans*, *Sulfolobus spp.*, and *Leptospirillum ferrooxidans* and fungi for example *Aspergillus niger*, *Penicillium spp* are often used to carry out the leaching process (Aung & Ting, 2005). In addition, micro-organisms that secrete specific organic and inorganic acids have also been used to recover various metals from specific low-

grade sources (Ilyas et al., 2007; Le et al., 2006; Shi & Fang, 2005). The heterotrophic microorganisms *Bacillus* and *Pseudomonas* often have the ability to remove metals from non-sulfidic materials. These heterotrophic bioleaching bacteria extract carbon and energy from organic carbon source generating a variety of metabolic by-products, including citric acid, oxalic acid, formic acid and other biochemical leaching reagents to leach metals from e-waste (Castro et al., 2000; Krebs et al., 1997; K. A. Natarajan & Deo, 2001).

Like most biological processes, microbial leaching is affected by both biotic and abiotic factors influencing bacterial behavior and metal extraction (Bosecker, 1997c; Choi et al., 2004; Krebs et al., 1997). The maximum removal of inhibitors, which hinder the growth of the microorganisms involved in the process, often improves metal solubilization. Biotic factors like microorganism species and form of culture, inoculum size, and abiotic factors such as growth temperature, pH, oxygen supply, aeration rate, humidity, particle size of extractable e-waste, incubation period and the composition of nutritional media often influence the bioleaching processes (Ilyas et al., 2007; J. Wang et al., 2009; T. Yang et al., 2009). (T. Yang et al., 2009) studied Cu recovery method with *Acidithiobacillus ferrooxidans* and observed the impact of Fe and inoculum concentrations, and pH as the controlling factors for Cu recovery. (Ilyas et al., 2007) managed to recover more than 80% of metals including Al, Cu, Ni, and Zn from PCBs utilizing acidophilic and heterotrophic bacteria. (Xiang et al., 2010) optimized the bioleaching conditions for Cu from waste PCBs and obtained a 95% dissolution within 5 days of bioleaching. (Zhu et al., 2011) studied the impact of variables such as initial pH, initial concentration of Fe(II), metal concentrate weight, particle size and inoculation quantity to determine the viability of bioleaching of PCB metal concentrates by mixed culture of acidophilic bacteria, resulting in 96.8% dissolution of Cu, 88.2% and 91.6% of Al and Zn, respectively. (Ilyas et al., 2013) researched the potential of a moderately thermophilic bacterial group, *Sulfobacillus thermosulfidooxidans* to remove metals through electronic scrap utilizing additional energy sources ( $\text{FeS}_2$ ,  $\text{S}^0$ ) in shake bottles and lab-scale column reactors and recovered nearly 74% of Zn, 68% Al, 85% Cu and 78% Ni in 165 days. (Faramarzi et al., 2004) used the cyanogenic microorganism *Chromobacterium violaceum* by to extract Au from PCBs during bioleaching. (Işıldar et al., 2016) produced a two-step bioleaching method for microbial recovery of Cu using chemolithotrophic acidophilic *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrivorans* and later Au together with heterotrophic cyanide-producing *Pseudomonas putida* and *Pseudomonas fluorescens* from scrap PCBs.

Biohydrometech methods have proved to be the most effective method for the recovery of precious metals from secondary resources such as e-waste. Bioleaching-based methods deliver a range of benefits compared to traditional techniques, such as low running costs, reducing the amount of chemical sludge production, compatible with the climate, fairly low energy usage and fast metal extraction technology (figure-9).

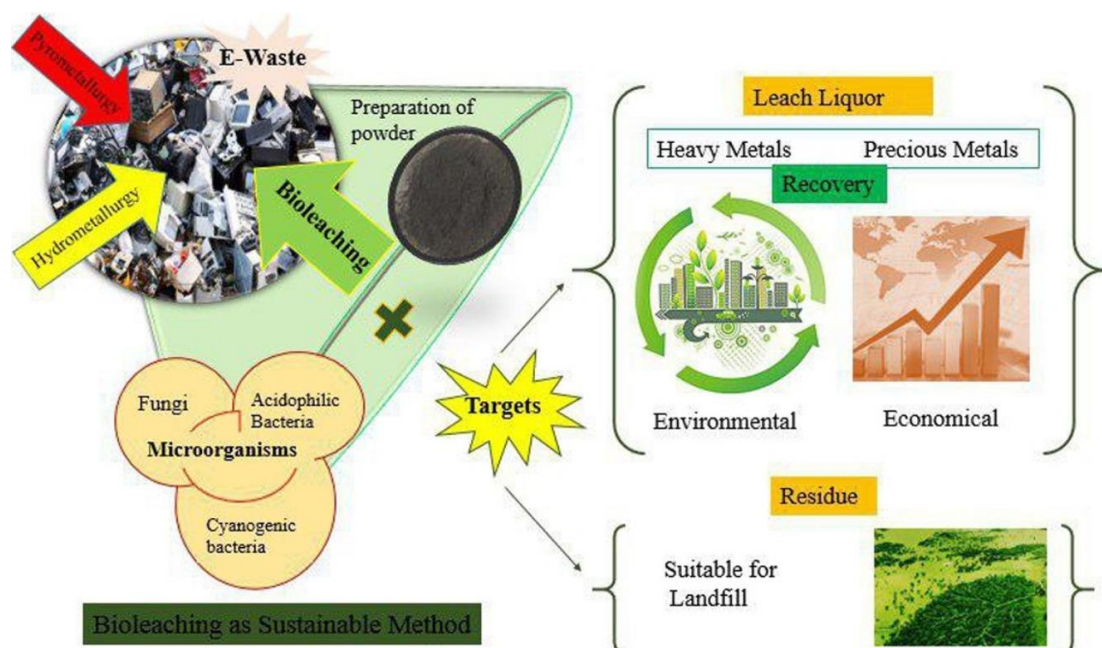


**Figure-8: Biohydrometallurgical product recovery model description from e-waste(Priya & Hait, 2017)**

**Table-3: Comparison of methods for the extraction and refining of metals(Işıldar et al., 2018)**

Parameters	Pyrometallurgy	Hydrometallurgy	Biohydrometallurgy
Scale of operation	Possible only on a large scale	Possible at smaller scale	Possible at smaller scale
Capital, operational and energy costs	Higher	Lower	Lower
Metal selectivity	Low, only a fraction of metals	High	High

<b>Capital and operating costs</b>	Capital intensive, low job creation	Low capital, high operating cost	Low investment and operating costs
<b>Environmental impact</b>	High, due to gaseous emissions	Moderate, due to toxic chemicals	Low
<b>Social acceptance</b>	Low	Medium, some toxic reagents and end-products	High, cleaner processes and auto-pollution control
<b>Final residues</b>	Slags, high	Low, circulated water	Low-to-none, circulated water
<b>Process conditions</b>	Harsh thermal treatment conditions	Harsh corrosive acids	Safe conditions, low-to-none toxic chemicals

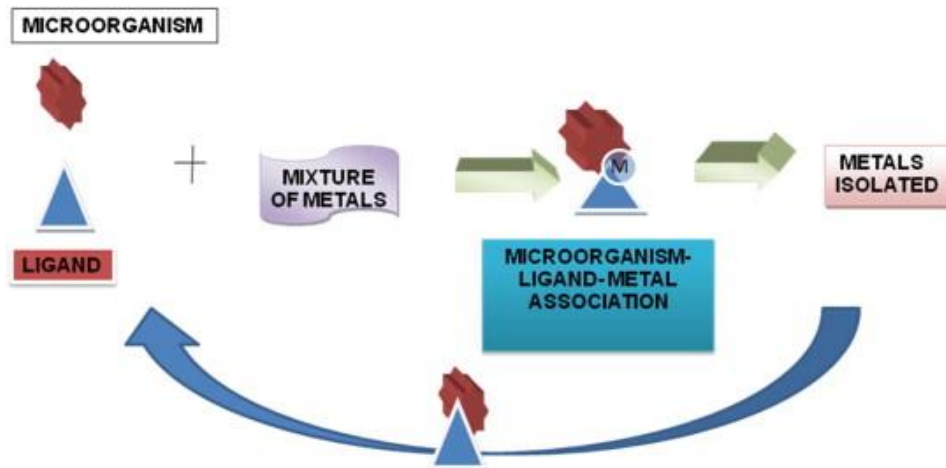


**Figure-09: Biorecovery as a sustainable method(Baniasadi et al., 2019)**

### 1.3.4 Hybrid technologies

Recently, hybrid technologies that combine chemical (more efficient) with biological (more environmentally friendly) processes have also been introduced, in order to take advantage of both chemical and biological leaching (Ilyas & Lee, 2015; Pant et al., 2012). Biological leaching is typically a cost-efficient yet time-consuming procedure and the absolute recovery

of metal by biological leaching alone is in most situations not feasible. On the other side, chemical leaching is fairly fast and effective but has its own environmental concerns. If a hybrid method is implemented, it involves a combination of chemical (safer chemicals) and biological leaching so that both will support each other (figure-10) for an efficient and improvised extraction method of metals.



**Figure-10: hybrid method (Pant et al., 2012)**

Biogenic leachate excretion is enhanced, catalyzed or supplemented by chemical processes in these applications. In situations where there is insufficient biological leaching, chemical leaching tries to compensate the oxidative capacity that required to leach metals. A hybrid process can include a sequence in which the main or secondary material is biologically processed, followed by a chemical process or vice versa (Ilyas & Lee, 2015). In this way a double benefit will be obtained (i) reducing the use of chemical reagents and (ii) improving the environmental profile.

## **1.4 Bioleaching microorganisms**

### **1.4.1 Chemolithoautotrophic acidophilic bacteria**

In the case of chemolithoautotrophs, biogenic acid formed by microorganisms such as sulfuric acid can solubilize the metals from the solid matrix through acidolysis and redoxolysis (Baniasadi et al., 2019) As a source of energy, chemolithotrophic organisms use atmospheric carbon dioxide (CO<sub>2</sub>) and inorganic compounds such as ferrous iron (Fe<sup>2+</sup>), elemental sulfur (S<sup>0</sup>) or reduced sulfur compounds (Donati, 2007; Peiravi et al., 2017). The most prevalent species of bacteria facilitating metal solubilization are *Leptospirillum ferroxidans*, *A. Ferrooxidans*, *A. thiooxidans* etc. (Adhapure et al., 2014; H. Brandl et al., 2001). Most chemolithoautotrophs have a high tolerance for toxicity of heavy metals which

is making them the most widely used group of microorganisms for processing WEEE (Orell et al., 2010). The two main functions of this type of bacteria are  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  and S to  $\text{H}_2\text{SO}_4$  oxidation, which is involved in leaching (Lee & Pandey, 2012). Such acidophiles grow on ores containing iron and sulfur such as pyrite, pentlandite  $[(\text{Fe}, \text{Ni})_9\text{S}_8]$  and chalcopyrite ( $\text{CuFeS}_2$ ) at temperatures varying from 45 to 75°C (Işıldar et al., 2019).

Based on their optimal temperature range, the acidophilic microorganisms are classified into two categories,- mesophilic and thermophilic microorganisms.

#### **1.4.1.1 Thermophilic bacteria**

*Thiobacillus*-like bacteria, so-called Th-bacteria, are moderately thermophilic bacteria that can grow at temperatures about 50°C on pyrite, pentlandite, and chalcopyrite. Ferrous iron is used as the basis of energy but growth is observed only in the presence of yeast extract (James A Brierley, 1978). Extremely thermophilic bacteria can grow at temperatures 60°C and above (Bosecker, 1997b). Microorganisms such as *Acidianus brierleyi*, *Sulfobacillus thermosulfidooxidans*, and *Metallosphaera sedula* are active in thermophilic processes (Du Plessis et al., 2007). *Sulpholobus species*, such as *S. acidocaldarius*, *S. solfataricus* and *S. brierleyi*, are examples of extreme thermophiles that can be used up to 70°C.(Lee & Pandey, 2012)

#### **1.4.1.2 Mesophilic bacteria**

In the moderate temperature range, mesophiles develop from around 20°C (or below) to 45°C. *T. thiooxidans*, *T. ferrooxidans* and *L. ferrooxidans* are some examples of mesophilic bacteria with optimum growth temperature 25–35°C (Bosecker, 1997b). The most extensively studied mesophilic microorganisms in bioleaching communities are *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* (Modin et al., 2017)

#### **1.4.2 Heterotrophic bacteria**

Heterotrophs such as *Sulfolobus*, *Bacillus*, and *Pseudomonas* have been often used for the degradation of metal containing materials (Ilyas et al., 2007). They gain their carbon and energy requirements from organic carbon to produce several metabolites as well as organic acids as by-products which enhance metal leaching from e-waste (Burgstaller & Schinner, 1993; Liang et al., 2013). The chemoorganotrophic bacteria are also known as heterotrophic bacteria.

Heterotrophic bioleaching is usually focused on microorganisms that produce a lixiviant solution consisting of organic acids (e.g., citrate, gluconate, oxalate) or chelating agents (siderophores, cyanide) that release metals into the aqueous process (Hennebel et al., 2015). Heterotrophic bioleaching has been recommended for leaching primary sources of critical raw materials and products with strong relative economic importance (Al, Cr, Co, Mn, etc.)

### **1.4.3 Cyanogenic bacteria**

Precious metals, such as gold, are extracted through cyanogenic bacteria, such as *Cyanobacterium violaceum*, *B. megaterium*, *Pseudomonas fluorescens* which develops in an alkaline atmosphere containing cyanide and forming cyanide complex of respective metal ions (H. Brandl et al., 2001; Karwowska et al., 2014; Valix et al., 2001; J. Wang et al., 2009).

### **1.4.4 Reaction mechanism of bioleaching microorganisms**

The bioleaching of e-waste generally involves three mechanisms.

- i. acidolysis,
- ii. redoxolysis, and
- iii. complexolysis.

The protonation of oxygen atoms covering the metallic compound surface happens in acidolysis. Heterotrophic organic acid protons (malic, oxalic, gluconic, acetic, citric, succinic, pyruvic, and formic acids) and even bacterial inorganic acids (e.g. H<sub>2</sub>SO<sub>4</sub>) are capable for acidolysis. Redoxolysis is the process of metal solubilization by reactions of oxidation-reduction. The energy transfer needed for microbial growth occurs through redoxolysis by electron transfer. In acidophilic redoxolysis reactions, ferric ions are reduced enzymatically under anaerobic environments, where hydrogen or sulfur serve as an electron donor. Complexolysis is another process which is essential for the recovery of precious metals by cyanogenic bacteria. In the late stationary phase of growth of the microorganism, cyanide is formed by decarboxylation of glycine (Lu & Xu, 2016). Many cyanide-producing bacteria have the capability of  $\beta$ -cyanoalanine synthase by detoxifying the cyanide to  $\beta$ -cyanoalanine. This makes the method of biocyanidation an effective process in the wastewater streams (K. A. Natarajan, 2018)

### **1.4.5 Classification of bioleaching capable organisms**

Remarkably diverse classes of microbes such as bacteria, fungi and yeasts are used for bioleaching, however acidophilic sulfur-oxidizing bacteria and iron-oxidizing bacteria are

the most commonly utilized in this method. Bioleaching is performed by microorganisms with the capacity to secrete inorganic or organic acids or cyanides that improve enzymatic oxidation-reduction, proton-promoted pathways or ligand and complex creation (Vakilchap et al., 2016; Xiang et al., 2010). Specifically, microorganisms capable for bioleaching can be classified into three main classes (Table 4) (Pandey & Natarajan, 2015):

- a) chemolithoautotrophic bacteria function by acidolysis and redoxolysis mechanism. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* are the most well-known chemolithotrophs which catalyze the oxidation of ferrous iron ( $\text{Fe}^{2+}$ ) to ferric iron ( $\text{Fe}^{3+}$ ) ions;
- b) heterotrophic organic acid producing bacteria and fungi function by acidolysis and complexolysis mechanism. Species from the genus *Bacillus* as perhaps the most efficient bacteria and some other fungi such as *Aspergillus niger* and the genera *Penicillium simplicissimum* can be used in the bioleaching process;
- c) cyanogenic bacteria function by complexolysis mechanism. The most promising cyanogenic bacterial species are *Chromobacterium violaceum* and *Bacillus megaterium*.

**Table 4: Classification of microorganisms by their function**

Type of microorganism	Species	Leaching mechanism	Targeted materials	Targeted metals	References
<b>Chemolithoautotrophic acidophilic iron- and sulfur-oxidisers</b>	<i>Acidithiobacillus ferrooxidans</i> , <i>Acidithiobacillus thiooxidans</i>	Redoxolysis, acidolysis	PCB	Cu, Fe, Zn, Al, Zn, Pb, Ni	(Chen et al., 2015; Hong & Valix, 2014; Mäkinen et al., 2015)
<b>Heterotrophic acidophiles</b>	<i>Aspergillus niger</i> , <i>Penicillium simplicissimum</i>	Complexolysis, acidolysis	Spent batteries, PCB	Cu, Fe, Zn, Al, Zn, Mn, Cd, Pb, Ni	(H. Brandl et al., 2001; Faramarzi et al., 2004; Kim et al., 2016)
<b>Cyanide producers</b>	<i>Pseudomonas putida</i> , <i>Chromobacterium violaceum</i>	Complexolysis	PCB	Cu, Fe, Zn, Al, Ag, Au, Pd, Pt	(G. Natarajan & Ting, 2015; Pradhan & Kumar, 2012a)

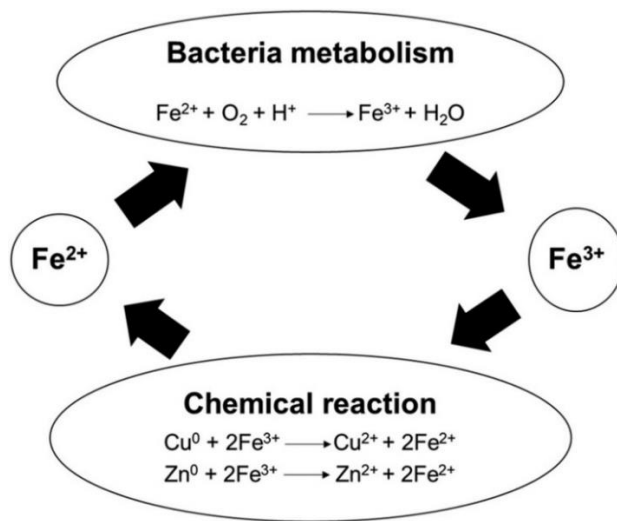
## 1.5 Principle of metal bioleaching

Content of metallic material is the main concern of e-waste. The organic fractions of e-waste are usually degraded and decomposed. Inorganic elements such as metals generated as part of waste from either the residential, manufacturing or commercial industries, are not decomposed, they are converted from one form to another. Availability and mobility of metals are of great concern due to their solubility at different pHs, intrinsic properties, potential for reduction and oxidation and their complex characteristics (H. Brandl et al., 2001). Environmentally discarded waste contains a range of poisonous and dangerous metals that pose a threat to both the environment and biota when converted into a mobile form. Typically this mobilization is mediated by microbial activity known as bioleaching. Microorganisms normally conduct various physiological reactions, which enable them to expand and develop. Microorganisms create various metabolic products during growth and metabolic processes, such as inorganic and organic acids, which help remove metals from wastes and minerals (Gehrke et al., 1998). Unlike traditional pyrometallurgical and hydrometallurgical methods, the biological solution offers advantages of cost competitiveness, energy efficiency, compatibility with the environment, and usefulness in metal recovery, including from degraded low-grade waste (Sand et al., 2001).

Bioleaching is classified into direct bioleaching and indirect bioleaching, on the basis of metal recovery mechanisms (Sand et al., 2001). Bacteria physically bind to the solid matrix in the direct process of bioleaching and enzymatically oxidize the metals while in indirect mechanisms, bacteria develop leaching agents such as ferric oxide, sulfuric acid, etc. that oxidize the metals and leach out of the solution (Bosecker, 1997a; Rawlings, 2002; Saidan et al., 2012; Sand et al., 2001). The role of bacteria is presumed to be in preserving high redox potential in the leaching medium by continuously oxidizing  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  (Bosecker, 1997a). Throughout the bioleaching phase of e-waste, iron and sulfur are typically added in the media in the form of ferrous sulfate and elemental sulfur in order to promote bacterial growth. This is mainly since e-wastes have no supply of energy (H. Brandl et al., 2001). The theory behind microbial leaching includes i) oxidation-reduction reaction; (ii) organic and inorganic acid production; and (iii) secretion of microbial metabolites, complexing agents, chelators. (Bosecker, 1997a; Choi et al., 2004; Jadhav et al., 2014)

Bioleaching utilizes the function of bacteria to be an oxidant for the  $\text{Fe}^{3+}$  regeneration as shown on Figure 12 (Lee & Pandey, 2012; Rawlings et al., 1999; Xiang et al., 2010). After  $\text{Fe}^{3+}$  has been reduced in metal dissolution in to  $\text{Fe}^{2+}$ , the latter is used as an energy supply

to expand the bacterial population and metabolism and make it ready for more metal oxidation (Figure-11) (Becci et al., 2020). This cyclical bioleaching mechanism is essential for the leaching reaction where  $\text{Fe}^{3+}$  plays an important role.



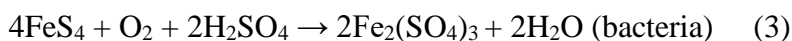
**Figure-11: Fe-ion cyclic process due to bacterial metabolism as supports bioleaching in the abiotic control (Becci et al., 2020)**

### 1.5.1 Direct and indirect mechanism

Physical interaction between the bacterial cell and the mineral sulfide surface arises in direct bacterial leaching, and oxidation to sulfate arises through many enzymatically catalyzed phases. The direct mechanism includes bacteria that are physically bound to the mineral sulfide, resulting in disproportionate oxidation in the mineral's crystal lattice, thereby inducing dissolution. It can be expressed by the following generalized overall reaction (Torma, 1977).

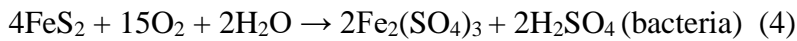


Here, MS considered as a metal sulfide. For more clarification, the two following reactions explain the "direct" oxidation of pyrite by acidophilic bacteria.

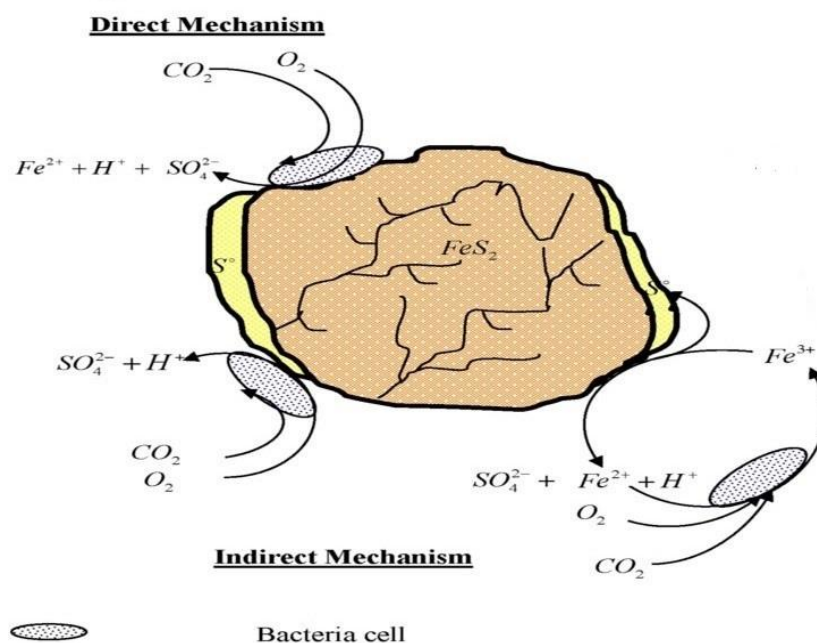


In earlier scientific literature, it was assumed that bacteria act as a trigger in the presence of water and oxygen to assist in the oxidation of sulfide to sulfate (Equation-2), thereby generating ferrous ions and creating sulfuric acid. The acid formed further reacts with ferrous ion and dissolves oxygen to create  $[\text{Fe}_2(\text{SO}_4)_3]$  ferric sulfate (Equation-3), which is

essential for the "indirect" oxidation process. In reaction (equation-4), pyrite is specifically attacked by oxidizing bacteria that explains "direct" bacterial oxidation, which is the sum of the bacteria-catalyzed oxidation of sulfur and iron by oxygen (Palencia et al., 1991)

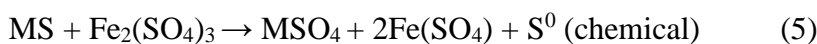


As per new findings from recent studies, this "direct" process (i.e., enzymatic attack) does not occur. Throughout their research (Sand et al., 2001) showed that the metals sulfides are degraded on the metal sulfide crystal lattice by a chemical attack of  $\text{Fe}^{3+}$  ions and/or protons agents. The main  $\text{Fe}^{2+}$  ions are provided by the bacterial extracellular polymeric substances (EPS), so the direct method is therefore an "indirect" mechanism dependent in a strict sense.

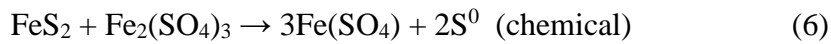


**Figure-12: Schematic of reactions involving the bacterial aided leaching of pyrite ( $\text{FeS}_2$ ). (Ahmadi et al., 2012; Y. Li et al., 2013)**

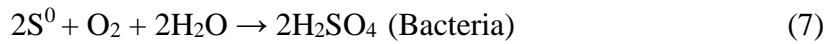
The "indirect" mechanism initially assumes that the produced bacterially lixiviant ( $\text{Fe}^{3+}$ ) plays a vital role in the bacterial assisted leaching of a range of minerals. The reduction potential of ferric ion ( $\text{Fe}^{+3}$ ) in acid solution (at  $\text{pH}=1.5$ ) makes it a powerful oxidizing agent for the solubilization of a number of minerals. The solubilization of the metal can therefore be defined as follows:



In the case of indirect oxidation, pyrite is chemically oxidized by ( $\text{Fe}^{+3}$ ) ions in the presence of an oxidant ( $\text{Fe}_2(\text{SO}_4)_3$ ) based on the reactions described below.

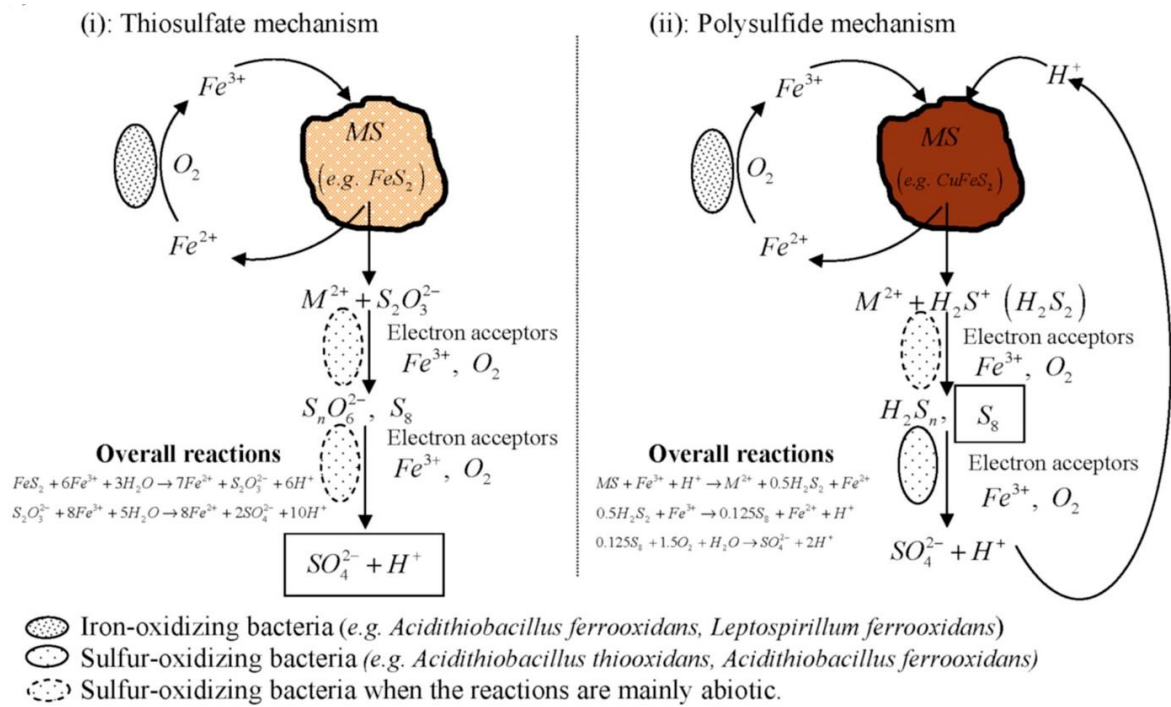


The bacteria do not need to be in contact with the mineral surface in "indirect" leaching. The oxidation of ferrous iron is around 10<sup>5</sup>-10<sup>6</sup> times faster than chemical oxidation, in the presence of bacteria and in the pH 2–3 of liquid phase of. (Ahonen & Tuovinen, 1989; Lacey & Lawson, 1970). In addition, the concurrently formed sulfur (Equation 6) can be oxidized to sulfuric acid in the presence of sulphur-oxidizing bacteria (Equation 7).



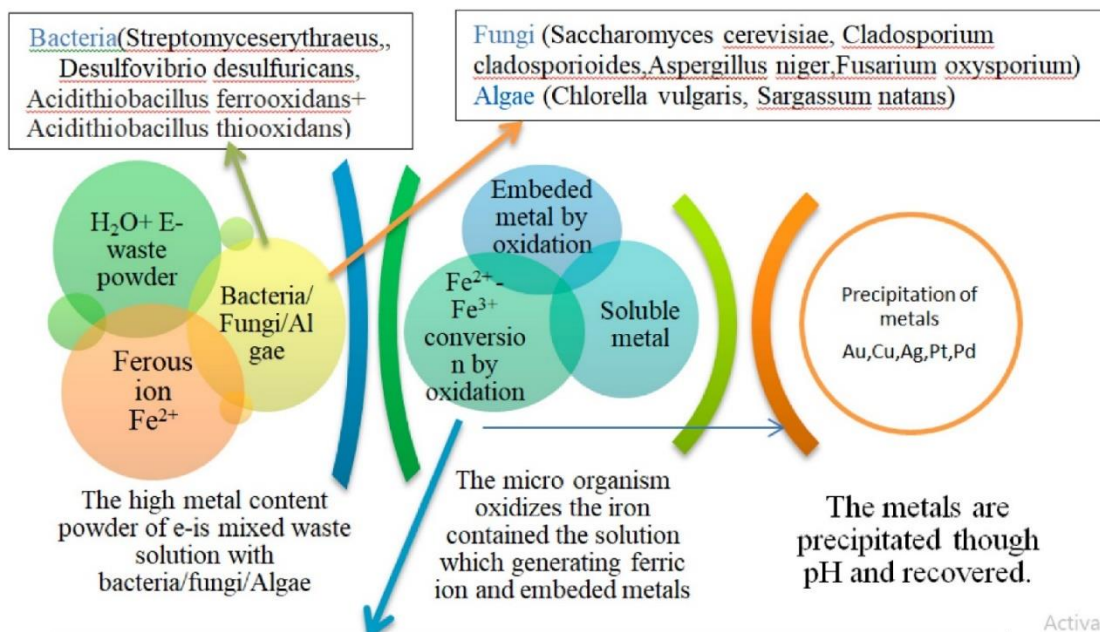
### **1.5.2 Thiosulfate and polysulfide mechanism**

There are two different reaction mechanisms that control the dissolution of metal sulfides – the thiosulfate pathway and the polysulfate pathway (Mishra et al., 2005). The crystal structure of monosulfide or disulfide does not control the path of dissolution, the reaction mechanism is rather determined by the minerals species (Schippers & Sand, 1999). Depending on their acid solubility, metal sulfides can be classified into (i) acid-insoluble metal sulfides (thiosulphate pathway) and (ii) acid-soluble metal sulfides (polysulphate pathway). In the thiosulfate mechanism (Figure 13(i)), metal sulfides such as pyrite, molybdenite and tungstenite (FeS<sub>2</sub>, MoS<sub>2</sub>, and WS<sub>2</sub> respectively) are oxidized entirely via ferric (Fe<sup>3+</sup>) ions. The chemical bonds between sulfur and metal moiety do not break in this group of metal sulfides until a total of six successive single-electron (Fe<sup>3+</sup> hexahydrate) oxidation steps have been performed and thiosulfate released. This mechanism takes its name from its first compound of free sulfur (Mishra et al., 2005)



**Figure-13: Scheme of the two bioleaching pathways for metal sulfide, the mechanism for (i) thiosulfate and (ii) polysulfide. Boxed, i.e. sulfuric acid in (i) and elemental sulfur in (ii) are the major reaction products that occur in the absence of sulfur oxidizer. (Mahmoud et al., 2017)**

In the mechanisms of polysulfide (Fig-13(ii)), metal sulfides such as sphalerite (ZnS), galena (PbS), arsenopyrite (FeAsS), chalcopyrite (CuFeS<sub>2</sub>), and hauerite (MnS<sub>2</sub>) are dissolved by the combined effect of electron extraction by ferric Fe<sup>3+</sup> ions and proton attack, i.e., the binding of protons by the sulfide moiety through valence bond electron. The chemical bonds between metal and sulfur moiety can be broken in this group of metal sulfides by proton attack and released hydrogen sulfide (H<sub>2</sub>S), after binding two protons. The first free sulfur compound here is most likely a sulfide cation (H<sub>2</sub>S<sup>+</sup>), which can rapidly dimerize into free disulfide (H<sub>2</sub>S<sub>2</sub>) and is further oxidized to elemental sulfur through use of higher polysulfides and polysulfide radicals (Mishra et al., 2005). Therefore this mechanism was called the "pathway of polysulfide"



**Direct mechanism**  
Physical contact of microorganism with metal sulphide  
 $MS + H_2SO_4 + 0.5O_2 \rightarrow MSO_4 + S^0 + H_2O$   
 $S^0 + 1.5 O_2 + H_2O \xrightarrow{\text{(Bacteria)}} H_2SO_4$

**Indirect mechanism**  
Involves ferrous to ferric cycle  
It involves ferric-ions (a strong oxidizing agent) which oxidizes minerals.  
Role of microbes is to regain Fe<sup>3+</sup> from Fe<sup>2+</sup>  
 $MS + 2Fe^{3+} \rightarrow M^{2+} + 2Fe^{2+} + S^0$   
 $2Fe^{2+} + .5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$

**Thiosulphate mechanism**  
Ferric ion attack on the acid insoluble metal sulphide (i.e., pyrite (FeS<sub>2</sub>)) and molybdenite (MoS<sub>2</sub>)  
 $FeS_2 + 6Fe^{3+} + 3H_2O \rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+$   
 $S_2O_3^{2-} + 8 Fe^{3+} + 5H_2O \rightarrow 2S_2O_4^{2-} + 8 Fe^{2+} + 10H^+$

**Polysulfide mechanism**  
Combined attack of protons and ferric ion on acid soluble metal sulphide [Like; sphalerite (ZnS), chalcopyrite (CuFeS<sub>2</sub>) of galena (PbS)]  
 $MS + Fe^{3+} + H^+ \rightarrow M^{2+} + 0.5 H_2S_n + Fe^{2+} \quad (n \geq 2)$   
 $0.5H_2S_n + Fe^{3+} \rightarrow 0.125S_n + Fe^{2+} + H^+$   
 $0.125S_n + 1.5 O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$

**Figure-14: Biological process for extracting metals from electronic waste (Islam et al., 2020)**

## 1.6 Factors affecting bioleaching

Leaching performance depends on the potency of the microorganisms and the chemical and mineral content of the material to be leached. Maximum amounts of metal extraction will only be obtained if the leaching conditions correspond to the optimal growth conditions of bacteria.

**pH.**

Leaching with most autotrophic bacteria happens at acidic pH, while heterotrophic biosolubilization of metals is possible at higher pH values (Ruan et al., 2014). pH of the medium has a close interaction with the growth of bacteria, which in effect influences the solubilization of metals. Most of the metals are extracted at acidic pH (Ilyas et al., 2007; T. Yang et al., 2009).  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  bacterial oxidation is usually hampered at higher pH. Hence, slower metal dissolution happens at the beginning of the leaching process, where the pH is high because of the alkaline nature of the e-waste. Leaching improves to optimum value with a decrease in pH. However, the leaching process slows down at a very low pH because of bacterial growth retardation and metabolism (J. Wang et al., 2009).

**Temperature.**

The optimal temperature for leaching varies with the growth requirement of various microbial cultures. The most of mesophilic acidophiles extract metals at a target temperature range of 25–30°C while thermophiles leach at an average temperature level of 40–45°C (Choi et al., 2004; Ilyas et al., 2007). The temperature must, therefore, be compatible with the effective growth and metabolism of the bacteria involved in the bioleaching process to solubilize metals.

**Nutrients.**

The content of nutrients of the leaching medium influences the oxidation of metals by facilitating or hindering the growth of cells. Autotrophs obtain carbon and energy from inorganic substances such as  $\text{CO}_2$ ,  $\text{Fe}^{2+}$ , reduced sulfur compounds while the heterotrophs get their nutrients from organic compounds (Choi et al., 2004; Krebs et al., 1997). Concentrations of  $\text{Fe}^{2+}$ ,  $\text{S}^0$ , and other inorganic/organic components significantly affect microbial growth, metabolism, and biodissolution of metals. Nonetheless, an increase in the concentration of  $\text{Fe}^{2+}$  in the nutrient medium favors the precipitation of  $\text{Fe}^{3+}$  forming jarosite, which causes passivation leading to the creation of shields over metal surfaces preventing any attacks (Zhu et al., 2011).

**Aeration.**

The bacteria present in the process of bioleaching are typically aerobic by nature. For their growth and metabolism, they need sufficient air supply to increase metal leaching (Bosecker, 1997a). Aeration is usually given in the laboratory by shaking in shaker incubators, calculated in terms of rotations per minute (rpm). For bacterial cultures, the optimal rotation

rate is 120–175 rpm (Beolchini et al., 2012; J. Li et al., 2007; T. Yang et al., 2009). Excessive agitation by sparking air or increasing rpm may cause conflict and abrasion, leading in pressure building up in bacteria, results in lower levels of metal extraction. Thus sufficient O<sub>2</sub> supply is a requirement for healthy microbial growth and efficient bioleaching of metals.

### **Substrate.**

Except metals, e-waste includes a large variety of materials such as plastics, ceramics, glass, etc.. The non-metallic element of e-waste can also lead to the toxicity for bacterial cultures (H. Brandl et al., 2001). The high metal content of e-wastes, their diverse and complex nature often have a negative effect on bacterial growth (Zhou et al., 2009). It has been found that the rate of metal bioleaching decreases at large PCB dosages or pulp densities (Choi et al., 2004). It may be due to the toxicity of metals above the allowable limit, or inadequacy of the rate of mass transfer of air and oxygen hindering bacterial development, thus limiting bioleaching. For bioleaching, the optimal pulp density should be in the region of 10–20 g/L

### **Chelators, surfactants, and complexing agents.**

Compared to traditional e-waste treatment methods, bioleaching requires a long operating period. To speed up the bioleaching process, metals may be leached using a combination strategy that blends healthier chemical substances such as chelators and surfactants with bioleaching microbes (Pant et al., 2012). By incorporating citric acid, it was found that Cu leaching in the leaching medium was increased from 37 to 80% (by weight). A mixture of sulfur-oxidizing bacteria and biosurfactant-producing bacteria will more easily solubilize metals compared with only sulfur-oxidizing bacteria (Karwowska et al., 2014).

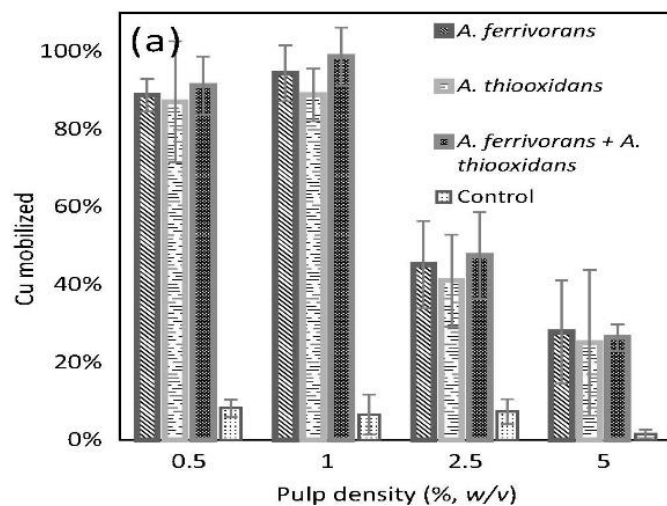
## **1.7 Best practices for metal leaching from PCBs**

### **1.7.1 Extraction of copper**

In order to improve the yield of metal mobilization, as well as to decrease the lixiviant consumption, a two-step approach was proposed by (Işildar et al., 2016) based on the different chemical properties and leaching mechanisms of base metals and precious metals. Base metal (copper) was extracted before the precious metal (gold) to reduce the toxicity of the medium after which the precious metal could be recovered by cyanogenic bacteria (Işildar et al., 2016). Copper was leached in the first step by acidophilic bacteria *A. ferrivorans* and *A. thiooxidans*.

On the other hand, copper can be released also in a one-step process using cyanogenic bacteria *P. aeruginosa* and *C. violaceum* (Pradhan & Kumar, 2012b). (Pradhan & Kumar, 2012b) experimented bioleaching of precious metal without removing base metal by acidophilic microorganisms. Base metals, e.g., copper, nickel, iron, and zinc form stable complexes with cyanide. Their presence at a high concentrations would interfere with precious metal cyanidation. This is why removal of base metal at the first stage provides higher percentage of precious metal (Ag, Au, Pt) mobilization.

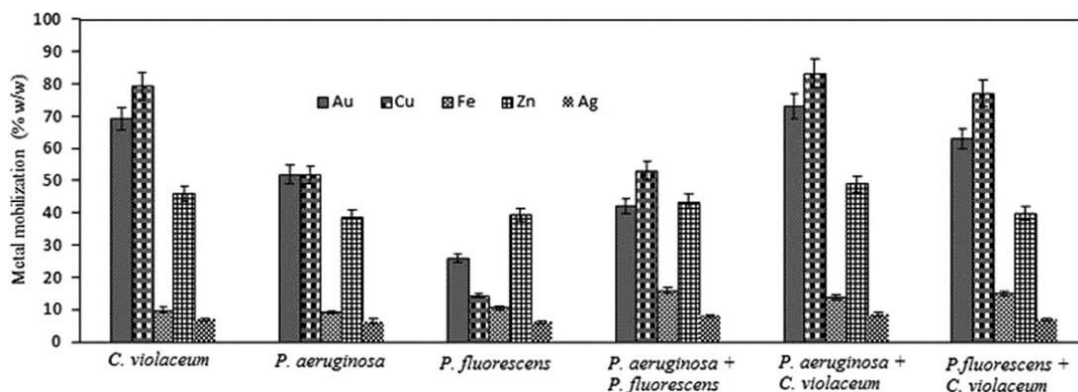
In both references, electronic waste (37 to 500  $\mu\text{m}$  particle size) as a substrate was used in the form of ground powder of printed circuit boards. Leaching was performed at ambient temperature. Pulp density and glycine concentration were maintained at 10 g/l and 5 g/l, respectively. For *C. violaceum* and *P. aeruginosa* bioleaching, the pH range was 8.0-9.0 (Pradhan & Kumar, 2012b), the mixed culture of *A. ferrivorans* and *A. thiooxidans* had pH 2.0 (Işıldar et al., 2016). With mixed culture of *A. ferrivorans* and *A. thiooxidans* at 1% w/v (10 g/l) pulp density, a higher mobilization with an efficiency of 98% copper was obtained (Figure 15). Higher pulp densities showed a lower bioleaching performance with a rise in pH. In terms of copper yield in solution and mobilization efficiency, pulp densities below 2.5% were more efficient (Figure 15) (Işıldar et al., 2016).



**Figure-15: Copper mobilization at various pulp densities of the bioleaching cultures(Işıldar et al., 2016)**

Bioleaching of e-waste without any pretreatment of base metal, with a single bacterial culture *C. violaceum* demonstrated 79.3% w/w metal recovery of Cu, preceding that of *P. ae-*

*ruginosa*. The mixture of *P. aeruginosa* and *C. violaceum* demonstrated the highest bioleachability among mixed cultures, with 83.46% w/w metal recovery of the total Cu (Pradhan & Kumar, 2012b).



**Figure-16: Percentage of metal solubilization after bioleaching of electronic waste with *C. violaceum*, *P. fluorescens*, *P. aeruginosa* and mixed cultures (Pradhan & Kumar, 2012b)**

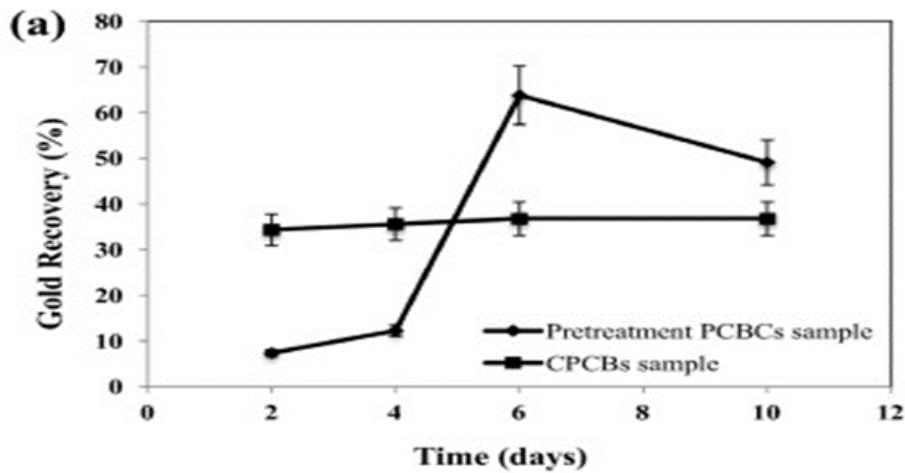
**Table 5: Bioleaching conditions for copper extraction.**

Microbes	Particle size	Pulp density	Temperature	Glycin concentration	pH	Metal (Cu) recovery%	References
<i>P. aeruginosa</i> + <i>C. violaceum</i>	37 -149 $\mu\text{m}$	10 g/l	30 $^{\circ}\text{C}$	5 g/l	8.0-9.0	83.46%	(Pradhan & Kumar, 2012b)
<i>A. Ferrivoran</i> + <i>A. Thiooxidans</i>	500 $\mu\text{m}$	10 g/l	25 $^{\circ}\text{C}$	5 g/l	2.0	98.0%	(Işıldar et al., 2016)

### 1.7.2 Extraction of gold

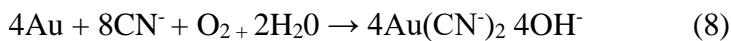
Bioleaching of gold from discarded PCBs can also be applied as a one-step or a two-step process. (Arshadi & Mousavi, 2015) first performed extraction of gold from PCBs solid waste utilizing *B. megaterium* as a bacterium-generating cyanide. Maximizing recovery of gold occurred at initial pH 10, pulp density 2 g/l, particle size 100  $\mu\text{m}$  and glycine concentration 0.5 g/l, resulting in gold production of 36.81% (Figure 17, Table 6). For decreasing the copper influence as an interfering factor in the leaching solution, a pretreatment strategy was used. Copper was first completely extracted using *Acidithiobacillus ferrooxidans* in the PCB powder under optimum conditions of pH 3,  $\text{Fe}^{+3}$  concentration 8.4 g/l, pulp density 20 g/l and particle size 95  $\mu\text{m}$ . Then the residual sediment was subjected to further gold recovery

by *B. megaterium*. Pretreatment of PCB waste using *A. ferrooxidans* resulted in the dissolution of gold up to 63.8% (fig.17) because of the depletion of copper from PCBs (Arshadi & Mousavi, 2015).



**Figure-17: Development characteristics by using *B. megaterium* on original and pretreated PCBs in optimum condition(Arshadi & Mousavi, 2015)**

The dissolution of Au can be represented as equation-8



In another study (Chi et al., 2011) *Chromobacterium violaceum*, a cyanide generating bacterium was used to leach gold and copper together from the mobile phone printed circuit boards (PCBs) without any pretreatment of base metals. Thus, the rate of gold extraction was very low. It was also noticed that a higher pulp density and the bigger particle size inhibited the gold leaching.

The author (Chi et al., 2011) tried to enhance gold leaching by adding extra  $\text{H}_2\text{O}_2$  which increased dissolved oxygen (DO) content without seriously affecting bacterial growth but improved gold recovery only marginally from 10.8% to 11.31% at pH 11.0 (Figure 18, table 6). Gold extraction was relatively low because of high concentration of copper present in the sample consuming cyanide produced at higher DO level. Thus it can be concluded that precious metals recovery could be more efficient, if base metals were removed.

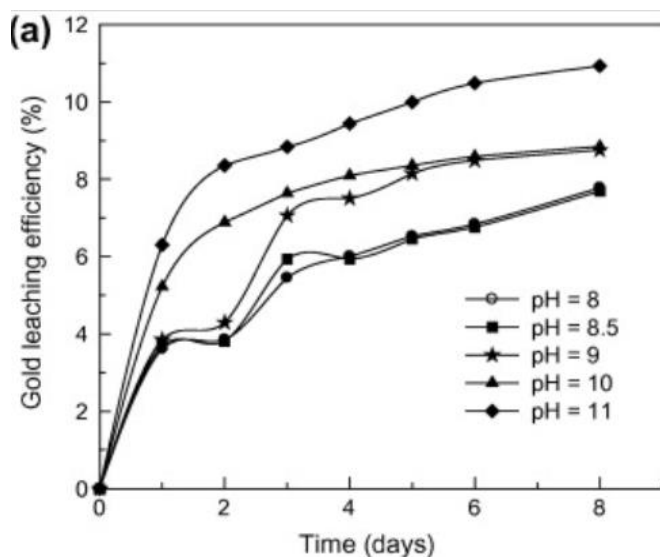


Figure-18: Gold bioleaching at different pH (Chi et al., 2011)

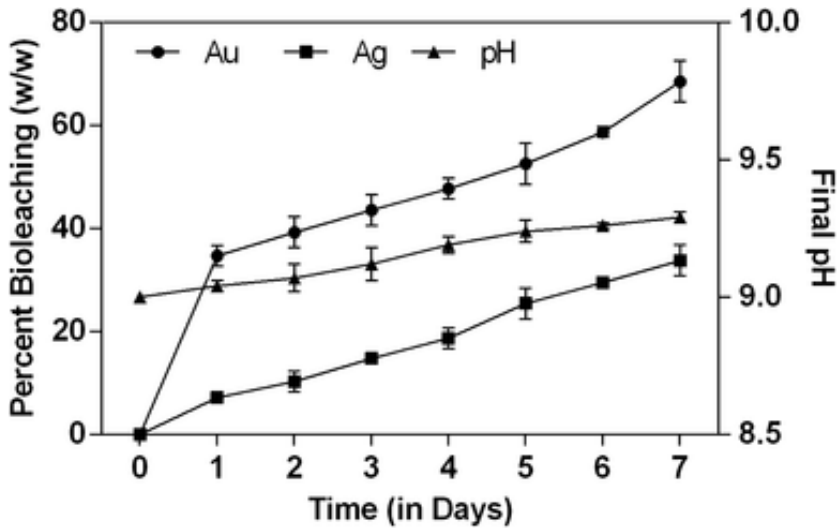
Table-6: Bioleaching conditions for gold extraction.

Microbes	Particle size (WEEE)	Pulp density	Temperature	Glycin concentration	pH	Metal (Au) recovery%	References
<i>B. megaterium</i>	100 $\mu\text{m}$	2 g/l	25-35 $^{\circ}\text{C}$	0.5 g/l	3.0	36.81%	(Arshadi & Mousavi, 2015)
<i>A. ferrooxidans</i> + <i>B. megaterium</i>	100 $\mu\text{m}$	2 g/l	25-35 $^{\circ}\text{C}$	0.5 g/l	10.0	63.8%	(Arshadi & Mousavi, 2015)
<i>C. violaceum</i>	1000 $\mu\text{m}$	15 g/l	30 $^{\circ}\text{C}$	0.5 g/l	8.0-11.0	11.31%	(Chi et al., 2011)

### 1.7.3 Extraction of silver

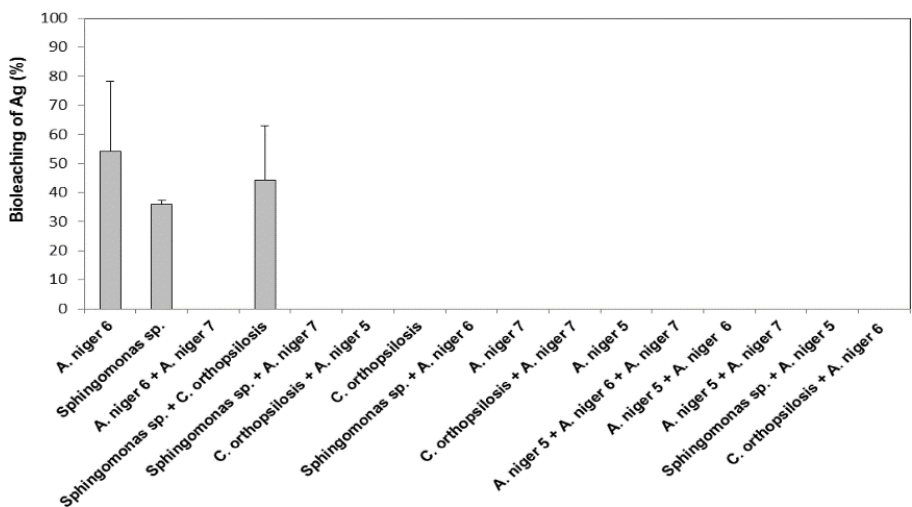
A two-stage bioleaching method can also be set up with the first stage with only bacterial culture and the second stage with e-waste added. In the experiment of (Kumar et al., 2018a) bacterial culture was inoculated into the sterile LB water in the absence of e-waste. After autoclaving sterilized e-waste was transferred to cultivation flasks and incubated for several days, the flasks were then tested for the presence of metal ions. In the analysis, the leaching factors were checked for variable ranges of pulp density (10, 50, and 100 g/l), glycine concentration (2.5, 5.0, 7.5, and 10.0 g/L), temperature (25, 30, 35, and 40 $^{\circ}\text{C}$ ), and initial pH (7, 8 and 9) to find optimum metal mobilization from waste PCBs (Kumar et al., 2018a).

Figure-19 shows the dissolution profile of Ag during the two-stage bioleaching experiment. Metal leaching increased until the seventh day; no mobilization was observed thereafter. Maximum Ag (33.8%) was mobilized at 10 g/L pulp density, 5 g/L glycine concentration, pH 9, temperature 30°C, 150 rpm, and particle size of waste PCBs 150 μm. *Pseudomonas balearica* SAE1 strain was used for the bioleaching of Ag from waste printed circuit boards because of their higher tolerance to e-waste toxicity (Kumar et al., 2018a)



**Figure-19: Bioleaching percentage of precious metals (primary axis) and modification of final pH (secondary axis) under optimized conditions by *P. balearica* SAE1(Kumar et al., 2018a)**

In (Díaz-Martínez et al., 2019), the highest leaching of Ag – 54% was reported with inoculation with *Aspergillus niger* MXPE6. On the other hand, 44.2% leaching of Ag was found with inoculation with consortium *Sphingomonas* sp. + *Candida orthopsilosis* (Figure 20, Table 7).



**Figure-20: Microbial leaching of Ag in contact with PCB (Díaz-Martínez et al., 2019)**

**Table-7: Bioleaching conditions for silver extraction.**

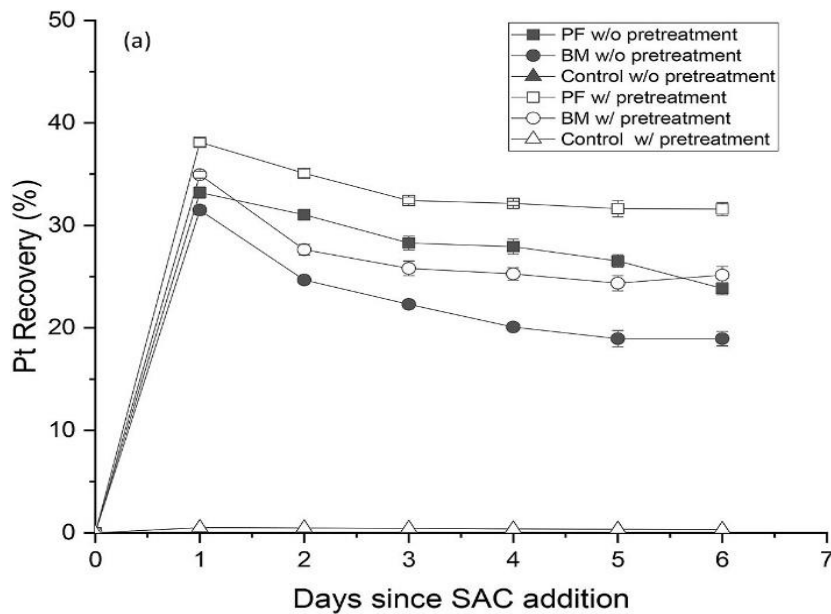
Microbes	Particle size	Pulp density	Temperature	Glycine concentration	pH	Metal (Ag) recovery%	References
<i>Pseudomonas balearica</i>	150 µm	10 g/l	30°C	7.5 g/l	9.0	33.8%	(Kumar et al., 2018a)
<i>A. niger</i>	60 µm	5 g/l	25-30°C	5 g/l	7.0 - 9.0	54.0%	(Díaz-Martínez et al., 2019)

#### 1.7.4 Extraction of platinum

As studies on platinum bioleaching from e-waste are rare, a recent paper on automotive catalysts (Karim & Ting, 2020) is analyzed instead. Platinum group metals (PGMs) are widely used in industrial catalysts because of their significant catalytic qualities (Mpinga et al., 2015). Catalysts used in vehicles are rich sources of platinum group metals (PGMs). The general conditions for Pt recovery from e-waste and SAC (spent automotive catalysts) are almost similar.

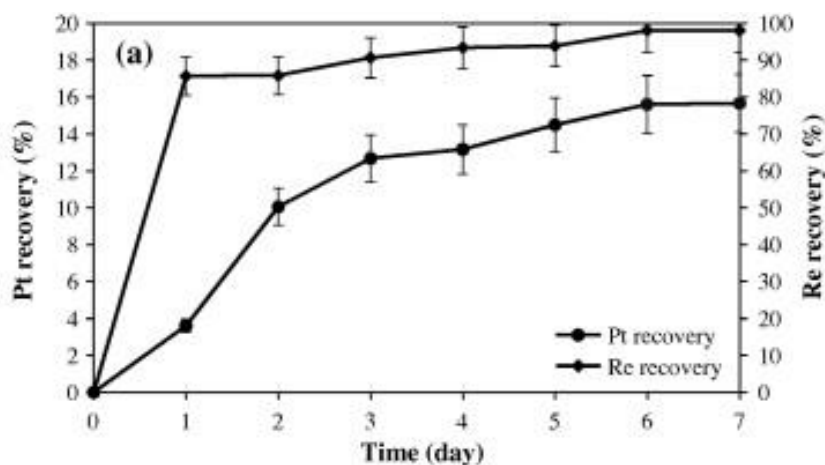
The impact of pretreatment with ultrasound-assisted nitric acid enhancing the leaching of cyanogenic bacteria of PGMs was explored in the experiment of (Karim & Ting, 2020). SAC samples were first pretreated using ultrasonic treatment and acid to remove competing metals that would otherwise interfere bioleaching in the subsequent PGM-cyanidation process. Ultrasound power 80%, nitric acid concentration 6 M, ultrasound period 50 min, and ultrasound frequency 37 kHz were considered to be the optimum parameters in the pretreatment. *Pseudomonas fluorescens* and *Bacillus megaterium* were used for the bioleaching of PGMs from untreated and pretreated SAC (Karim & Ting, 2020)

Pretreated samples of SACs demonstrated a better recovery of PGMs. Implementing a two-step bioleaching at a pulp density of 10 g/l and pH 9 for pretreated samples, *P. fluorescens* yielded maximum recovery of Pt (38%); for *B. megaterium* the corresponding value was Pt (35%) (Figure 21) (Karim & Ting, 2020).



**Figure-21: Pt recovery in two-step bioleaching by *P. Fluorescens* (□) and *B. Megaterium*(■) using untreated and pretreated SAC(Karim & Ting, 2020).**

*Bacillus megaterium* was also used in the study of (Motaghd et al., 2014) for releasing platinum from a spent catalyst. The study measured the ability of *Bacillus megaterium* as a to generate cyanide to solubilize platinum. Two main effective parameters including initial concentration of glycine and pulp density were studied. Maximum recovery of Pt at 15.7% and Re at 98% were obtained under optimal conditions of initial glycine concentration of 12.8 g/l and pulp density of 4% (w/v) after 7 days (Figure 22, Table 8). This efficiency is less than in the study of (Karim & Ting, 2020). The reasons could be higher pulp density and higher glycine concentration.



**Figure-22: Pt and Re recovery by bioleaching with *B. megaterium*(Motaghd et al., 2014)**

**Table-8: Bioleaching conditions for platinum extraction.**

<b>Microbes</b>	<b>Particle size</b>	<b>Pulp density</b>	<b>Temperature</b>	<b>Glycine concentration</b>	<b>pH</b>	<b>Metal(Pt) recovery%</b>	<b>References</b>
<i>P. fluorescens</i>	45 µm	10 g/l	30°C	5 g/l	9.0	38%	(Karim & Ting, 2020)
<i>Bacillus megaterium</i>	<75 µm	40 g/l	25°C	12.8 g/l	8.0 - 9.0	15.7%	(Motaghad et al., 2014)

## 2 THE AIMS OF THE THESIS

- To give an overview of contemporary bioleaching methods for extracting copper and platinum group metals (gold, silver and platinum)
- To propose a suitable bioleaching method for electronic waste material with pre-defined parameters

### 3 DISCUSSION

#### Pulp Density

Higher pulp density could be attributed to toxic effects of e-waste that can hinder bacterial metabolism (Ilyas et al., 2010), resulting in decreased growth rate and lower generation of lixivants. As a result, metal mobilization becomes low. It was found that at pulp density 10 g/l, *Pseudomonas balearica* was able to mobilize the maximum amount of metal (Kumar et al., 2018a). A rise in the pulp density from 10 g/l to 100 g/l ended in a substantial decrease in metal recovery (Kumar et al., 2018b). Pradhan and Kumar reported 69.3% mobilization of Au at a pulp density of 10 g/l and decrease to 20.28% at a pulp density of 100 g/l using *Chromobacterium violaceum* (Pradhan & Kumar, 2012b). So, pulp density should be about 10 g/l to get optimum result for bioleaching.

#### Temperature

It was found that the temperature influences production of cyanides by microorganisms. The perfect temperature for the production of cyanides varied from 25°C to 35°C (Zoidakis et al., 2005). (Kumar et al., 2018a) experimented bioleaching with temperatures varying from 25 to 40°C and Au & Ag maximum was observed at 30°C. The moderate temperature (30°C) has an advantage of reducing energy demands during industrial use, while eventually reducing production costs.

#### Glycine

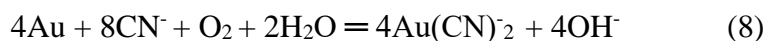
In the metal recovery process, adding glycine supports cyanide production capacity in cyanogenic microorganisms (Arshadi & Mousavi, 2015; Helmut Brandl et al., 2008). Therefore, specific glycine concentrations (2.5, 5.0, 7.5, and 10.0 g/l) were applied in an experiment to study their effects on PGM (platinum group metals) recovery (Kumar et al., 2018a). It was observed that increase of glycine concentration from 2.5 g/l to 7.5 g/l facilitated metals leaching capacity of the bacterial strain. Nevertheless, a further rise in the concentration of glycine from 7.5 to 10.0 g/l resulted in lowered mobilization of metals because inhibitory effects of glycine on bacteria leading to decreased bacterial growth (Faramarzi et al., 2004). Işıldar et al. and Shin et al. also documented inhibitory effect of glycine above 7.5 g/l utilizing *P. fluorescens* and *P. putida* during the bioleaching of metals from e-waste (Işıldar et al., 2016; Shin et al., 2013).

## pH

The alkaline pH raises the amount of aqueous cyanide ions (CN<sup>-</sup>) and thereby improves the efficiency of metal mobilization. Optimal bacterial growth occurs within the pH range of 8–9 (G. Natarajan & Ting, 2014). An equilibrium response to the production of bacterial cyanide may be represented as  $\text{HCN} \leftrightarrow \text{H}^+ + \text{CN}^-$  (G. Natarajan & Ting, 2015). Au & Ag mobilization increased with the pH growing from 8 to 9. The maximum mobilization of Ag was achieved at low pulp density (10 g/l) and high pH 8.6 (Kumar et al., 2018b) and also a higher metals leaching (Au 68.5%; Ag 33.8%) was observed at pH 9 (Kumar et al., 2018a), whereas low mobilization of metals was observed at pH 7. This is because changes in equilibrium: at low pH (pH 7) more HCN gas which is volatile and less water-soluble is generated. At alkaline pH (pH 9), the balance changes towards aqueous cyanide ions (CN<sup>-</sup>), allowing them to be readily usable for solubilization / complexation of metals (G. Natarajan & Ting, 2015). The leaching process using alkaline cyanidation provides optimum conditions and the highest recovery of metals (Hernandez et al., 2014).

## Cyanide Production

Cyanogenic microorganisms such as the species *Pseudomonas* and *Chromobacterium violaceum* generate HCN as a secondary metabolite (İşıldar et al., 2016; G. Natarajan & Ting, 2015), creating a water-soluble complex (i.e., dicyanoaurate with PGM). For example, the Au dissolution can be described as Eq-8



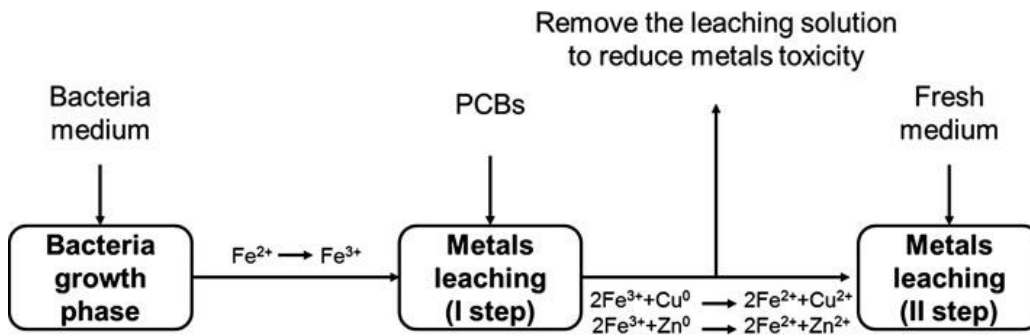
Ag and Pt cyanidation also demonstrates similar reactions and has been apparently reported by different researchers as dicyanoargentate during cyanogenic bioleaching (Helmut Brandl et al., 2008; Faramarzi et al., 2004). Higher mobilization of PGM may be directly linked to e-waste heterogeneity. Studies overviewed in earlier chapters (Arshadi & Mousavi, 2015; Pradhan & Kumar, 2012b; Ruan et al., 2014) reported high concentrations of Cu (10.00%, 12.06% and 90.43% respectively) in their e-waste, which interferes with leaching of precious metals by consuming cyanide which is produced during cyanogenic bioleaching, resulting in poor precious metal mobilization. In the study of (Kumar et al., 2018a) amount of Cu is lower (2.4%), which makes cyanide available for precious metals, resulting in higher mobilization of Ag and Au even though a high level of dicyanoargentate is toxic to bacteria (Helmut Brandl et al., 2008).

The decline in PGM recovery over time is attributed to many reasons: depletion of cyanide after metal complexation, bacterial biosorption or bioaccumulation (Harris & Knowles, 1983).

### Two step bioleaching for PGM

In the case of PGM recovery from PCBs, which contain a high amount of copper,  $\text{Cu}^{2+}$  ions can interfere with PGM recovery due to the development of  $\text{Cu}^-$  cyanide complexes which are produced faster and at a higher concentration than  $\text{Au}^-/\text{Ag}^-/\text{Pt}^-$  cyanide complexes. In other words, the occurrence of Cu dissolution dominates in PGM due to the following reasons (Işıldar et al., 2016):

- i) base metals like Cu ingest free cyanide and make it unavailable for PGM complexation;
- ii) the standard gold ( $\text{Au}^0/\text{Au}^+$ ) electrode potential ( $E^0$ ) is equivalent to  $-1,83$  V which makes it less reactive than base metals such as Ni ( $E^0 = -0,67$  V), Cu ( $E^0 = -0,34$  V).



**Figure-23: Two step bioleaching scheme(Becci et al., 2020)**

Therefore, the bioleaching of e-waste should be envisaged for the removal of base metals in the first step and precious metals in the second step (Figure 23). Thus, in the first step (pretreatment), all authors used a novel method to separate the base metals by chemolithoautotrophic acidophilic bacteria and the precious metals were leached by cyanogenic bacteria in the second step.

However, in each situation, the microbial activity might be different. The method of metal bioleaching from e-waste is often affected by the nature of toxic metal substances in them and the microbe's adaptability to e-waste toxicity (Gadd, 2004). Reportedly, the computer PCBs waste is alkaline in nature (H. Brandl et al., 2001). Therefore, for optimal dissolution and microbial viability, the proper acclimatization and adaptation of microbial culture is needed prior to the processing of metal recovery. The rate of bioleaching is therefore maximized by getting accustomed or adjusting the microbes to the liquid phase before the bioleaching process (Ilyas et al., 2007, 2010).

## RECOMMENDATION

The data for the recommendation was presented by the project LIFE17/ENV/ES/000216 Bi-oleaching of WEEE wastes for the recovery of valuable metals (Table 9).

**Table-9: Metal percentages on different given samples. Each sample containing 10 tons (10 000kg) of the total non-metallic fraction of WEEE.**

Sample	Cu%	Ag%	Au%	Ni%	Pb%	Sn%	Zn%	Al%	Fe%	Cr%	Pd%	Pt%
Sample 1, (F15 Initial)	<12.3	0.005	0.0032	n.d.	n.d.	n.d.	-	n.d.	0.14	-	n.d.	<0.005
Sample 2, F15(1ª Vuelta)	7.68	0.0068	0.0039	0.06	0.27	0.38	0.39	6.44	0.36	0.06	0.00	0.000
Sample 3, F5(1ª Vuelta)	7.30	0.0061	0.0040	0.04	0.15	0.31	0.32	6.47	0.31	0.02	0.00	0.000
Sample 4, (Filter Dust Zig-Zag)	8.18	0.0498	0.0390	0.40	1.57	2.62	1.09	5.18	3.77	0.08	0.00	n.d.
<b>Total average amount of metal from each sample</b>	<b>8.8% (880kg)</b>	<b>0.063% (6.3kg)</b>	<b>0.012% (1.2kg)</b>									<b>0.003% (0.3kg)</b>
<b>Recommended recovery amount by bioleaching from each sample (kg) approx.</b>	<b>882kg</b>	<b>2.12kg</b>	<b>0.76kg</b>	-	-	-	-	-	-	-	-	<b>0.12kg</b>

According to the given data, 10 tons (10 000kg) per year of non-metallic fraction of WEEE was produced from each sample. The amount of Cu, Ag, Au and Pt in each sample is almost similar. For Cu extraction using the mixture of microorganisms *A. Ferrivorans* and *A. Thi-oxidans* with 10g/l pulp density, maintaining pH 2.0 with ambient temperature, we can expect to get 98% (862 kg) of Cu recovery on the 10<sup>th</sup> day of bioleaching (Işıldar et al., 2016).

As regards to gold, with using pretreatment of electronic waste for Cu depletion, the efficacy of Au extraction could be increased to a large extent. So after removing the highest proportion of copper and other base metals from the e-waste, *B. megaterium* can be used as a bioleaching microorganism with pH 10, a pulp density of 2 g/l and glycine concentration 0.5 g/l and gold can be recovered maximum up to 63.8% (0.76kg) from each sample (Arshadi & Mousavi, 2015).

On the other hand, using *Pseudomonas balearica* SAE1 with 10 g/l e-waste pulp density, 150 µm particle size, 5 g/L glycine concentration at pH 9, 30°C temperature maximum 33.8% (2.12kg) of silver can be recovered by bioleaching (Kumar et al., 2018a).

As to platinum extraction, pretreatment of given e-waste can increase the platinum mobilization. At a temperature of 30<sup>0</sup>C, glycine concentration 5g/l with particle size  $\leq 45$   $\mu\text{m}$  and pulp density 10 g/l, maintaining pH 9 and utilizing *P. fluorescens* the maximum Pt recovery from the e-waste sample can be 38% (0.12kg) (Karim & Ting, 2020).

## **SUMMARY**

Worldwide generation of e-waste and implementation of multiple technologies in the recovery of e-waste, specifically, the different applications of the biological method which utilizes microorganisms to extract the platinum group metals were reviewed in this thesis. The mechanisms as well as influencing factors for the biological treatment of e-waste were discussed, some examples of the method were introduced.

Base metals (i.e., Cu) should be removed as much as possible before bioleaching of PGM (platinum group metals) due to their toxic effects on the bacterial culture and in order to increase the amount of free cyanide ions. Thus a novel two step bioleaching process is needed for metal bioleaching from e-waste. The higher efficiency of base metals extraction is achieved at low pH; on the other hand the higher efficiency for PGM ( Au, Ag, Pt ) is achieved at high pH. Acidophilic bacteria are more suitable for Cu bioleaching and cyanogenic bacteria for the precious metals bioleaching. Cyanide production and optimum pulp density are the most important parameters for platinum group metals bioleaching. Also hybrid bioleaching combining biological methods with physical and chemical methods could be a promising recycling option for the maximum efficiency for metal extraction in the future.

E-waste is the fastest growing waste source than any other waste production in the current world. Bioleaching method for e-waste processing is the most cost-effective, environmentally friendly as well as able to participate in economical development in a significant way. The use of optimal environments, the right mixed culture of bacteria and the right method will further enhance the recovery of metals and their production.

## **ACKNOWLEDGEMENT**

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## REFERENCES

- Adhapure, N. N., Dhakephalkar, P. K., Dhakephalkar, A. P., Tembhurkar, V. R., Rajgure, A. V., & Deshmukh, A. M. (2014). Use of large pieces of printed circuit boards for bioleaching to avoid “precipitate contamination problem” and to simplify overall metal recovery. *MethodsX*, *1*, e181–e186. <https://doi.org/10.1016/j.mex.2014.08.011>
- Ahmadi, A., Ranjbar, M., & Schaffie, M. (2012). Catalytic effect of pyrite on the leaching of chalcopyrite concentrates in chemical, biological and electrobiochemical systems. *Minerals Engineering*, *34*, 11–18. <https://doi.org/10.1016/j.mineng.2012.03.022>
- Ahonen, L., & Tuovinen, O. H. (1989). Microbiological Oxidation of Ferrous Iron at Low Temperatures. *Applied and Environmental Microbiology*, *55*(2), 312–316.
- Arshadi, M., & Mousavi, S. M. (2015). Enhancement of simultaneous gold and copper extraction from computer printed circuit boards using *Bacillus megaterium*. *Bioresource Technology*, *175*, 315–324. <https://doi.org/10.1016/j.biortech.2014.10.083>
- Assembly, P. (2019). *FR-4 Printed Circuit Board Provider | Fuchuangke Technology*. 2019. <https://pcbboardassembly.com/fr-4-pcb/>
- Aung, K. M. M., & Ting, Y. P. (2005). Bioleaching of spent fluid catalytic cracking catalyst using *Aspergillus niger*. *Journal of Biotechnology*, *116*(2), 159–170. <https://doi.org/10.1016/j.jbiotec.2004.10.008>
- Baniasadi, M., Vakilchap, F., Bahaloo-Horeh, N., Mousavi, S. M., & Farnaud, S. (2019). Advances in bioleaching as a sustainable method for metal recovery from e-waste: A review. In *Journal of Industrial and Engineering Chemistry* (Vol. 76, pp. 75–90). Korean Society of Industrial Engineering Chemistry. <https://doi.org/10.1016/j.jiec.2019.03.047>
- Barontini, F., & Cozzani, V. (2006). Formation of hydrogen bromide and organobrominated compounds in the thermal degradation of electronic boards. *Journal of Analytical and Applied Pyrolysis*, *77*(1), 41–55. <https://doi.org/10.1016/j.jaap.2006.01.003>
- Becci, A., Amato, A., Fonti, V., Karaj, D., & Beolchini, F. (2020). An innovative biotechnology for metal recovery from printed circuit boards. *Resources, Conservation and Recycling*, *153*, 104549. <https://doi.org/10.1016/j.resconrec.2019.104549>
- Beolchini, F., Fonti, V., Dell’Anno, A., Rocchetti, L., & Vegliò, F. (2012). Assessment of biotechnological strategies for the valorization of metal bearing wastes. *Waste Management*, *32*(5), 949–956. <https://doi.org/10.1016/j.wasman.2011.10.014>
- Bosecker, K. (1997a). Bioleaching: Metal solubilization by microorganisms. *FEMS Microbiology Reviews*, *20*(3–4), 591–604. [https://doi.org/10.1016/S0168-6445\(97\)00036-3](https://doi.org/10.1016/S0168-6445(97)00036-3)
- Bosecker, K. (1997b). Bioleaching: metal solubilization by microorganisms. *FEMS Microbiology Reviews*, *20*(3–4), 591–604. <https://doi.org/10.1111/j.1574-6976.1997.tb00340.x>
- Bosecker, K. (1997c). Bioleaching: Metal solubilization by microorganisms. *FEMS Microbiology Reviews*, *20*(3–4), 591–604. [https://doi.org/10.1016/S0168-6445\(97\)00036-3](https://doi.org/10.1016/S0168-6445(97)00036-3)

- Brandl, H., Bosshard, R., & Wegmann, M. (2001). Computer-munching microbes: Metal leaching from electronic scrap by bacteria and fungi. *Hydrometallurgy*, 59(2–3), 319–326. [https://doi.org/10.1016/S0304-386X\(00\)00188-2](https://doi.org/10.1016/S0304-386X(00)00188-2)
- Brandl, Helmut, Lehmann, S., Faramarzi, M. A., & Martinelli, D. (2008). Biomobilization of silver, gold, and platinum from solid waste materials by HCN-forming microorganisms. *Hydrometallurgy*, 94(1–4), 14–17. <https://doi.org/10.1016/j.hydromet.2008.05.016>
- Brierley, J. A., & Brierley, C. L. (2001). Present and future commercial applications of biohydrometallurgy. *Hydrometallurgy*, 59(2–3), 233–239. [https://doi.org/10.1016/S0304-386X\(00\)00162-6](https://doi.org/10.1016/S0304-386X(00)00162-6)
- Brierley, James A. (1978). Thermophilic Iron-Oxidizing Bacteria Found in Copper Leaching Dumps. In *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*. <http://aem.asm.org/>
- Buffalo, U. (2020). *Characterizing the Environmental Burden of E-waste Recycling/Repair Workers in Bangladesh - COE: Community for Global Health Equity - University at Buffalo*. <http://www.buffalo.edu/globalhealthequity/global-projects/exposome/characterizing-the-environmental-burden-of-e-waste-recycling-repair-workers-in-bangladesh.html>
- Burgstaller, W., & Schinner, F. (1993). Leaching of metals with fungi. In *Journal of Biotechnology* (Vol. 27, Issue 2, pp. 91–116). Elsevier. [https://doi.org/10.1016/0168-1656\(93\)90101-R](https://doi.org/10.1016/0168-1656(93)90101-R)
- Castro, I. M., Fietto, J. L. R., Vieira, R. X., Trópia, M. J. M., Campos, L. M. M., Paniago, E. B., & Brandão, R. L. (2000). Bioleaching of zinc and nickel from silicates using *Aspergillus niger* cultures. *Hydrometallurgy*, 57(1), 39–49. [https://doi.org/10.1016/S0304-386X\(00\)00088-8](https://doi.org/10.1016/S0304-386X(00)00088-8)
- Chen, S., Yang, Y., Liu, C., Dong, F., & Liu, B. (2015). Column bioleaching copper and its kinetics of waste printed circuit boards (WPCBs) by *Acidithiobacillus ferrooxidans*. *Chemosphere*, 141, 162–168. <https://doi.org/10.1016/j.chemosphere.2015.06.082>
- Chi, T. D., Lee, J. C., Pandey, B. D., Yoo, K., & Jeong, J. (2011). Bioleaching of gold and copper from waste mobile phone PCBs by using a cyanogenic bacterium. *Minerals Engineering*, 24(11), 1219–1222. <https://doi.org/10.1016/j.mineng.2011.05.009>
- Chmielewski, A. G., Urbański, T. S., & Migdał, W. (1997). Separation technologies for metals recovery from industrial wastes. *Hydrometallurgy*, 45(3), 333–344. [https://doi.org/10.1016/s0304-386x\(96\)00090-4](https://doi.org/10.1016/s0304-386x(96)00090-4)
- Choi, M.-S., Cho, K.-S., Kim, D.-S., & Kim, D.-J. (2004). Microbial Recovery of Copper from Printed Circuit Boards of Waste Computer by *Acidithiobacillus ferrooxidans*. *Journal of Environmental Science and Health, Part A*, 39(11–12), 2973–2982. <https://doi.org/10.1081/LESA-200034763>
- Choubey, P. K., Panda, R., Jha, M. K., Lee, J. C., & Pathak, D. D. (2015). Recovery of copper and recycling of acid from the leach liquor of discarded Printed Circuit Boards (PCBs). *Separation and Purification Technology*, 156, 269–275. <https://doi.org/10.1016/j.seppur.2015.10.012>

- Creamer, N. J., Baxter-Plant, V. S., Henderson, J., Potter, M., & Macaskie, L. E. (2006). Palladium and gold removal and recovery from precious metal solutions and electronic scrap leachates by *Desulfovibrio desulfuricans*. *Biotechnology Letters*, 28(18), 1475–1484. <https://doi.org/10.1007/s10529-006-9120-9>
- Cui, J., & Forssberg, E. (2003). Mechanical recycling of waste electric and electronic equipment: A review. In *Journal of Hazardous Materials* (Vol. 99, Issue 3, pp. 243–263). Elsevier. [https://doi.org/10.1016/S0304-3894\(03\)00061-X](https://doi.org/10.1016/S0304-3894(03)00061-X)
- Cui, J., & Zhang, L. (2008). Metallurgical recovery of metals from electronic waste: A review. In *Journal of Hazardous Materials* (Vol. 158, Issues 2–3, pp. 228–256). Elsevier. <https://doi.org/10.1016/j.jhazmat.2008.02.001>
- Dalrymple, I., Wright, N., Kellner, R., Bains, N., Geraghty, K., Goosey, M., & Lightfoot, L. (2007). An integrated approach to electronic waste (WEEE) recycling. In *Circuit World* (Vol. 33, Issue 2, pp. 52–58). Emerald Group Publishing Limited. <https://doi.org/10.1108/03056120710750256>
- Das, A., Vidyadhar, A., & Mehrotra, S. P. (2009). A novel flowsheet for the recovery of metal values from waste printed circuit boards. *Resources, Conservation and Recycling*, 53(8), 464–469. <https://doi.org/10.1016/j.resconrec.2009.03.008>
- Díaz-Martínez, M. E., Argumedo-Delira, R., Sánchez-Viveros, G., Alarcón, A., & Mendoza-López, M. R. (2019). Microbial Bioleaching of Ag, Au and Cu from Printed Circuit Boards of Mobile Phones. *Current Microbiology*, 76(5), 536–544. <https://doi.org/10.1007/s00284-019-01646-3>
- Dominguez-Benetton, X., Varia, J. C., Pozo, G., Modin, O., Ter Heijne, A., Fransaer, J., & Rabaey, K. (2018). Metal recovery by microbial electro-metallurgy. In *Progress in Materials Science* (Vol. 94, pp. 435–461). Elsevier Ltd. <https://doi.org/10.1016/j.pmatsci.2018.01.007>
- Donati, E. R. . W. S. (2007). *Microbial Processing of Metal Sulfides*. <https://link.springer.com/content/pdf/10.1007/1-4020-5589-7.pdf>
- Du Plessis, C. A., Batty, J. D., & Dew, D. W. (2007). Commercial applications of thermophile bioleaching. In *Biomining* (pp. 57–80). Springer-Verlag Berlin Heidelberg. [https://doi.org/10.1007/978-3-540-34911-2\\_3](https://doi.org/10.1007/978-3-540-34911-2_3)
- Duan, H., Hou, K., Li, J., & Zhu, X. (2011). Examining the technology acceptance for dismantling of waste printed circuit boards in light of recycling and environmental concerns. In *Journal of Environmental Management* (Vol. 92, Issue 3, pp. 392–399). Academic Press. <https://doi.org/10.1016/j.jenvman.2010.10.057>
- Ebert, J., & Bahadir, M. (2003). Formation of PBDD/F from flame-retarded plastic materials under thermal stress. *Environment International*, 29(6), 711–716. [https://doi.org/10.1016/S0160-4120\(03\)00117-X](https://doi.org/10.1016/S0160-4120(03)00117-X)
- European Commission, 2010. (2009). Being wise with waste: the EU's approach to waste management. In *European Commission*. <https://doi.org/10.2779/93543>
- Faramarzi, M. A., Stagars, M., Pensini, E., Krebs, W., & Brandl, H. (2004). Metal solubilization from metal-containing solid materials by cyanogenic *Chromobacterium violaceum*. *Journal of Biotechnology*, 113(1–3), 321–326.

<https://doi.org/10.1016/j.jbiotec.2004.03.031>

- Gadd, G. M. (2004). Microbial influence on metal mobility and application for bioremediation. *Geoderma*, 122(2-4 SPEC. IIS.), 109–119. <https://doi.org/10.1016/j.geoderma.2004.01.002>
- Gehrke, T., Telegdi, J., Thierry, D., & Sand, W. (1998). Importance of Extracellular Polymeric Substances from *Thiobacillus ferrooxidans* for Bioleaching Downloaded from. In *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* (Vol. 64, Issue 7). <http://aem.asm.org/>
- Hadi, P., Xu, M., Lin, C. S. K., Hui, C. W., & McKay, G. (2015). Waste printed circuit board recycling techniques and product utilization. *Journal of Hazardous Materials*, 283, 234–243. <https://doi.org/10.1016/j.jhazmat.2014.09.032>
- Hagelüken, C. (2006a). Recycling of Electronic Scrap at Umicore's Integrated Metals Smelter and Refinery. In *researchgate.net*. [https://www.researchgate.net/profile/Christian\\_Hagelueken/publication/240629115\\_Recycling\\_of\\_Electronic\\_Scrap\\_at\\_Umicore's\\_Integrated\\_Metals\\_Smelter\\_and\\_Refinery/links/54c9f6d40cf298fd26275196/Recycling-of-Electronic-Scrap-at-Umicores-Integrated-Metals-Sm](https://www.researchgate.net/profile/Christian_Hagelueken/publication/240629115_Recycling_of_Electronic_Scrap_at_Umicore's_Integrated_Metals_Smelter_and_Refinery/links/54c9f6d40cf298fd26275196/Recycling-of-Electronic-Scrap-at-Umicores-Integrated-Metals-Sm)
- Hagelüken, C. (2006b). *Recycling of Electronic Scrap at Umicore's Integrated Metals Smelter and Refinery*.
- Hall, W. J., & Williams, P. T. (2007). Separation and recovery of materials from scrap printed circuit boards. *Resources, Conservation and Recycling*, 51(3), 691–709. <https://doi.org/10.1016/j.resconrec.2006.11.010>
- Harris, R., & Knowles, C. J. (1983). Isolation and growth of a *Pseudomonas* species that utilizes cyanide as a source of nitrogen. *Journal of General Microbiology*, 129(4), 1005–1011. <https://doi.org/10.1099/00221287-129-4-1005>
- Hennebel, T., Boon, N., Maes, S., & Lenz, M. (2015). Biotechnologies for critical raw material recovery from primary and secondary sources: R&D priorities and future perspectives. *New Biotechnology*, 32(1), 121–127. <https://doi.org/10.1016/j.nbt.2013.08.004>
- Herat, S., & Agamuthu, P. (2012). E-waste: a problem or an opportunity? Review of issues, challenges and solutions in Asian countries. *Waste Management & Research: The Journal of the International Solid Wastes and Public Cleansing Association, ISWA*, 30(11), 1113–1129. <https://doi.org/10.1177/0734242X12453378>
- Hernandez, J., Patino, F., Rivera, I., Reyes, I. A., Flores, M. U., Juarez, J. C., & Reyes, M. (2014). Leaching kinetics in cyanide media of Ag contained in the industrial mining-metallurgical wastes in the state of Hidalgo, Mexico. *International Journal of Mining Science and Technology*, 24(5), 689–694. <https://doi.org/10.1016/j.ijmst.2014.07.003>
- Hino, T., Agawa, R., Moriya, Y., Nishida, M., Tsugita, Y., & Araki, T. (2009). Techniques to separate metal from waste printed circuit boards from discarded personal computers. *Journal of Material Cycles and Waste Management*, 11(1), 42–54. <https://doi.org/10.1007/s10163-008-0218-0>
- Hong, Y., & Valix, M. (2014). Bioleaching of electronic waste using acidophilic sulfur

- oxidising bacteria. *Journal of Cleaner Production*, 65, 465–472. <https://doi.org/10.1016/j.jclepro.2013.08.043>
- Huang, K., Guo, J., & Xu, Z. (2009). Recycling of waste printed circuit boards: A review of current technologies and treatment status in China. In *Journal of Hazardous Materials* (Vol. 164, Issues 2–3, pp. 399–408). Elsevier. <https://doi.org/10.1016/j.jhazmat.2008.08.051>
- Ilyas, S., Anwar, M. A., Niazi, S. B., & Afzal Ghauri, M. (2007). Bioleaching of metals from electronic scrap by moderately thermophilic acidophilic bacteria. *Hydrometallurgy*, 88(1–4), 180–188. <https://doi.org/10.1016/j.hydromet.2007.04.007>
- Ilyas, S., & Lee, J. C. (2015). Hybrid leaching: An emerging trend in bioprocessing of secondary resources. In *Microbiology for Minerals, Metals, Materials and the Environment* (pp. 359–383). CRC Press. <https://doi.org/10.1201/b18124-15>
- Ilyas, S., Lee, J. C., & Chi, R. A. (2013). Bioleaching of metals from electronic scrap and its potential for commercial exploitation. *Hydrometallurgy*, 131–132, 138–143. <https://doi.org/10.1016/j.hydromet.2012.11.010>
- Ilyas, S., Ruan, C., Bhatti, H. N., Ghauri, M. A., & Anwar, M. A. (2010). Column bioleaching of metals from electronic scrap. *Hydrometallurgy*, 101(3–4), 135–140. <https://doi.org/10.1016/j.hydromet.2009.12.007>
- Işıldar, A., Rene, E. R., van Hullebusch, E. D., & Lens, P. N. L. (2018). Electronic waste as a secondary source of critical metals: Management and recovery technologies. *Resources, Conservation and Recycling*, 135, 296–312. <https://doi.org/10.1016/j.resconrec.2017.07.031>
- Işıldar, A., van de Vossenberg, J., Rene, E. R., van Hullebusch, E. D., & Lens, P. N. L. (2016). Two-step bioleaching of copper and gold from discarded printed circuit boards (PCB). *Waste Management*, 57, 149–157. <https://doi.org/10.1016/j.wasman.2015.11.033>
- Işıldar, A., van Hullebusch, E. D., Lenz, M., Du Laing, G., Marra, A., Cesaro, A., Panda, S., Akcil, A., Kucuker, M. A., & Kuchta, K. (2019). Biotechnological strategies for the recovery of valuable and critical raw materials from waste electrical and electronic equipment (WEEE) – A review. In *Journal of Hazardous Materials* (Vol. 362, pp. 467–481). Elsevier B.V. <https://doi.org/10.1016/j.jhazmat.2018.08.050>
- Islam, A., Ahmed, T., Awual, M. R., Rahman, A., Sultana, M., Aziz, A. A., Monir, M. U., Teo, S. H., & Hasan, M. (2020). Advances in sustainable approaches to recover metals from e-waste-A review. In *Journal of Cleaner Production* (Vol. 244, p. 118815). Elsevier Ltd. <https://doi.org/10.1016/j.jclepro.2019.118815>
- Ivanus, R., International, D. I.-M., & 2009, undefined. (n.d.). Metal leaching from electronic scrap by fungi. *CALEA GRIVITEI, NR 83, SECTOR 1 ....*
- Jadhav, U., Science, H. H.-C., & 2014, U. (2014). Use of *Aspergillus niger* 34770 culture supernatant for tin metal removal. Elsevier. <https://www.sciencedirect.com/science/article/pii/S0010938X14000262>
- Jung, L. B., & Bartel, T. J. (1999). Computer take-back and recycling: An economic analysis for used consumer equipment. *Journal of Electronics Manufacturing*, 9(1), 67–77.

<https://doi.org/10.1142/S0960313199000295>

- Kahhat, R., & Williams, E. (2009). Product or waste? Importation and end-of-life processing of computers in Peru. *Environmental Science and Technology*, *43*(15), 6010–6016. <https://doi.org/10.1021/es8035835>
- Karim, S., & Ting, Y. P. (2020). Ultrasound-assisted nitric acid pretreatment for enhanced biorecovery of platinum group metals from spent automotive catalyst. *Journal of Cleaner Production*, *255*, 120199. <https://doi.org/10.1016/j.jclepro.2020.120199>
- Karwowska, E., Andrzejewska-Morzuch, D., Łebkowska, M., Tabernacka, A., Wojtkowska, M., Telepko, A., & Konarzewska, A. (2014). Bioleaching of metals from printed circuit boards supported with surfactant-producing bacteria. *Journal of Hazardous Materials*, *264*, 203–210. <https://doi.org/10.1016/j.jhazmat.2013.11.018>
- Kaya, M. (2016). Recovery of metals and nonmetals from electronic waste by physical and chemical recycling processes. In *Waste Management* (Vol. 57, pp. 64–90). Elsevier Ltd. <https://doi.org/10.1016/j.wasman.2016.08.004>
- Kim, M. J., Seo, J. Y., Choi, Y. S., & Kim, G. H. (2016). Bioleaching of spent Zn-Mn or Ni-Cd batteries by *Aspergillus* species. *Waste Management*, *51*, 168–173. <https://doi.org/10.1016/j.wasman.2015.11.001>
- Krebs, W., Brombacher, C., Bosshard, P. P., Bachofen, R., & Brandl, H. (1997). Microbial recovery of metals from solids. *FEMS Microbiology Reviews*, *20*(3–4), 605–617. <https://doi.org/10.1111/j.1574-6976.1997.tb00341.x>
- Kumar, A., Saini, H. S., & Kumar, S. (2018a). Bioleaching of Gold and Silver from Waste Printed Circuit Boards by *Pseudomonas balearica* SAE1 Isolated from an e-Waste Recycling Facility. *Current Microbiology*, *75*(2), 194–201. <https://doi.org/10.1007/s00284-017-1365-0>
- Kumar, A., Saini, H. S., & Kumar, S. (2018b). Enhancement of gold and silver recovery from discarded computer printed circuit boards by *Pseudomonas balearica* SAE1 using response surface methodology (RSM). *3 Biotech*, *8*(2), 1–11. <https://doi.org/10.1007/s13205-018-1129-y>
- Lacey, D. T., & Lawson, F. (1970). Kinetics of the liquid-phase oxidation of acid ferrous sulfate by the bacterium *Thiobacillus ferrooxidans*. *Biotechnology and Bioengineering*, *12*(1), 29–50. <https://doi.org/10.1002/bit.260120104>
- LaDou, J. (2006). Printed circuit board industry. *International Journal of Hygiene and Environmental Health*, *209*(3), 211–219. <https://doi.org/10.1016/j.ijheh.2006.02.001>
- Le, L., Tang, J., Ryan, D., & Valix, M. (2006). Bioleaching nickel laterite ores using multi-metal tolerant *Aspergillus foetidus* organism. *Minerals Engineering*, *19*(12), 1259–1265. <https://doi.org/10.1016/j.mineng.2006.02.006>
- Lee, J. chun, & Pandey, B. D. (2012). Bio-processing of solid wastes and secondary resources for metal extraction - A review. In *Waste Management* (Vol. 32, Issue 1, pp. 3–18). Pergamon. <https://doi.org/10.1016/j.wasman.2011.08.010>
- Lee, J. chun, Song, H. T., & Yoo, J. M. (2007). Present status of the recycling of waste electrical and electronic equipment in Korea. In *Resources, Conservation and*

- Recycling* (Vol. 50, Issue 4, pp. 380–397). Elsevier. <https://doi.org/10.1016/j.resconrec.2007.01.010>
- Li, J., Lu, H., Guo, J., Xu, Z., & Zhou, Y. (2007). Recycle technology for recovering resources and products from waste printed circuit boards. *Environmental Science and Technology*, 41(6), 1995–2000. <https://doi.org/10.1021/es0618245>
- Li, Y., Kawashima, N., Li, J., Chandra, A. P., & Gerson, A. R. (2013). A review of the structure, and fundamental mechanisms and kinetics of the leaching of chalcopyrite. In *Advances in Colloid and Interface Science* (Vols. 197–198, pp. 1–32). Elsevier B.V. <https://doi.org/10.1016/j.cis.2013.03.004>
- Liang, G., Tang, J., Liu, W., & Zhou, Q. (2013). Optimizing mixed culture of two acidophiles to improve copper recovery from printed circuit boards (PCBs). *Journal of Hazardous Materials*, 250–251, 238–245. <https://doi.org/10.1016/j.jhazmat.2013.01.077>
- Lu, Y., & Xu, Z. (2016). Precious metals recovery from waste printed circuit boards: A review for current status and perspective. In *Resources, Conservation and Recycling* (Vol. 113, pp. 28–39). Elsevier B.V. <https://doi.org/10.1016/j.resconrec.2016.05.007>
- Mahmoud, A., Cézac, P., Hoadley, A. F. A., Contamine, F., & D’Hugues, P. (2017). A review of sulfide minerals microbially assisted leaching in stirred tank reactors. *International Biodeterioration and Biodegradation*, 119, 118–146. <https://doi.org/10.1016/j.ibiod.2016.09.015>
- Mäkinen, J., Bachér, J., Kaartinen, T., Wahlström, M., & Salminen, J. (2015). The effect of flotation and parameters for bioleaching of printed circuit boards. *Minerals Engineering*, 75, 26–31. <https://doi.org/10.1016/j.mineng.2015.01.009>
- Mishra, D., Kim, D.-J., Ahn, J.-G., & Rhee, Y.-H. (2005). Bioleaching: A microbial process of metal recovery; A review. *Metals and Materials International*, 11(3), 249–256. <https://doi.org/10.1007/bf03027450>
- Modin, O., Fuad, N., & Rauch, S. (2017). Microbial electrochemical recovery of zinc. *Electrochimica Acta*, 248, 58–63. <https://doi.org/10.1016/j.electacta.2017.07.120>
- Motaghed, M., Mousavi, S. M., Rastegar, S. O., & Shojaosadati, S. A. (2014). Platinum and rhenium extraction from a spent refinery catalyst using *Bacillus megaterium* as a cyanogenic bacterium: Statistical modeling and process optimization. *Bioresource Technology*, 171, 401–409. <https://doi.org/10.1016/j.biortech.2014.08.032>
- Mpinga, C. N., Eksteen, J. J., Aldrich, C., & Dyer, L. (2015). Direct leach approaches to Platinum Group Metal (PGM) ores and concentrates: A review. In *Minerals Engineering* (Vol. 78, pp. 93–113). Elsevier Ltd. <https://doi.org/10.1016/j.mineng.2015.04.015>
- Murugan, R. V., Bharat, S., Deshpande, A. P., Varughese, S., & Haridoss, P. (2008). Milling and separation of the multi-component printed circuit board materials and the analysis of elutriation based on a single particle model. *Powder Technology*, 183(2), 169–176. <https://doi.org/10.1016/j.powtec.2007.07.020>
- Nakajima, K., Takeda, O., Miki, T., Matsubae, K., Nakamura, S., & Nagasaka, T. (2010). Thermodynamic analysis of contamination by alloying elements in aluminum

- recycling. *Environmental Science and Technology*, 44(14), 5594–5600. <https://doi.org/10.1021/es9038769>
- Natarajan, G., & Ting, Y. P. (2014). Pretreatment of e-waste and mutation of alkali-tolerant cyanogenic bacteria promote gold biorecovery. *Bioresource Technology*, 152, 80–85. <https://doi.org/10.1016/j.biortech.2013.10.108>
- Natarajan, G., & Ting, Y. P. (2015). Gold biorecovery from e-waste: An improved strategy through spent medium leaching with pH modification. *Chemosphere*, 136, 232–238. <https://doi.org/10.1016/j.chemosphere.2015.05.046>
- Natarajan, K. A. (2018). *Biotechnology of Metals: Principles, Recovery Methods and Environmental Concerns* - K.A. Natarajan - Google Books. Elsevier. [https://books.google.ee/books?hl=en&lr=&id=uOJgDwAAQBAJ&oi=fnd&pg=PP1&ots=KdNI45Z7Rm&sig=f\\_KDKWNVjDpzBdXRYUIYLJX8I7c&redir\\_esc=y#v=onepage&q&f=false](https://books.google.ee/books?hl=en&lr=&id=uOJgDwAAQBAJ&oi=fnd&pg=PP1&ots=KdNI45Z7Rm&sig=f_KDKWNVjDpzBdXRYUIYLJX8I7c&redir_esc=y#v=onepage&q&f=false)
- Natarajan, K. A., & Deo, N. (2001). Role of bacterial interaction and bioreagents in iron ore flotation. *International Journal of Mineral Processing*, 62(1–4), 143–157. [https://doi.org/10.1016/S0301-7516\(00\)00049-1](https://doi.org/10.1016/S0301-7516(00)00049-1)
- Ni, M., Xiao, H., Chi, Y., Yan, J., Buekens, A., Jin, Y., & Lu, S. (2012). Combustion and inorganic bromine emission of waste printed circuit boards in a high temperature furnace. *Waste Management*, 32(3), 568–574. <https://doi.org/10.1016/j.wasman.2011.10.016>
- Norgate, T. E., Jahanshahi, S., & Rankin, W. J. (2007). Assessing the environmental impact of metal production processes. *Journal of Cleaner Production*, 15(8–9), 838–848. <https://doi.org/10.1016/j.jclepro.2006.06.018>
- Orell, A., Navarro, C. A., Arancibia, R., Mobarec, J. C., & Jerez, C. A. (2010). Life in blue: Copper resistance mechanisms of bacteria and Archaea used in industrial biomining of minerals. In *Biotechnology Advances* (Vol. 28, Issue 6, pp. 839–848). Elsevier. <https://doi.org/10.1016/j.biotechadv.2010.07.003>
- Palencia, I., Wan, R. Y., & Miller, J. D. (1991). The electrochemical behavior of a semiconducting natural pyrite in the presence of bacteria. *Metallurgical Transactions B*, 22(6), 765–774. <https://doi.org/10.1007/BF02651153>
- Pandey, B., & Natarajan, K. (2015). *Microbiology for minerals, metals, materials and the environment*. [https://books.google.com/books?hl=en&lr=&id=Q4mbBgAAQBAJ&oi=fnd&pg=PP1&ots=HaWJKns6Zr&sig=NA4cH7\\_mq57i90pFtZYehOLv3qE](https://books.google.com/books?hl=en&lr=&id=Q4mbBgAAQBAJ&oi=fnd&pg=PP1&ots=HaWJKns6Zr&sig=NA4cH7_mq57i90pFtZYehOLv3qE)
- Pant, D., Joshi, D., Upreti, M. K., & Kotnala, R. K. (2012). Chemical and biological extraction of metals present in E waste: A hybrid technology. *Waste Management*, 32(5), 979–990. <https://doi.org/10.1016/j.wasman.2011.12.002>
- Peiravi, M., Mote, S. R., Mohanty, M. K., & Liu, J. (2017). Bioelectrochemical treatment of acid mine drainage (AMD) from an abandoned coal mine under aerobic condition. *Journal of Hazardous Materials*, 333, 329–338. <https://doi.org/10.1016/j.jhazmat.2017.03.045>
- Pradhan, J. K., & Kumar, S. (2012a). Metals bioleaching from electronic waste by

- Chromobacterium violaceum* and *Pseudomonads* sp. *Waste Management & Research*, 30(11), 1151–1159. <https://doi.org/10.1177/0734242X12437565>
- Pradhan, J. K., & Kumar, S. (2012b). Metals bioleaching from electronic waste by *Chromobacterium violaceum* and *Pseudomonads* sp. *Waste Management & Research*, 30(11), 1151–1159. <https://doi.org/10.1177/0734242X12437565>
- Priya, A., & Hait, S. (2017). Comparative assessment of metallurgical recovery of metals from electronic waste with special emphasis on bioleaching. *Environmental Science and Pollution Research*, 24(8), 6989–7008. <https://doi.org/10.1007/s11356-016-8313-6>
- Quora. (2016). *Why is CO a better reducing agent than carbon at high temperature?* - Quora. <https://www.quora.com/Why-is-CO-a-better-reducing-agent-than-carbon-at-high-temperature>
- Rath, S. S., Nayak, P., Mukherjee, P. S., Roy Chaudhury, G., & Mishra, B. K. (2012). Treatment of electronic waste to recover metal values using thermal plasma coupled with acid leaching - A response surface modeling approach. *Waste Management*, 32(3), 575–583. <https://doi.org/10.1016/j.wasman.2011.11.001>
- Rawlings, D. E. (2002). Heavy Metal Mining Using Microbes. *Annual Review of Microbiology*, 56(1), 65–91. <https://doi.org/10.1146/annurev.micro.56.012302.161052>
- Rawlings, D. E., Rawlings, D. E., Tributsch, H., & Hansford, G. S. (1999). Reasons why 'Leptospirillum'-like species rather than *Thiobacillus ferrooxidans* are the dominant iron-oxidizing bacteria in many commercial processes for the biooxidation of pyrite and related ores. *Microbiology*, 145, 5–13. <https://doi.org/10.1099/13500872-145-1-5>
- Reck, B. K., & Graedel, T. E. (2012). Challenges in metal recycling. In *Science* (Vol. 337, Issue 6095, pp. 690–695). American Association for the Advancement of Science. <https://doi.org/10.1126/science.1217501>
- Ruan, J., Zhu, X., Qian, Y., & Hu, J. (2014). A new strain for recovering precious metals from waste printed circuit boards. *Waste Management*, 34(5), 901–907. <https://doi.org/10.1016/j.wasman.2014.02.014>
- Saidan, M., Brown, B., & Valix, M. (2012). Leaching of electronic waste using biometabolised acids. *Chinese Journal of Chemical Engineering*, 20(3), 530–534. [https://doi.org/10.1016/S1004-9541\(11\)60215-2](https://doi.org/10.1016/S1004-9541(11)60215-2)
- Sand, W., Gehrke, T., Jozsa, P. G., & Schippers, A. (2001). (Bio)chemistry of bacterial leaching - direct vs. indirect bioleaching. *Hydrometallurgy*, 59(2–3), 159–175. [https://doi.org/10.1016/S0304-386X\(00\)00180-8](https://doi.org/10.1016/S0304-386X(00)00180-8)
- Schippers, A., & Sand, W. (1999). Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. *Applied and Environmental Microbiology*, 65(1), 319–321. <https://doi.org/10.1128/aem.65.1.319-321.1999>
- Shi, S., & Fang, Z. (2005). Bioleaching of marmatite flotation concentrate by adapted mixed mesoacidophilic cultures in an air-lift reactor. *International Journal of Mineral Processing*, 76(1–2), 3–12. <https://doi.org/10.1016/j.minpro.2004.05.005>

- Shin, D., Jeong, J., Lee, S., Pandey, B. D., & Lee, J. C. (2013). Evaluation of bioleaching factors on gold recovery from ore by cyanide-producing bacteria. *Minerals Engineering*, *48*, 20–24. <https://doi.org/10.1016/j.mineng.2013.03.019>
- Silvas, F. P. C., Jiménez Correa, M. M., Caldas, M. P. K., de Moraes, V. T., Espinosa, D. C. R., & Tenório, J. A. S. (2015). Printed circuit board recycling: Physical processing and copper extraction by selective leaching. *Waste Management*, *46*, 503–510. <https://doi.org/10.1016/j.wasman.2015.08.030>
- Torma, A. E. (1977). *The role of Thiobacillus ferrooxidans in hydrometallurgical processes* (pp. 1–37). Springer, Berlin, Heidelberg. [https://doi.org/10.1007/3-540-08363-4\\_1](https://doi.org/10.1007/3-540-08363-4_1)
- Tsydenova, O., & Bengtsson, M. (2011). Chemical hazards associated with treatment of waste electrical and electronic equipment. In *Waste Management* (Vol. 31, Issue 1, pp. 45–58). Pergamon. <https://doi.org/10.1016/j.wasman.2010.08.014>
- Tuncuk, A., Stazi, V., Akcil, A., Yazici, E. Y., & Deveci, H. (2012). Aqueous metal recovery techniques from e-scrap: Hydrometallurgy in recycling. *Minerals Engineering*, *25*(1), 28–37. <https://doi.org/10.1016/j.mineng.2011.09.019>
- Vakilchah, F., Mousavi, S. M., & Shojaosadati, S. A. (2016). Role of *Aspergillus niger* in recovery enhancement of valuable metals from produced red mud in Bayer process. *Bioresource Technology*, *218*, 991–998. <https://doi.org/10.1016/j.biortech.2016.07.059>
- Valix, M., Tang, J. Y., & Malik, R. (2001). Heavy metal tolerance of fungi. *Minerals Engineering*, *14*(5), 499–505. [https://doi.org/10.1016/S0892-6875\(01\)00037-1](https://doi.org/10.1016/S0892-6875(01)00037-1)
- Veit, H. M., Diehl, T. R., Salami, A. P., Rodrigues, J. S., Bernardes, A. M., & Tenório, J. A. S. (2005). Utilization of magnetic and electrostatic separation in the recycling of printed circuit boards scrap. *Waste Management*, *25*(1), 67–74. <https://doi.org/10.1016/j.wasman.2004.09.009>
- Wang, J., Bai, J., Xu, J., & Liang, B. (2009). Bioleaching of metals from printed wire boards by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* and their mixture. *Journal of Hazardous Materials*, *172*(2–3), 1100–1105. <https://doi.org/10.1016/j.jhazmat.2009.07.102>
- Wang, X., Lu, X., & Zhang, S. (2013). Study on the waste liquid crystal display treatment: Focus on the resource recovery. *Journal of Hazardous Materials*, *244–245*, 342–347. <https://doi.org/10.1016/j.jhazmat.2012.11.059>
- Widmer, R., Oswald-Krapf, H., Sinha-Khetriwal, D., Schnellmann, M., & Böni, H. (2005). Global perspectives on e-waste. *Environmental Impact Assessment Review*, *25*(5 SPEC. ISS.), 436–458. <https://doi.org/10.1016/j.eiar.2005.04.001>
- Xiang, Y., Wu, P., Zhu, N., Zhang, T., Liu, W., Wu, J., & Li, P. (2010). Bioleaching of copper from waste printed circuit boards by bacterial consortium enriched from acid mine drainage. *Journal of Hazardous Materials*, *184*(1–3), 812–818. <https://doi.org/10.1016/j.jhazmat.2010.08.113>
- Xie, F., Cai, T., Ma, Y., Li, H., Li, C., Huang, Z., & Yuan, G. (2009). Recovery of Cu and Fe from Printed Circuit Board waste sludge by ultrasound: Evaluation of industrial application. *Journal of Cleaner Production*, *17*(16), 1494–1498. <https://doi.org/10.1016/j.jclepro.2009.06.012>

- Xin, B., Zhang, D., Zhang, X., Xia, Y., Wu, F., Chen, S., & Li, L. (2009). Bioleaching mechanism of Co and Li from spent lithium-ion battery by the mixed culture of acidophilic sulfur-oxidizing and iron-oxidizing bacteria. *Bioresource Technology*, *100*(24), 6163–6169. <https://doi.org/10.1016/j.biortech.2009.06.086>
- Yamane, L. H., de Moraes, V. T., Espinosa, D. C. R., & Tenório, J. A. S. (2011). Recycling of WEEE: Characterization of spent printed circuit boards from mobile phones and computers. *Waste Management*, *31*(12), 2553–2558. <https://doi.org/10.1016/j.wasman.2011.07.006>
- Yang, H., Liu, J., & Yang, J. (2011). Leaching copper from shredded particles of waste printed circuit boards. *Journal of Hazardous Materials*, *187*(1–3), 393–400. <https://doi.org/10.1016/j.jhazmat.2011.01.051>
- Yang, T., Xu, Z., Wen, J., & Yang, L. (2009). Factors influencing bioleaching copper from waste printed circuit boards by *Acidithiobacillus ferrooxidans*. *Hydrometallurgy*, *97*(1–2), 29–32. <https://doi.org/10.1016/j.hydromet.2008.12.011>
- Zhan, L., & Xu, Z. (2008). Application of vacuum metallurgy to separate pure metal from mixed metallic particles of crushed waste printed circuit board scraps. *Environmental Science and Technology*, *42*(20), 7676–7681. <https://doi.org/10.1021/es801205b>
- Zhang, S., & Forssberg, E. (1997). Mechanical separation-oriented characterization of electronic scrap. *Resources, Conservation and Recycling*, *21*(4), 247–269. [https://doi.org/10.1016/S0921-3449\(97\)00039-6](https://doi.org/10.1016/S0921-3449(97)00039-6)
- Zhou, H. B., Zeng, W. M., Yang, Z. F., Xie, Y. J., & Qiu, G. Z. (2009). Bioleaching of chalcopyrite concentrate by a moderately thermophilic culture in a stirred tank reactor. In *Bioresource Technology* (Vol. 100, Issue 2, pp. 515–520). Elsevier. <https://doi.org/10.1016/j.biortech.2008.06.033>
- Zhu, N., Xiang, Y., Zhang, T., Wu, P., Dang, Z., Li, P., & Wu, J. (2011). Bioleaching of metal concentrates of waste printed circuit boards by mixed culture of acidophilic bacteria. *Journal of Hazardous Materials*, *192*(2), 614–619. <https://doi.org/10.1016/j.jhazmat.2011.05.062>
- Zoidakis, J., Loaiza, A., Vu, K., & Abu-Omar, M. M. (2005). Effect of temperature, pH, and metals on the stability and activity of phenylalanine hydroxylase from *Chromobacterium violaceum*. *Journal of Inorganic Biochemistry*, *99*(3), 771–775. <https://doi.org/10.1016/j.jinorgbio.2004.12.017>

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