BIOLEACHING OF METALS FROM WASTE COMPUTER PRINTED CIRCUIT BOARDS (CPCBs) USING INDIGENOUS BACTERIAL ISOLATES FROM METAL CONTAMINATED SOIL

Thesis submitted in fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY
IN
BIOTECHNOLOGY

BY

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DEDICATED TO MY
BELOVED MOTHER

TABLE OF CONTENT

DECLARATION BY THE SCHOLAR

I, **Pooja Thakur (Enrollment No. 186553)**, hereby declare that the work reported in the Ph.D. thesis entitled **"Bioleaching of metals from waste computer printed circuit boards (CPCBs) using indigenous bacterial isolates from metal contaminated soil"** submitted at the **Jaypee University of Information Technology, Waknaghat, Solan (HP), India** is an authentic record of my work carried out under the supervision of **Professor (Dr.) Sudhir Kumar**. I have not submitted this work elsewhere for any other degree or diploma. I am fully responsible for the contents of my Ph.D. thesis.

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SUPERVISOR’S CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled **“Bioleaching of metals from waste computer printed circuit boards (CPCBs) using indigenous bacterial isolates from metal contaminated soil”** submitted by **Ms. Pooja Thakur (Enrollment No. 186553)** at **Jaypee University of Information Technology, Waknaghat, Solan (HP), India** is a bonafide record of her original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

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ABSTRACT

The industrial economy and technology have rapidly released electronic products, accompanied by significant advancements. This surge has resulted in a substantial increase in outdated and discarded electronic equipment, contributing to the global issue of e-waste. The unsustainable consumption of natural resources for the continuous advancement of electric and electronic equipment designates e-waste as the core of 'urban mining.' Thus, this metal reservoir has prompted our focus on addressing this solid waste stream, explicitly directing our efforts toward bioleaching e-waste.

In the current study, the shredded dust from waste computer printed circuit boards (CPCBs) generated during mechanical dismantling is subjected to metal content analysis and recovery via bioleaching. The presence of various base metals, including copper, aluminum, nickel, iron, and precious metals such as silver, gold, and platinum in the shredded dust of CPCBs designates this waste as a viable 'secondary source' for a variety of metals within the 'urban mining' strategy. Despite the numerous cyanogenic microorganisms previously documented for their metal solubilization potential in biohydrometallurgical techniques, constraints such as low toxicity tolerance and limited efficiency persist. These limitations necessitate ongoing research efforts to identify novel microbial strains acclimated to metal-rich environments, intending to enhance the efficacy of the bioleaching process for industrial-scale e-waste management. Consequently, we aimed to investigate the bacterial community that thrives within the metal-acclimated environment for their application in bioleaching. The present study identified *Bacillus sporothermodurans* ISO1 as a novel *Bacillus* strain exhibiting higher toxicity tolerance (EC_{50} = 425 g/L) and leaching potential than earlier reported bacterial strains. It is imperative to optimize various parameters for maximum recovery of metals, as these significantly influence bacterial growth and the efficiency of the bioleaching process. Response Surface Methodology (RSM), a statistical technique, was employed to optimize various culture variables, including temperature, pH, glycine concentration, pulp density, and time. The interactive effects of critical factors in the RSM approach resulted in up to 95% Cu and 44% Ag recovery.

Furthermore, a chemo-biohydrometallurgy approach with suitable chemical lixiviant ($FeCl_3$) was utilized for Cu recovery before bioleaching by *Bacillus sporothermodurans* ISO1 and *Pseudomonas balearica* SAE1. This strategy improved the leaching of precious metals, such as Ag (57%) and Au (67%). Furthermore, to reduce the dependence on chemical lixiviants and to enhance process economy, we endeavored to stimulate the availability of biogenic

cyanide for efficient metal recovery using a suitable inducer. A concentration of 1 mg/L of inducer (i.e., methionine) efficiently promoted glycine-utilized cyanide production, resulting in an 86% solubilization of Cu and a 75% solubilization of Au.

Additionally, the metal solubilization and operating conditions were explored at an increased volume (i.e., 3 L working volume) of the bioleaching medium to assess the industrial-scale potential of this potent bacterial strain. The substantial recovery of Cu (>60%) and other metals at this increased volume suggests the feasibility of implementing the bioleaching process with this potent bacterial strain at a large-scale operation. Thus, the present study has provided a potential bacterial strain and a suitable chemo-biohydrometallurgy approach, which could be a proficient strategy for metals recovery from e-waste.

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
Ag	Silver
Al	Aluminium
Ala	Alanine
As	Arsenic
Au	Gold
BFR	Brominated Flame Retardant
B. sporothermodurans	*Bacillus sporothermodurans*
BLAST	Basic Local Alignment Search Tool
C	Carbon
CFU	Colony Forming Unit
CCD	Central Composite Design
CPCBs	Computer Printed Circuit Boards
CN^-	Cyanide ion
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cys	Cystein
DNA	Deoxyribonucleic Acid
oC	Degree Celesius
EEE	Electrical and Electronic Equipment
E-waste	Electronic waste
E-scrap	Electronic scrap
EDS	Energy Dispersive Spectroscopy
Eq	Equation
EC_{50}	50% Effective Concentration
Fe	Iron
$FeCl_3$	Ferric chloride
$FeSO_4$	Ferrous Sulfate
g	Gram
Gly	Glycine

h	Hour
HCN	Hydrogen Cyanide
HCl	Hydrochloric acid
HNO_3	Nitric acid
H_2SO_4	Sulphuric acid
Kg	Kilogram
L	Litre
M	Molar
Mg	Magnesium
Met	Methionine
mL	Milliliter
mg	Milligram
min	Minute
Mt	Million Metric Tons
µ	Micro
NA	Nutrient Agar
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NB	Nutrient Broth
NCBI	National Centre for Biotechnology Information
Ni	Nickle
nm	Nanometer
OFAT	One Factor at a Time
O.D.	Optical density
O	Oxygen
p	Probability
%	Percent
Pb	Lead
P. balearica	*Pseudomonas balearica*
PBDEs	Poly Brominated Diphenyl Ethers
PCBs	Printed Circuit Boards
PCBEs	Poly Chlorinated Biphenyl Ethers
PCR	Polymerase Chain Reaction

Phe	Phenylalanine
P.D	Pulp density
Pd	Palladium
ppm	Part Per Million
psi	Per square inch
Pt	Platinum
R^2	Coefficient of determination
REEs	Rare Earth Elements
rpm	Rotation per minute
RSM	Response Surface Methodology
RNA	Ribonucleic Acid
sp.	Species
Si	Silicon
SEM	Scanning Electron Microscopy
TSDF	Treatment Storage Disposal Facility
UV	Ultra Violet
v/v	Volume by volume
w/v	Weight by volume
WEEE	Waste Electrical and Electronic Equipment
XRD	X-Ray Diffraction
Zn	Zinc

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CHAPTER 1

INTRODUCTION

INTRODUCTION

The shift to advanced industrialization has been accompanied by the fast growth of technology and contemporary lifestyle, compelling us to integrate an increasing number of electronic devices into various aspects of our lives. However, this advancement has created a new solid waste stream comprising outdated electrical and electronic products. Waste electronic and electrical equipment (WEEEs) and electronic waste (e-waste) are the most commonly used terms that refer to electronic equipment discarded at the end of its useful life or due to equipment upgrades. The widespread use of electrical and electronic equipment (EEE) is intricately linked to broad-based economic growth, urbanization, and advanced industrialization. These factors collectively drive the substantial production of EEE characterized by a short life cycle and limited repair options [1]. Every year, the global consumption of all EEEs increases by 2.5Mt and ends up as e-waste. With an average generation of 7.3kg per person, the current total e-waste volume has reached 57.4 Mt, rising from 53.6 Mt in 2019 [3,4,5]. According to the Solving the E-waste Problem (StEP) Initiative, Asia primarily contributes to e-waste, producing a substantial 24.9 million metric tons. China takes the lead in Asia with 12.2 million metric tons [6]. Furthermore, a survey by the United Nations Environmental Program (UNEP) reported that approximately 4 million waste personal computers (PCs) from China alone contribute to the annual global generation of 20-50 Mt e-waste. Thus, e-waste solid waste streams threaten human well-being and the ecosystem [7].

The problem of e-waste is not limited to developed nations; India, as a developing nation, stands as the world's third-largest contributor with a volume of 3.2 million metric tons [6,8]. According to the Associated Chambers of Commerce of India (ASSOCHAM), the WEEE assets in India are experiencing a Compound Annual Growth Rate (CAGR) of 25% [6]. The Central Pollution Control Board (CPCB) reported a substantial increase in e-waste generation in India, reaching 1,014,961 tonnes in 2019-2020, marking a 32% rise from 2018-2019. Moreover, the report found that only 3.6% and 10% of the generated e-waste was collected in 2018 and 2019, respectively [7, 9]. Additionally, the anticipation of more advanced microprocessors continually reshaping products implies an inevitable increase in electrical and electronic equipment in India [7].

In addition to the relentless growth of e-waste, the disposal and treatment of this solid waste stream have become significant concerns worldwide due to its abundance and complex composition. The hazardous components of WEEEs pose a threat to the environment if disposed of alongside regular household garbage [10]. Landfilling and incineration are two commonly employed methods for managing e-waste, which can adversely impact humans and the environment. These methods release noxious effluents that can seep into the soil and emit harmful gases into the surrounding environment. Brominated flame retardants (BFR), which often constitute 5 to 10% of the weight of e-waste, are among the harmful components of WEEEs [11,12].

Printed circuit boards (PCBs) and other electronic gadgets contain flammable plastic parts. When incinerating these components, they generate brominated flame retardants, dioxins, and furans, which can harm human health and the environment [12]. The disposal of cathode ray tubes (CRT) as e-waste is expected to release various hazardous elements, such as lead (Pb), cadmium (Cd), arsenic (As), and others. This process poses a significant environmental risk as these substances can leach into the groundwater, endangering aquatic systems. Moreover, it is projected that a single mobile phone battery has the potential to release an amount of cadmium (Cd) significant enough to pollute 600 cubic meters of water [12,13,14]. In addition to the toxic components and adverse impacts of e-waste mentioned above, the inclusion of valuable metals such as copper (Cu), iron (Fe), nickel (Ni), gold (Au), silver (Ag), renders this waste valuable and suitable for the circular economy approach [14]. Compared to their natural resources, the concentration of various valuable metals in e-waste is elevated, making e-waste the "heart of urban mining." The quantity of Cu and Au metal in e-waste is estimated to exceed their natural ores by 13-26 times and 35-50 times, respectively. [11,14,15]. In 2019, the estimated value of raw materials derived from secondary waste was projected to be $57 billion, and Cu and Au are anticipated to be worth $10,960 and $ 9,481 million, respectively [15]. Hence, it would be worthwhile for economic and environmental benefits to transform this waste into wealth. However, using unconventional and informal methods to extract these precious metals has also led to environmental issues and health risks [16]. The principal impediment to e-waste recycling is the safety risks faced by individuals who receive inadequate compensation for their labor, encompassing men, women, and children. In informal recycling practices, working without proper protective gear in confined spaces with insufficient ventilation exposes individuals to inhaling hazardous and potentially poisonous substances [14,16,17].

Additionally, the lack of immediate medical attention implies that common injuries sustained by untrained workers may lead to the exposure of wounds to toxic substances. Furthermore, the unintentional release of acid during the recovery of precious metals into nearby areas may give rise to long-term neurological, reproductive, excretory, or endocrine issues, as well as an increased risk of cancer. Thus, these environmental and health hazards emphasize the critical need for enhanced safety measures and regulatory frameworks in e-waste recycling processes [17, 18, 19].

In formal methods, no metallurgical process is entirely satisfactory for this complex and heterogeneous mineral substance in isolation. Therefore, executing the metal recovery process in distinct phases, encompassing physico-mechanical procedures, segregation steps, and metallurgical treatments, is preferable [20,21]. Pyrometallurgy and hydrometallurgy are two commonly employed conventional processes for metal extraction due to their speed and efficiency. However, these processes exhibit drawbacks, including losing precious metals, high costs, energy demands, human health concerns, and environmental footprints [21,22]. Despite the widespread adoption and industrialization of these conventional techniques, their associated disadvantages have spurred researchers to explore alternative methods that are both economical and eco-friendly [22].

Biohydrometallurgy, recognized as a 'green technology,' has proven to be a proficient alternative to conventional metallurgical methods for metal extraction [23]. Biohydrometallurgy utilizes the potential of microorganisms to catalyze the dissolution of metals into an aqueous solution, a technique known as 'bioleaching.' This approach allows for the recovery of metals in an energy-efficient, cost-effective, and environmentally responsible manner [24,25,26,27]. It is an eminent method for extracting metals from various primary mineral ores/concentrates and secondary metal resources [28,29,30]. In bioleaching, the direct dissolution of targeted base metals such as Cu, Ni, Zn, and Fe occurs, while bio-oxidation is another aspect of the process targeting interfering mineral sulfides to dissolve precious metals (e.g., Pt, Pd, Au, and Ag) [31]. The acidophilic chemolithotrophs, notably *Acidithiobacillus* sp., are the foremost exploited microorganisms extensively used for extracting base metals from natural mines and secondary sources like e-waste. Additionally, cyanogenic microorganisms (*Chromobactrium violeacium, Bacillus megatarium, Pseudomonas* sp.) form another major group used to catalyze the dissolution of both base and precious metals in a liquid solution [31, 32].

In recent years, PCBs, integral components of all Electrical and Electronic Equipment (EEE), accounting for 3-6% of all WEEE, have drawn more attention due to the abundance of various valuable metals [17,18]. PCBs are estimated to hold a 30% share in the global technology field, with a production rate of 1.1×10^{9} m^{2} [20]. Furthermore, PCBs contribute to over 40% of the total valuable metals in e-waste, with an economic potential of $21,200 per ton [18,19,20]. Cu is a prevalent metal, constituting 10-20% of the PCBs' composition, numerous valuable constituents (i.e., Au, Ag, Pt), and hazardous elements like Cd, Cr, and Ni [20, 21]. The concentration of Ag and Au in e-waste derived from PCBs is anticipated to be 1000g and 250g per ton, respectively, exceeding their concentrations in natural occurrence sources (<10 g per ton). Thus, the presence of these valuable metals positions this waste as the core of 'urban mining' and can be utilized as a 'secondary ore' [16,20,21]. In the metallurgical processing of WEEEs, Cu is the second-most imperative metal to be recovered after Au [33]. However, its higher abundance in PCBs poses challenges to the dissolution of precious metals, such as Au, and thus, Cu is prioritized over Au [22,33]. Many researchers have employed bio-cyanide ions (CN^{-}) as a lixiviant, produced by various cyanogenic microorganisms, for Au extraction from PCBs [22]. However, most of the cyanide lixiviant is consumed by Cu, as it forms a more stable complex with CN than Au, thereby affecting its recovery rate [20,21,22]. Thus, to enhance the leaching efficiency of other metals, prior recovery of Cu is necessary for the bioleaching process [23]. Researchers have explored various approaches to improve the recovery rate of the bioleaching process, and the synergistic nature of microorganisms has demonstrated enhancement in the bioleaching process. Several studies suggest using a mixed inoculum of iron and sulfur-oxidizing bacteria for Cu recovery, followed by bio-cyanide leaching for Au recovery by cyanogenic microorganisms [34]. Furthermore, chemo-biohydrometallurgy has enhanced the dissolution rate of precious metals such as Au, Ag, and base metals (Cu, Fe, Al). This method uses a chemical lixiviant to recover Cu before the bioleaching of Au and Ag [16,18].

The present state of the biohydrometallurgy process is notably promising; nevertheless, exploration in this field is still in its early stages. Researchers are persistently working to identify new approaches that can enhance the bioleaching potential of recognized chemoautotrophs (e.g., *A. thiooxidans*, *A. ferrooxidans*) and cyanogenic microorganisms (e.g., *C. violacium*, *P. aeruginosa*, *P. fluorescence*). Despite these efforts, certain limitations, including slow leaching kinetics, metal and substrate toxicity, reduced recovery at higher pulp density (PD), and jarosite precipitation, persist as challenges for the industrial-scale implementation of these bacterial strains [26,27]. Thus, to achieve profitable metals recovery with this green technology, intense bioleaching research is needed to explore more novel microbial strains and optimize parameters that can tolerate higher toxicity of WEEE constituents [11]. So, this 'secondary resource' of metals has motivated us to focus on the metallurgical process of this lucrative solid waste stream through the biohydrometallurgy technique. This method is environmentally friendly, with low operational costs and energy requirements. Considering all aspects of the bioleaching process, the current study aims to explore novel microbial communities adapted to metal-rich environments. The goal is to harness their bioleaching potential and address challenges such as low toxicity tolerance at higher pulp density and specificity limitations observed in previously reported microbial strains.

Additionally, dose-response analysis is utilized to evaluate toxicity tolerance because it is crucial in establishing the viability of bacterial growth and their bioleaching capacity for large-scale application. Furthermore, optimizing the factors that affect bacterial growth and metal leaching potential is a vital step in the bioleaching process, as slight differences in the parameters can deviate from the final yield of the process. Thus, we employ a conventional One-Factor-at-a-Time (OFAT) approach to systematically assess the range and impact of various abiotic parameters, including temperature, pH, a suitable precursor and its concentration, incubation time, and pulp density. Subsequently, a statistical Response Surface Methodology (RSM) approach is employed to optimize these parameters and attain efficient leaching potential. Chemo-biohydrometallurgy has also been used with a cost-effective and environmentally acceptable chemical lixiviant (ferric chloride), resulting in increased leaching efficiency of potential bacterial strains for various base metals (Cu, Fe, Ni, and Co) and precious metals (Au and Ag).

Hence, our research introduced a novel bacterial isolate, *B. sporothermodurans* ISO1, with promising bioleaching capabilities and also identified a suitable chemical lixiviant (i.e., ferric chloride) for an enhanced chemo-biohydrometallurgical process. The successful implementation of optimized conditions at the pilot scale underscores the potential applicability of these findings for industrial-scale bioleaching operations, marking a significant contribution to sustainable e-waste management.

Considering the aspects mentioned above, the hypothesis of this study posits that the exploration and selection of microbial strains exhibiting heightened toxicity tolerance and superior bioleaching potential will significantly enhance the efficacy of the bioleaching process for e-waste treatment. Additionally, we hypothesize that developing hybrid leaching methods tailored for improved efficiency and reduced time requirements will contribute to overcoming the current constraints of the bioleaching process. Furthermore, we anticipate that gaining insights into the implications of bioleaching at an industrial scale will pave the way for its successful integration into large-scale applications for sustainable and effective e-waste management. So, the current study has been designed for the recovery of base and precious metals from waste "computer printed circuit boards" (CPCBs) with the following objectives:

Objective 1: To characterize the waste CPCBs and metal-tolerant bacterial isolates.

Objective 2: To optimize the parameters for efficient metals leaching from waste CPCBs.

Objective 3: To implement the chemo-biohydrometallurgy approach to augment the leaching efficiency of potent bacterial isolates.

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 Electrical or Electronic Equipment (EEEs) as e-waste

The extensive range of equipment that operates with electromagnetic fields or electric currents is considered electrical and electronic equipment (EEEs). However, for statistical purposes, EEE is classified into three categories: White Goods, which includes domestic appliances, such as ACs, freezers, washing machines, and dryers. Grey Goods includes IT equipment, like disk drivers, computers, CD-ROM, LCDs, and monitors, and Green Goods displays small household appliances and audio or visual equipment, such as televisions, mobile phones, recorders, cameras, and projectors). The categorization of products is determined by their functional similarities, composition analysis, overall weight, and comparable end-of-life attributes. Each category possesses a distinct material composition, necessitating different disposal and recycling methods, and if not managed responsibly, these products can have numerous negative impacts on the environment and human health [1-4].

Regular upgrading with immense advancement to all EEEs results in many obsolete items known as e-waste or waste electric and electronic equipment (WEEEs) [5-7]. The categorization of e-waste is based on its source of origin, composition, and decomposition mechanism [3],[4],[8]. Although e-waste has been defined in various ways, the definition that has prompted the most widespread acceptance is that of any equipment dependent on electric power for its operation and has reached the end of its useful life" [5],[6],[9]. E-waste is a vast and expanding category of electronic items, including oversized household appliances (refrigerators, air conditioners, mobile phones, personal stereos) and laptops that users have abandoned. “European Waste Electrical and Electronic Equipment (WEEE) Directives” 2002/96/ “European Commission” (EC) and 2012/ 19/ “European Union” (EU) classified the various electronic equipment into ten different e-waste categories, which are enlisted in Table 2.1. E-waste originates from numerous sorts of products, and it has been suggested that garbage from grey goods (computers, printers, fax machines, scanners) is more harmful than waste from white and brown goods [4],[5],[10].

Table 2.1 Classification of various electronic and electrical equipment under different WEEEs categories.

S. no.	**Equipment**	**Waste category**	**Label**
1	Video game consoles, alarm clocks, CD and DVD players, television, grinder–juicer–mixers, electric kettles, electric chimneys.	Minor household appliances	Small HH
2	Microwave oven, dishwasher, refrigerator, air conditioner, washing machines, induction cooker.	Major household appliances	Large HH
3	Radio receivers, televisions, camcorders, personal computers, digital cameras, MP3 players, DVD players.	User implements	CE
4	Telecommunication sources, teleprinters, communication satellites, LAN, and modem.	IT and telecommunication gadgets	ICT
5	LED and compact fluorescent lamps, halogen, neon, and ballast lamp,	Illumination devices	Lighting
6	Transistors, diodes, ICs, motors, batteries, relays, transformers, wires, switches and generators,	Electrical and electronic devices	E and E tool
7	Medical thermometers and biomedical engineering instruments	Medical instruments	Medical equipment
8	Batteries in the transportation sources	Sports gadgets and leisure	Toys
9	Relays, thermostats, and microcontrollers	Monitoring and control instruments	M and C
10	Automatic dispensing systems such as soap, water, and spray.	Automatic dispensers	Dispensers

2.2 Constituent of WEEEs and their detrimental effects

e-waste is predicted to consist of a complex mixture of approximately 1000 hazardous and non-hazardous materials divided into three broad groups: ceramics, metals, and organic chemicals. This challenging, diverse, and heterogeneous solid waste comprises 50% iron steel, 13% nonferrous metals, 21% polymers, and 16% miscellaneous materials such as ceramics, glass, and rubber [11-14]. Base and precious metals, including Cu, Fe, Al, Ni, Au, Pt, and Pd, are among the nonferrous metals that are found most abundantly in WEEEs. However, claiming the explicit composition of WEEEs is challenging since the metallic composition of e-waste varies substantially depending on the equipment typically used for investigation [15-17]. Figure 2.1 displays a typical e-waste component of metallic and non-metallic materials. Other than metallic constituents, plastic components such as PAH (polycyclic aromatic hydrocarbons), PBDD (polybrominated dibenzo-p-dioxin), organic material including BFR (brominated flame retardants), dioxins, and glass fibers also contribute to the part of WEEEs. Moreover, ceramics such as alkaline earth oxides, titanate, alumina, barium, and silica [14, 18-21] are all harmful additives of WEEEs that contribute to the toxicity of e-waste [3, 8, 12, 22]. It is estimated that > 6% Pb by weight is consumed in a standard computer, and 50 tons of Hg and 71 kg of BFR plastics are emitted yearly as the unrecorded flow of e-waste worldwide [7, 10, 23, 24]. The low collection rates and non-environmentally sound disposal and treatment pose significant environmental and human health threats and cause a primary concern for this hazardous waste stream. According to some researchers, in the landfilling of e-waste, the toxic constituents infiltrate the groundwater and subsequently undergo bioaccumulation and biomagnification in the food chain, thereby posing a considerable environmental hazard [25], [[26]. For example, Hg and Pb get leached during the disposal and recycling of circuits and broken glass of CRTs from televisions and monitors, respectively; in addition, landfilling of BFR plastics or plastics containing Cd results in leakage of PBDE and Cd into the soil and groundwater[21, 27-31]. Informal recycling of this toxic waste is accompanying to causes numerous health problems, most notably cancer and neurological diseases[31-35].

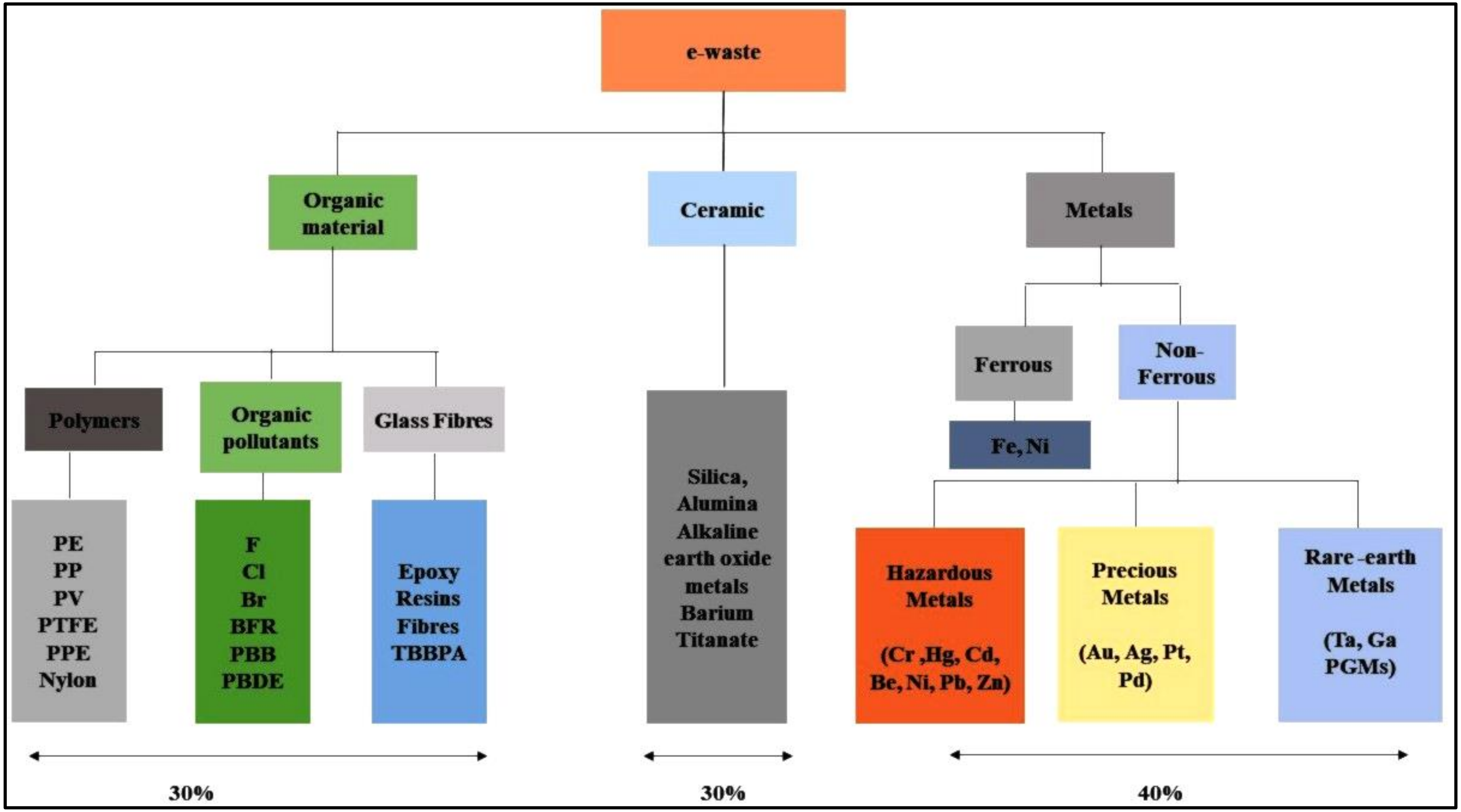


Figure 2.1 Mass fraction of various constituents majorly present in WEEEs.

Table 2.2 outlines the potential physiological consequences of many hazardous substances detected in WEEEs. This evidence manifests a serious occupational danger to humans and the ecosystem if treated through informal recycling [20, 36, 37]. Implementing comprehensive safety management strategies is necessary to avoid the adverse effects of WEEEs on individuals and the environment [2, 38].

Table 2.2: Hazardous substances that cause human health issues and their component sources in WEEEs.

Source	Element	Human Health Consequences
CRT screens, PCB diodes,	Arsenic (As)	Cause of malignancy and skin ailments. Impairment of the respiratory, cardiovascular, and reproductive systems.
Protector and radiators	Barium (Ba)	Cerebral edema, muscular debility, and impairment of cardiac, hepatic, and spleen function.
Motherboards	Beryllium (Be)	Skin ailments and lung cancer
Flame retardant for electronic equipment	Brominated flame retardant (BFR)	Affects the endocrine system and the liver. Also, it causes a child neurodevelopmental impairment.
PCBs	Copper (Cu)	Responsible for liver disorders and diarrhea.
Computer housing, CRTs, batteries and diodes,	Cadmium (Cd)	Responsible for the destruction of the neurovascular system, respiratory system, and kidneys.
Corrosion protector of galvanized steel plates	Chromium (Cr)	DNA damage, skin sensitivity, and kidney impairment.
Batteries	Lithium (Li)	Impact on nerve and intestinal activity.
Computer monitor	Lead (Pb)	Disrupt the central nervous, blood, and reproductive system.
Switches and lamps	Mercury (Hg)	Disrupt central nervous system and kidney
Batteries	Nickel (Ni)	Accountable for skin and lung diseases. Also, it causes numerous cancers.
Combustion by-products	Polychlorinated dibenzodioxins (PCDDs)	It disrupts the neurological system and contributes to many malignancies.

2.3 The abundance and economic value of e-waste

The persistent expansion of WEEEs has emerged as a global prime concern. At present, approximately 59.4 Mt (i.e., 7.3 kg per inhabitant) of staggering e-waste has been generated around the world; this has increased by 9.2 Mt since 2014 and is continuously projected to rise to 74.7 Mt by 2030—an almost twofold upsurge in only 16 years [7, 13, 19]. The progression in industrialization and urbanization upsurges the EEE consumption rates, short life cycles, and fewer repair alternatives that ultimately lead to an increase in the volume of e-waste [11, 25]. Since the inception of e-waste generation, Asia has continuously remained the top e-waste producer, accounting for a staggering 24.9 Mt, whereas current statistical analysis indicates that China leads the globe with a total of 10.1 Mt production. India ranks second and third among Asia and the world, respectively, with a 3.2 Mt production rate [8, 39]. Furthermore, America (13.1 Mt) holds the second position around the continents, followed by Europe and then Africa with a production of 12 and 2.9 Mt, respectively. Oceania is the least among all, with 0.7 Mt e-waste production. Figure 2.2 depicts the countries around the globe that contribute majorly to e-waste production. Additionally, in 2020, only 9.3 Mt (i.e., 17.4%) of the total 53.6 WEEEs was officially recorded for collection and recycling. The reported rate of collection and recycling increased by 1.8 Mt since 2014, or about 0.4 Mt annually, while the yearly growth rate of over 2 Mt led to a 9.2 Mt rise in the total amount of e-waste generated [13, 20, 39] . Hence, the global surge in e-waste is not being adequately addressed by recycling endeavors.

This staggering waste represents an accumulation of many rare and precious metals, and a continual upsurge in this solid waste stream might serve as a center for secondary resource production [18,20,23]. According to the United Nations University (UNU), the assessment for 2019 indicates a secondary raw material worth exceeding $60 billion in 53.6 Mt of e-waste [13, 19, 39]. Table 2.3 provides information on the quantity and cost of various metals used in 2019 to produce electronic equipment. The Environmental Protection Agency (EPA) report reveals that 320 tons Au and 7500 tons Ag are consumed each year to create new electronic items that further end up as a stack of metal sources. Surprisingly, the concentration of Cu and Au in WEEEs is 13-26 and 35-50 times greater, respectively, than their natural deposits, making them "sleeping mines" or the "heart of urban mining." According to an additional EPA report, 10 tons of Cu, 0.01 tons of Pb, 0.275 tons of Ag, and 0.025 tons of Au can be recycled from just 1 million cell phones [2, 3, 40, 41].

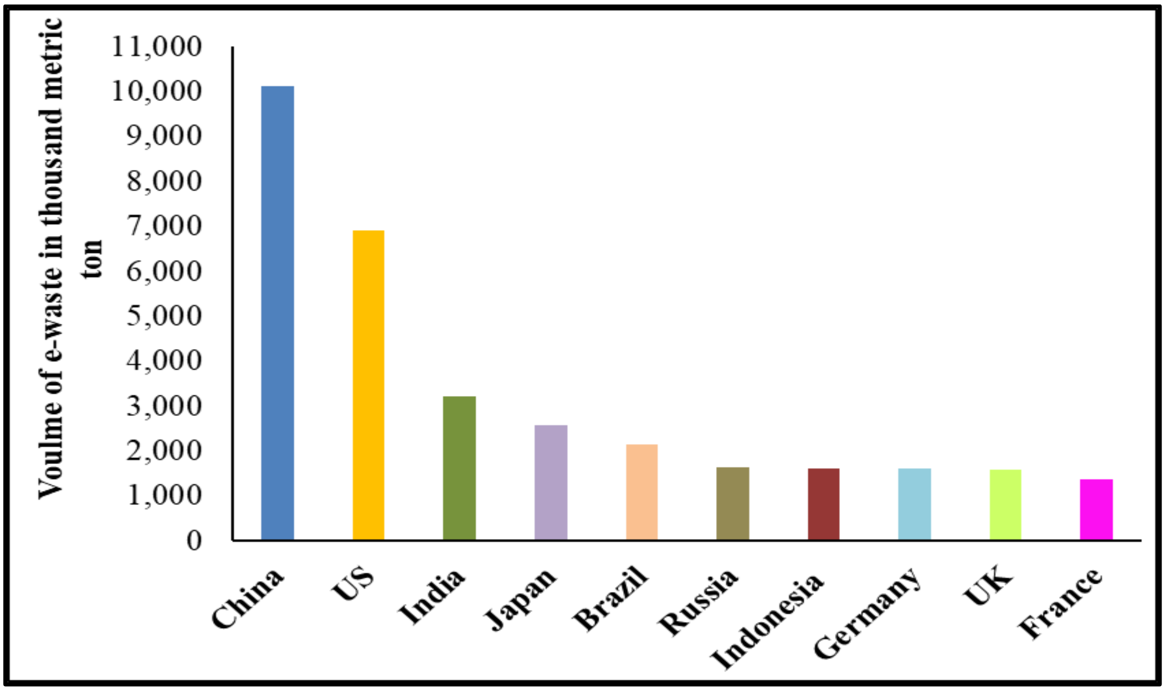


Figure 2.2 Leading e-waste producing nations. Source: Statista.

The rapid expansion of the technology industry has raised concerns about its repercussions on primary mining, including material scarcity, limited availability, and restricted resource access. Thus, this emphasizes the immediate need to improve secondary resources' recovery and decrease reliance on virgin materials [7, 42]. In the context of a circular economy, it is crucial to acknowledge the potential of e-waste as a 'sleeping mine,' representing a valuable source of various secondary raw materials. This recognition is essential for nations to effectively and sustainably reduce their material demand [6]. However, the recycling industries are frequently plagued with high energy and economic demands, environmental and human health, and material recycling issues. For example, retrieving elements like germanium (Ge) and indium (In) is challenging due to their scattered placement in the equipment. This difficulty arises because the designs don't incorporate intentional provisions for reprocessing considerations. However, the economic feasibility of e-waste collection and recycling can be potentially viable, mainly when conducted manually, with a minimal reduction in material losses (<5%) for electronic devices exhibiting high concentrations of valuable metals [8, 22, 43, 44]. In 2019, within an official documentation framework, only 25 Mt of the total Fe, Cu, and Al present in electronic waste (e-waste) were recycled despite a consumption rate of 39 Mt. Therefore, it can be inferred that approximately 14 Mt of Fe, Cu, and Al from primary sources would still be required for the production of new technological devices [22, 41]. Hence, there is a sizable difference between the demand for secondary metals and their supply in new EEEs [19, 39, 45]. Consequently, restoring these volumes and material values is contingent upon the predominant portion of the raw materials employed in WEEEs being recycled economically and environmentally sound.

Table 2.3 Estimated value of the raw materials exhibited in WEEEs.

Metals	Amount (kt)	Value ($ millions)
Ag	1.2	579
Cu	1808	10960
Al	3064	6062
Co	13	1036
Au	0.2	9481
Fe	20466	24645
Pd	0.1	3532
Pt	0.002	71

2.4 E-waste as a source of "urban mining"

Urban mining is a concept and a strategy to preserve environmental resources and advance the conservation of natural resources through the '3R' (Reuse, Recycling, and Recovery) strategy via a circular economy approach [46-48]. According to research done by UNU in 2018, roughly 40 critical metals may be extracted from a single smartphone, with Au accounting for approximately 25-30 times more purity than primary Au ore [39, 49]. China pioneered adapting this strategy and is highly regarded for its ability to render a substitute that can thrive on the country's resources necessary for manufacturing EEEs [7, 50]. Moreover, according to the "US Geological Survey," in 2018, the worldwide yield of Rare Earth Elements (REE) was predominantly attributed to China, which accounted for nearly 81% of the total output [18, 49]. Furthermore, according to UNU, Europe can excavate almost 2 Mt of EoL batteries annually

(90% lead-based and 10% Ni- hydride, Zn, and Li-based), consisting of the secondary raw material of approximately 78,000 tons of lithium (Li), 21,000 tons cobalt (Co), and 114,000 tons manganese (Mn) [50, 51]. The ProSUM "Prospecting Secondary Raw Materials in the Urban Mine and Mining Wastes" project in Europe has stated that it has integrated and endeavored to accomplish the urban mining strategy. According to the study, with the current documentation and formal recycling flow of 17.4% global e-waste in 2019, a primary material value of $10 billion can be retrieved in an environmentally sound manner, and 4 Mt of may be made accessible for recycling [6, 39, 46, 50]. This recycling rate of metals contributed not only to resource conservation but also to environmental preservation. Metals extraction from low-grade primary ore necessitates the extraction of a substantial amount of material through numerous processes that pose significant environmental impact due to the emission of greenhouse gases[52]. For example, mining 1 ton of gold is estimated to release approximately 17,000 tons of CO2, while producing palladium (Pd) and platinum (Pt) contributes about 10,000 and 14,000 tons of CO2, respectively. [19, 43]. Hence, implementing a suitable framework for the regulated recovery of numerous metals from the significant volume of WEEEs can offer advantages from both ecological and economic perspectives [19, 53].

2.5 Processing routes of e-waste at regional or cross-border level

Despite various valuable metals in WEEEs, issues such as excessive production, insufficient management procedures, and a lack of consumer awareness contribute to the unregulated processing of this substantial waste [43]. Figure 2.4 illustrates that there are four alternative ways to treat WEEE: (1) informal landfilling and incineration, (2) transboundary transportation, (3) recycling, and (4) reuse/remanufacturing.

2.5.1 Route 1

Even though e-waste poses numerous significant threats to humans and the ecosystem (mentioned in section 2.2), it continues to be disposed improperly through landfilling and incineration. The study by the US "Environmental Protection Agency" (EPA) revealed that merely 15-20 % of the total generated waste is recycled, whereas the remainder ends up in informal disposals such as landfills and incineration [30, 43]. WEEEs landfilling has become a significant concern throughout the world since it leads to the release of its hazardous constituents into the food web and causes substantial acute and long-term health consequences

[30, 37, 54-56]. In addition, the unauthorized collection of WEEEs with municipal solid waste streams in rural regions and its subsequent burning without segregation to minimize the volume before final disposal at unlined landfills is a serious concern. It facilitates the percolation of various toxic metals enclosed within the plastic grid of WEEEs and released into the environment after burning but not accessible during landfilling [31, 32, 36]. High-temperature open burning and burning in the combustion chamber during incineration cause the emission of harmful gases to the surrounding environment. Moreover, using hot shredder equipment during the processing of WEEE plastic decreases the particle size, producing more dioxins and furans[57]. Using modern incinerators that can purify the gas stream and recover energy is a safe alternative treatment solution to this open-burning process, which must be forbidden entirely [58, 59].

2.5.2 Route 2

Global e-waste trading has always been a source of contention, driven by the compelling prospect of interconnecting the digital divide and potentially dangerous health consequences of informal recycling in underdeveloped nations [60-62]. Lower labor costs and lack of stringent environmental regulations uphold illegal cross-border transit from developed to developing countries, where India and China potentially receive 50-80% unlawful transboundary e-waste exports, as illustrated in Figure 2.3 [59, 63]. Some studies claim that the bulk of e-waste is transferred from the Northern American hemisphere to underdeveloped nations for informal disposal [64]. Although assessing the precise quantity of e-waste in circulation is consistently challenging because most of it is exported illegally or with the false claim that it is intended for reuse or scrap, it is widely accepted that the volume is enormous. A substantial portion goes through alternative channels [65]. The Basal Action Network (BAN) is a critical substitute that strives to examine the cross-border movement of WEEEs [20, 66]. According to the Global E-waste Monitor 2017, nearly 4000 tons of WEEEs are thought to be discarded globally every hour. BANs have deployed 205 trackers to scrutinize cross-border movement and revealing that 34% of e-waste is exported through the coastline to developing countries, with the majority (93%) to Asia and Africa, where there are no suitable reutilizing facilities, and 7% shipped to nations like Mexico and Canada [1, 7, 20, 39].

Transboundary e-waste flows from industrialized to developing countries have drawn attention and become a big concern because they increase the environmental burden in destination countries. Due to e-waste treatment being an environmentally unsound way in developing nations that poses severe threats to health and the environment, many conventions and stringent legislations have been enforced against transboundary movements [66]. The Basel Convention, the most important international initiative, was established in 1992 to limit the flow of hazardous wastes, including WEEE, across the nations. The critical element of the Basel Convention is the notion of EPR (Extended Producer Responsibility), which asserts that the producer is solely responsible for the appropriate disposal of hazardous waste [20, 40, 67, 68]. Furthermore, the transfer of hazardous materials to developing nations is forbidden unless the acquiring government provides prior informed consent". Several other laws, including the Restriction of Hazardous Substances Directive 2002/95/EC, (RoHS 1) directive, WEEE directive (2002/96/EC), RoHS Recast Directive, 2011 (RoHS 2), "StEP (Solving the WEEE Problem)," and other regional initiatives, have been implemented to restrict the illicit overseas exportation and to divulge the concept of "reverse supply chain" of the WEEEs stream [20, 39, 47, 69].

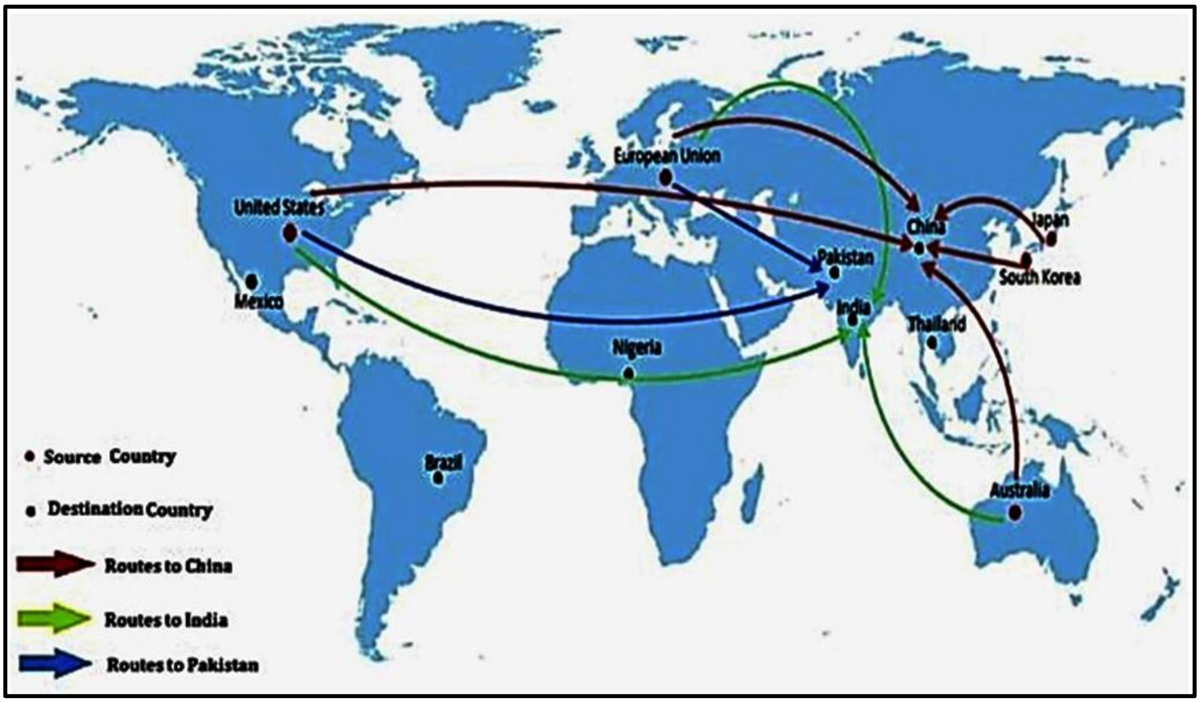


Figure 2.3 Transboundary flow of e-waste from developed countries to developing nations.

2.5.3 Route 3

WEEE recycling entails formal and informal economic activities such as dismantling, manual sorting, and various physical processes to recover materials. 'Formal' recycling is regarded as a safe and impenetrable practice as employed in specially designed facilities that pose minimal environmental and human health risks; however, the high cost of facilities and equipment limits the widespread acceptance of formal recycling [19, 43, 70]. Unlike formal recycling, "Informal" recycling is unregistered, illegal, and performed by unskilled labor under uncontrolled condition that causes health issues to the worker involved [32, 71]. In developing nations such as India (Bengaluru), Nigeria (Lagos), China (Guiyu and Taizhou), Ghana (Accra and Agbogbloshie), Thailand, Philippines, and Vietnam, many informal sectors have sprouted up that process e-waste in an uncontrolled manner regardless the environmental and human health concern [29, 38, 61, 72]. Open burning, labor health issues, pollutant emissions into the air, and the discharge of different leachates negate any beneficial aspect of reprocessing or reutilization and shift ecological advantage impact balance to the adverse side [73]. However, various transnational and non-governmental organizations (NGOs) have been developed to increase apprehension about safe management and recycling [28, 58, 62, 68]. Some of the organizations that actively scrutinize international WEEEs status, advocacy, and regulation are Toxics Link (India), UNU, SCOPE, Greenpeace (China), Nature Conservation and Nuclear Safety (Germany), PACE, the Federal Ministry for the Environment (Germany), and the National Institute of Environmental Health Sciences: (US) [42-44].

2.5.4 Route 4

The upgrading, reselling, repair, reconditioning, and remanufacturing are all manifestations of WEEE reusability. This strategy decreases the e-waste burden that must be recycled while encouraging the reuse of old components in remanufacturing and repairing other products [7, 43]. While this represents the most favored approach for reducing WEEEs, its implementation poses challenges due to customer reluctance toward reusability and high rates of product obsolescence. Addressing this challenge emphasizes enhancing public awareness about reusability, as it significantly contributes to energy conservation during recycling processes [39, 68, 69].

An increasing amount of WEEEs, their economic value, improper and hazardous processing methods, landfill or incinerator disposal, and other factors compel us to pay attention to this overwhelming trash and mandate its management under long-term development objectives [25, 39, 65]. According to the “United Nations General Assembly” (New York), the management of e-waste is inextricably linked to the attainment of the 2030 Agenda for

Sustainable Development Goals (SDGs) as enlisted in Table 2.4 [39, 74, 75]. It entails the optimization of natural resource conservation, attaining economic benefits, and establishing robust facilities to facilitate a proficient and acknowledged e-waste management framework. Thus, adopting urban mining as a circular economy approach is a target to accomplish the (SDG's) 2030 agenda [6, 39, 50].

2.6 Metallurgical techniques to exploit e-waste as a mining source

The information mentioned above about the economic value of continuously expanding e-waste notes that reckless disposal leads to environmental harm and loss of its richness in various valuable metals. Hence, implementing specific metallurgical processes would be worthwhile in converting this substantial waste into a valuable resource by retrieving its metallic constituents and preserving environmental resources. [15, 19, 43]. Hence, for efficient metal recovery, the process must be carried out in several combined phases, including physical/mechanical procedures, segregation methods, and metallurgical treatment, because a single strategy is found inadequate to obtain efficient recovery [44, 76]. The three metallurgical approaches (i.e., pyrometallurgy, hydrometallurgy, and bio-hydrometallurgy) that have been implemented after the segregation and dismantling of metallic and non-metallic fractions for retrieving metals from e-waste are described here in a subsequent section.

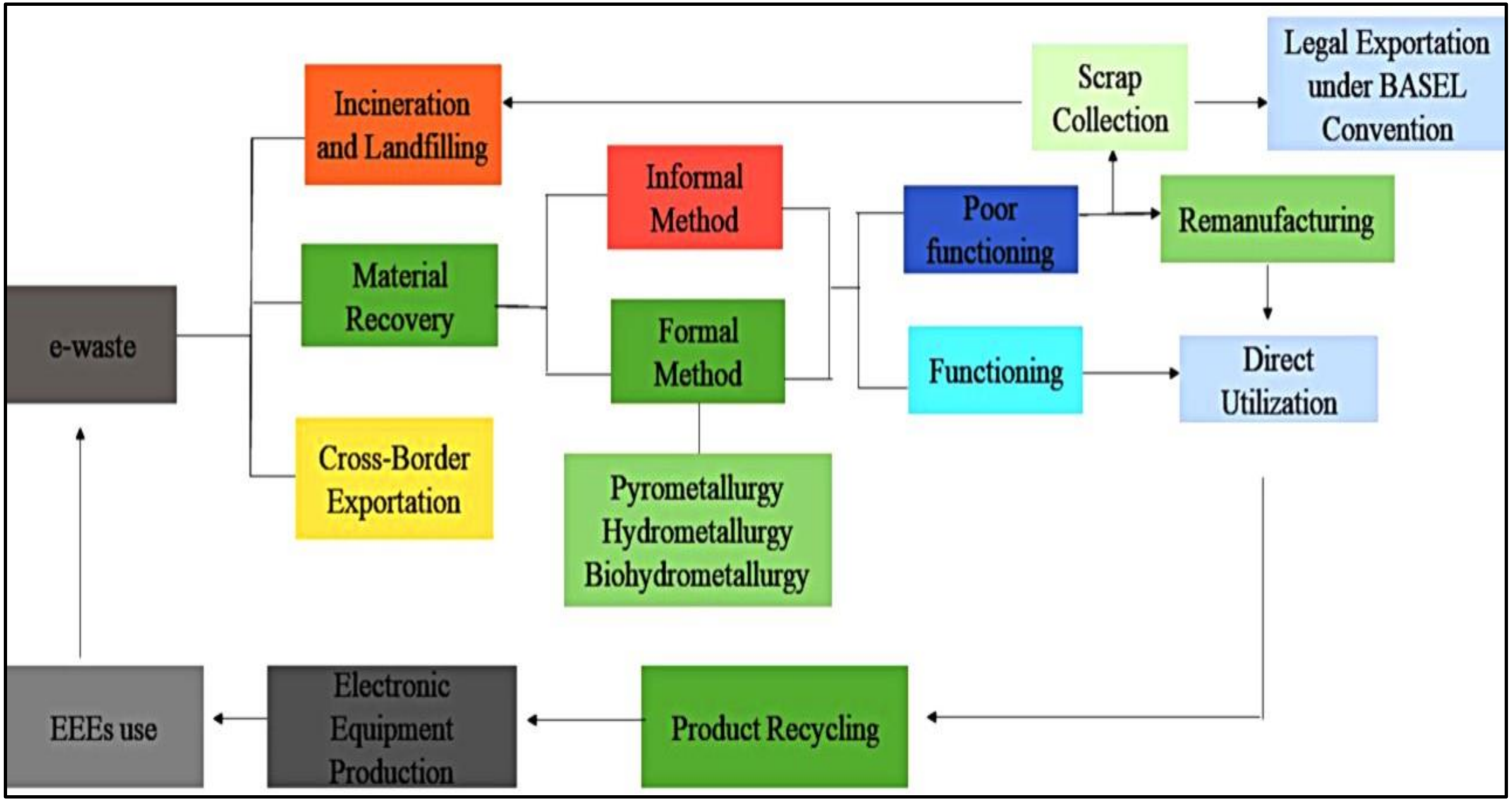


Figure 2.4 Flow diagram represents the route of e-waste generation and its processing at regional and national levels.

Table 2.4 List of sustainable development goals and targets associated with e-waste that need to be addressed by 2030.

Targets	Agenda
Target 3.9	Significant reduction in fatalities and illnesses due to exposure to detrimental pollutants and contamination of the air, water, and soil sources.
Target 8.3	Encourage the formalization and expansion of micro, small, and medium-sized firms, including through access to economic supports, and promote development-oriented policies that support productive activities, decent jobs, entrepreneurship, creativity, and innovation.
Target 8.8	Safeguard employment rights and ensure secure and safe working conditions for all workers, especially migrating women and uncertain workers.
Target 11.6	Limit cities' negative per capita ecological effect, with a particular emphasis on air quality and other waste management.
Target 12.4	Attain the eco-friendly management of chemicals and waste throughout their life cycles in compliance with established global standards and considerably reduce their discharge to air, water, and soil to minimize their detrimental effects.
Target 12.5	Reduce trash production significantly through repair, recycling, and reuse. Compared to primary excavation and processing, mining e-waste is expected to emit less CO2 than 80% less CO2 per unit of gold, so focus on recycling more waste.

2.6.1 Pyrometallurgy

Pyrometallurgy is a widely accepted conventional approach that uses high temperatures to extract metals. Incineration, roasting, and smelting are the most prevalent pyrometallurgical methods performed at high-temperature melting furnaces to separate many valuable metals [11, 43, 70]. Some of the globally available thermal operational plants that perform formal melting processes for resource recovery from WEEEs include “Aurubis smelter” in Germany, “Noranda copper smelting” in Canada, and “Ronnskar smelter” in Sweden, “Umicore” [41, 77, 78]. Umicore is noted for recovering a wide range of metals, including seven precious metals, by heating samples in an inert atmosphere at 400–700°C [77]. This process also decomposes other components of WEEEs, such as rubber, plastics, paper, and wood, followed by their conversion into volatile substances that are attained as oils or gases [14, 36]. It is estimated that the PCBs of a waste computer generate approximately 22.7% and 4.7% of oils and gases, which further utilize a raw compound in various chemical industries [79].

Furthermore, in the “Noranda” process, WEEEs are exposed to melt at a higher temperature (i.e., 1250°C) under a stable oxidizing condition. The Cu and other metals consisting of fractions are separated from other non-metallic components and transferred to produce pure Cu blisters (99.1% purity), followed by electrorefining to recover other precious metals. Thus, the smelting process facilitates pure metal recovery, yet a mixture of metals and their alloys further challenges the final upgrading, as it requires very high energy [79-81]. In addition, pyrolysis of e-waste can effuse numerous toxic dioxins and other gases from the plastic part of WEEEs that cause many environmental concerns. Higher energy and economic demand to manage poisonous gas emissions, deprivation, and trouble in the recovery of many metals are other challenges that restrict the further implementation of pyrometallurgical methods [2, 19, 34, 38, 63, 82, 83].

2.6.2 Hydrometallurgy

This metallurgical process uses strong acids and inorganic solvents as a chemical leaching method. The hydrometallurgical process employs an electrochemical mechanism to selectively extract valuable metals from WEEEs. The limitations of a pyrometallurgical process necessitate adopting this technology [84]. The production of less harmful gases, low energy demand, ease of achievement in the laboratory setting, cheaper operating expenses, and greater efficiency are some advantages of hydrometallurgy over the pyrometallurgy technique [49, 69, 70]. Some of the chemical lixiviants that have been used for the recovery of numerous metals include nitric acids (HNO_3), hydrochloric acids (HCl), sulfuric acids (H_2SO_4), cyanide (CN), halide, thiosulfate ($Na_2S_2O_3$), thiourea (CH_4N_2S), and aqua regia (HCl: HNO_3). This process has been deemed more selective for metal recovery, notably Cu from PCBs, since it is available in the elemental form [43, 70, 73].

Cyanide is a well-known chemical lixiviant, predominantly used to solubilize precious metals such as Au Ag. It is found that the utmost of the free cyanide resides as CN^- ion at alkaline pH; hence, leaching at pH greater than 10.5 favors an ionic form of cyanide with limited loss, efficiency, and fewer environmental causes. Cyanidation leaching of metals is initiated with the oxidation of cyanide to cyanate, followed by complex formation with metals [85, 86]. Inadequate handling of effluent streams that contain residual cyanide content may result in the infiltration of this noxious chemical into the groundwater and may lead to persistent health consequences in the long run. [29, 60, 62]. Despite the improved understanding of safe cyanide handling, environmental concerns continue to limit its usage in industrial practice [30, 87]. Hence, researchers have focused on less harmful chemical reagents such as halides, thiourea, and ferrous sulfate. Thiosulfate is another lixiviant that has supplanted the role of cyanide for the recovery of Au metal.

Moreover, using copper ions and ammonia as catalysts facilitates the efficiency of thiosulfate lixiviant for Au metal [88, 89]. Several researchers have also demonstrated the importance of iodide in recovering Au and Ag from PCBs. They asserted that the iodine (I_2) to iodide (I^-) ratio influences leaching proficiency because low I_2 concentration causes reduced I^- production and poor metal complexation capacity, resulting in metal iodide complex precipitation and less metal recovery. Conversely, higher (I_2) concentration hinders precious metal recovery by forming complexes with base metals at the same time [90] [91] [17, 92, 93].

While hydrometallurgy offers various advantages over pyrometallurgy, the processing risks and environmental impacts of strong acids underscore the process's limitations [8, 28, 44, 76]. The toxicity of chemicals employed in the process remains a concern as applying strong chemical lixiviants results in significant amounts of hazardous by-products with possible environmental effects. Furthermore, due to the substantial energy consumption, these techniques are not considered affordable for extracting valuable components from WEEE [30, 43, 77, 87].

2.6.3 Biohydrometallurgy

Environmental protection has become a global concern that has forced researchers to incorporate green technology in every aspect, and the adoption of biohydrometallurgy is one of the prime examples of this initiative [43, 70]. For centuries, ancient civilizations have employed oxidizing bacteria to extract diverse resources from mineral ores, although this practice may not have been widely recognized. [50, 94]. However, this technique has also gained attention over the last two decades for recovering various metals from WEEEs. This microorganism-mediated leaching process has been suggested as a novel technique that unveils notable economic benefits compared to conventional metallurgical methods.

Moreover, this methodology minimizes infrastructure expenditures and necessitates minimal energy consumption by functioning at ambient conditions [46, 95]. It is alternatively called biomining, bio-extraction, and bioleaching [96]. The underlying principle of this biohydrometallurgical process is based on harnessing the potential of microorganisms to dissolve metals from solid wastes and subsequently recover them from the solution [19, 80, 97]. Depending on the microorganisms engaged in the leaching process, mineral dissolution is followed by one or more methods such as “complexolysis,” “acidolysis,” and “redoxolysis”[98, 99]. Acidophilic bacteria, i.e., *Acidithiobacillus ferrooxidans* and *A. thiooxidans,* are the first and most extensively exploited microorganisms in biomining from natural mines as well as secondary sources of metals [19, 63, 100]. In 1999, Breed and Hansford's investigation unveiled pivotal insights into the bioleaching kinetics and mechanisms executed by acidophilic bacteria. Their findings underscored three fundamental processes: the oxidative transformation of sulfide minerals, the enzymatic generation of ferric ions by acidophilic bacteria, and the strategic modulation of redox potential. These steps play a critical role in regulating the efficiency and mechanisms of bioleaching processes employed by acidophiles [101]. *Thiobacillus* sp., an acidophilic bacterium, mediates the conversion of ferrous to ferric ions, a primary leaching lixiviant. This process occurs under specific parameter conditions, such as the ratio of O_2 production to CO_2 consumption, which indirectly influences the kinetics of the sub-processes involved in the leaching mechanism [90, 100]. Numerous studies have been reported using thermophilic acidophiles to dissolve Cu, Fe, Zn, Ni, Au, and Pt [102]. In 2001, Ilyas and co-workers demonstrated bioleaching of e-waste using a moderate thermophile *Sulfobacillus thermosulfidooxidans* and obtained 80% solubilization of Ni, Cu, Zn, and Al. Their findings claimed moderate thermophiles' bioleaching efficiency is higher than that of mesophiles and extreme thermophiles [103]. Liang et al. further demonstrated a bioleaching process using a mixed culture of *A. thiooxidans* and *A. ferrooxidans* to enhance the extraction of metals from PCBs. They obtained 94, 89, 90, and 86% of Cu, Ni, Zn, and Pb, respectively [104]. Although abundant literature summarizes the efficient leaching of base metals using *Thiobacillus* bacteria, the role of these bacteria in the recovery of precious metals has not been defined much [76, 97]. Moreover, some microalgae-based systems have also been exploited as an environmentally safe alternative to chemical oxidation methods to abolish various emerging contaminants in the environment, so microalgae-based systems can also be investigated for the remediation of various toxic pollutants of e-waste [105].

Table 2.5 Advantages and disadvantages of the metallurgical process utilized for metals recovery from natural mines and low-grade secondary sources.

Approach	Advantages	Disadvantages
Pyrometallurgy	High-efficiency rate, Low time requirement	High economic and energy demand and the release of toxic fumes that cause environmental footprints.
Hydrometallurgy	Low energy requirement, rapid accomplishment, high-efficiency rate	Highly corrosive, releases toxic by-products and fumes associated with various health and environmental issues.
Biohydrometallurgy	Simple, inexpensive, eco-friendly, no use of strong chemicals, low energy demand	Low efficiency, slow process, and less availability of molecular tool kits for genomic and proteomic studies. The scale-up process is complex and time-consuming.

2.6.3.1 Role of cyanogenic microorganism in biohydrometallurgy

Cyanide is a well-known conventional chemical lixiviant that exhibits high stability and solubility to form metal-cyanide complexes during cyanidation reactions with various precious metals (mentioned above). Despite its toxic behavior, this lixiviant is employed in 90% of mining procedures to recover Au metal [85]. Recently, biogenic cyanide has substituted the role of inorganic cyanide to implement the bioleaching process to recover precious metals [87]. Many bacteria, especially cyanogenic microorganisms such as *C. violaceum*, *P. fluorescens*, *P. aeruginosa*, *P. putida*, and *B. megaterium*, have the potential to produce hydrogen cyanide (HCN) as secondary metabolites by utilizing glycine as a primary precursor through oxidative decarboxylation reaction through HCN synthase enzyme at the early stationary phase [34, 93, 106, 107]. The bioleaching of e-waste using biogenic cyanide produced by cyanogenic microorganisms is known as “biocyanidation” or “alkaline bioleaching” as alkaline pH enhances more cyanide ion (CN^-) production. At neutral pH, cyanide exists as hydrogen cyanide (HCN) with a pKa value of 9.3; therefore, alkaline pH favors the formation of more CN^- that reacts with metals in solid residues [80, 81]. In addition to cyanide production, cyanogenic microorganisms possess another enzyme known as β-cyanoalanine synthase that can enable cyanogenic bacteria to detoxify excessive cyanide by themselves at the late stationary phase by converting into β-cyanoalanine, making biocyanidation leaching safer and more preferable [65, 108, 109].

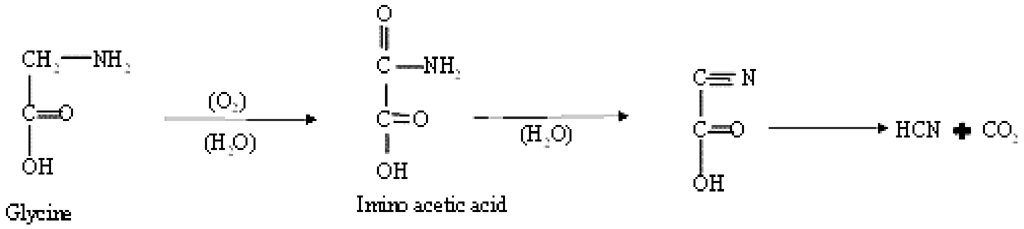
Farmarzi et al. established the biocyanidation leaching of metals from PCBs using cyanogenic bacteria. The authors observed Ni solubilization as tetracyanonickelate from Ni powder and Au as dicyanoaurate from e-waste (PCBs) through metal cyanide complex formation [110]. Elsner’s Equation (2.1) can represent the Au- CN complex. Meanwhile, in 2002, another researcher reported the potential of Pseudomonas sp. for the sequestration of uranium and thorium by the mechanism of bio-oxidation [111].

$$4Au + 8CN^- + O_2 + 2H_2O \xrightarrow{\hspace{3em}} 4Au\,(CN)^-_2 + 4OH^- \qquad (2.1)$$

Furthermore, many studies have been accomplished to recover precious metals through biocyanidation reactions, whereas some have demonstrated the development of methods to enhance the efficiency of cyanogenic microorganisms. Brandl et al. examined the bioleaching trend for metals from shredded PCB e-waste samples using *C. violaceum* and observed 68.5% Au solubilization as dicyanoaurate. They also demonstrated the recovery of Cu due to its higher content in PCBs, which causes a hindrance to the recovery of Au metal [109]. Chi et al. further tried to enhance the leaching efficiency of *C. violaceum* by supplementing the media with H2O2 and increasing 11% Au mobilization from waste mobile phones [112]. Moreover, some researchers investigated the bioleaching potential of individual cyanogenic strains and mixed cultures. Their results showed higher metal solubilization with *C. violaceum* and *P. aeruginosa* mixed cultures than individual cultures [106]. However, the quantitative analysis of HCN production by cyanogenic bacteria has not been explained yet, and most studies report qualitative information. However, the amount produced is limited, e.g., a maximum of 20 mg/L with a concentration of lmM of bacterial culture for *C. violaceum[113, 114]*. Therefore, in 2013, Tay et al. stepped towards genetic engineering to enhance the lixiviant production by *C.violaceum* and thereby increase Au recovery [115]. In addition, as reported earlier, Cu presence interferes with the Au cyanidation process. Therefore, Natarajan and Ting applied chemical pretreatment using nitric acid (HNO_3) to remove Cu from e-waste. The pre-treated waste was subjected to bioleaching using mutated *C. violaceum* and enhanced Au recovery compared to wild *C. violaceum* [116]. Numerous studies in chemo-biohydrometallurgy methods involved various chemical treatments before the bioleaching process to address the specificity and low-efficiency rate of the biohydrometallurgy method [117]. In addition, in 2008 another researcher suggested the extent of many cyanogenic bacteria such as *Ralstonia eutropha* MTCC 2487, *Pseudomonas putida* MTCC 1194 and *Bacillus indicus* MTCC 4374 for tolerance of toxic component like As metal that compel the use of these microbial strains for treatment of e-waste [118].

2.6.3.2 Biochemical mechanism of the biocyanidation leaching process

Cyanide is a chemical compound that exhibits a distinctive triple-bonded carbon-nitrogen moiety with +2 oxidation of carbon and -3 of nitrogen [87]. Cyanogenic microorganisms, such as *C. violacium, P. aeruginosa,* and *B. megatherium,* can produce this chemical compound as a secondary metabolite during mid-to-late log growth stages [119, 120]. The enzyme HCN synthase, which is associated with the inner cell membrane, is accountable for the production of cyanide and is also known by the name of glycine dehydrogenase; it is a flavoenzyme that comprises iron cofactors and is encoded in most cyanogenic microorganisms via hcnABC operon [50, 114] (shown in figure 2.5). The HCN synthase enzyme transforms precursor glycine to iminoacetic acid through an “oxidative decarboxylation” reaction and is subsequently converted to HCN. During the process, the HCN synthase enzyme generates four electrons that necessitate oxygen as an electron acceptor and then provides it to the electron transport chain. [121]. The genetic control of the hcnABC operon and the cyanogenesis process is complex, so the biocyanidation process occurs only under specific environmental conditions. The bacterial biocyanidation process is strictly regulated, so cyanide concentration is usually 1 mM, which many living cells tolerate [122].



(2.2)

Under physiological pH 7, CN is mainly in the form of HCN, a volatile gas rapidly diffusing into the environment [123, 124]. The cyanide equilibrium favors the formation of cyanide ions in alkaline conditions (Eq. 4), which forms various metal complexes. Based on the cyanide amount and pH conditions, anionic complexes of Cu are formed in various forms such as Cu $(CN)^{2-}$, Cu $(CN)^{2}$; Cu $(CN)^{3-,}$ and Cu $(CN)^{4-}$. Moreover, the formation of dicyanoaurate anions [Au $(CN)^{2-}$] and others, such as [Pt $(CN)_4]^{2-}$, [Pd $(CN)_4]^{2-}$ and [Ni $(CN)^{2-}$] are stable metal-complexes over a wide range of redox and pH conditions [125-127]. Since the pKa HCN is 9.3, conducting a metal dissolution reaction under alkaline conditions increases its availability for metal solubilization complexation.

$$HCN \longrightarrow H^+ + CN^- \qquad (2.3)$$

$$4Cu + 8CN^- + O_2 + H_2O \longrightarrow 4[Cu\,(CN)_2]^- + 4OH- \qquad (2.4)$$

$$4Au + 8CN^- + O_2 + H_2O \longrightarrow 4\,[Au\,(CN)_2]^- + 4OH^- \qquad (2.5)$$

$$4Ni + 8CN^- + O_2 + H_2O \longrightarrow 4\,[Ni\,(CN)_2\,]^- + 4OH^- \qquad (2.6)$$

2.6.3.4 The classification of bioleaching processes

Microbes utilized in biomining typically exhibit the bioleaching mechanisms mentioned above directly or indirectly, as elaborated upon in the subsequent section.

Microbes used in biomining often display the bioleaching methods in either direct or indirect manners that are described in the following section:

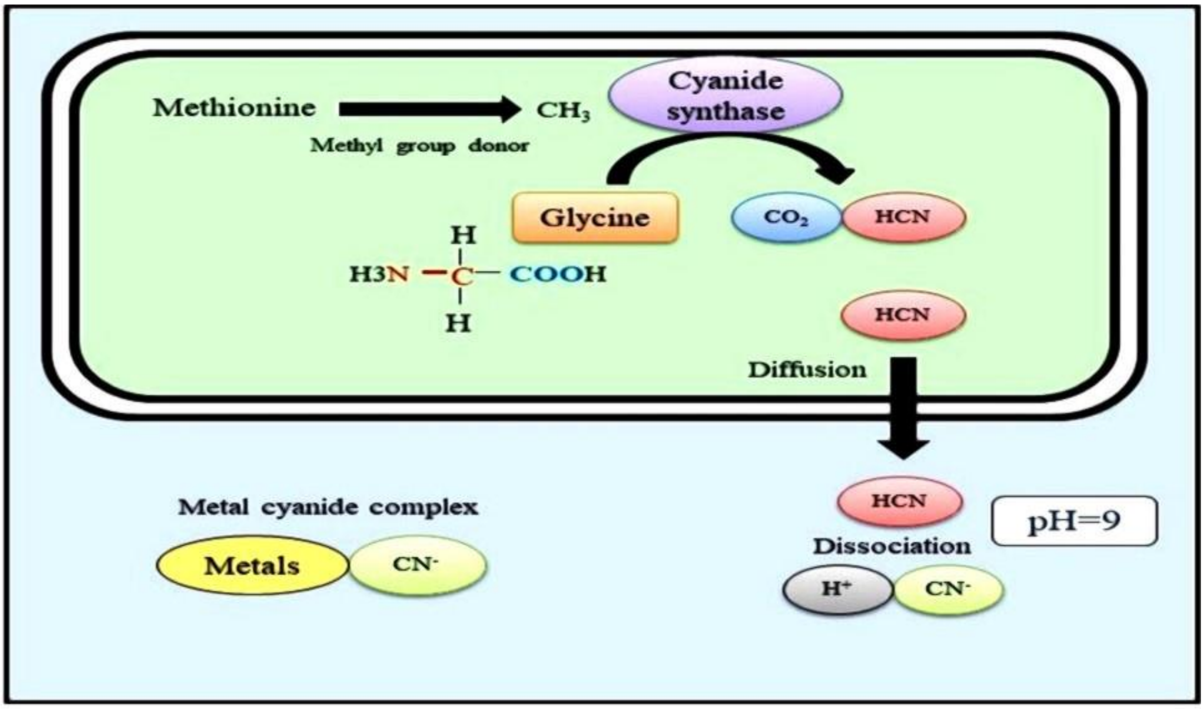


Figure 2.5 Illustration of cyanide production mechanism by HCN synthase enzyme in the cyanogenic microorganism.

(i) Direct leaching

The process of bioleaching that involves the direct contact of microorganisms with the metal ore followed by oxidation of the metallic element through a series of enzymatically catalyzed steps is known as direct bioleaching[96]. The example of a chemical reaction exhibited by chemolithoautotrophic bacteria is illustrated in the equations below [78].

$$4FeS_2+14O_2+4H_2O \xrightarrow{\text{bacteria}} FeSO_4 +4H_2SO_4 \tag{2.7}$$

$$4FeSO_4+O_2+2H_2SO_4 \xrightarrow{\text{bacteria}} 2Fe_2(SO_4)_3+2H_2O \tag{2.8}$$

Based on the interaction between microorganisms and e-waste, two unique approaches were recognized in the direct bioleaching process.

(a) One-step bioleaching

(b) Two-step bioleaching

(a) One-step bioleaching

The basic procedure for one step-bioleaching entails the growth of the microbe and the solubilization of the metals simultaneously. The culture is tolerable to grow aseptically in the presence of e-waste. The growth of microorganisms under optimized conditions is attributed to the production of suitable lixiviant that gradually dissolves the metals from a solid matrix [128]. Equations 2.8-2.11 represent the mechanism of *Acidithiobacillus ferrooxidans* that utilizes iron as an energy source for producing ferric ions as a lixiviant to recover Cu metal from e-waste. The solubilization of other metals, such as Zn, Ni, and Al, through bioleaching depends on their thermodynamic reactions, as indicated in Equations 2.9-2.12 [4]. The complex structure and composition of amalgamated elements, particularly heavy metals present in e-waste, are recognized for hindering the growth of microorganisms. As a result, this leads to a decrease in the rate of metal dissolution [101]. Thus, the one-step bioleaching process imposes certain challenges for efficient leaching of metals at industrial scale implementation.

$$4\,Fe^{2+} + O_2{}^{\text{Fe-Oxidizer}} \longrightarrow 4Fe^{3+} + H_2O \qquad (2.9)$$

$$2\,Fe^{3+} + Cu^{0}2Fe^{2+} + Cu^{2+}\ \Delta G^{0} = -82.9\ \text{kL/mol} \qquad (2.10)$$

$$2\,Fe^{3+} + Zn^{0}2Fe^{2+} + Zn^{2+}\ \Delta G^{0} = -295.4\ \text{kL/mol} \qquad (2.11)$$

$$2\,Fe^{3+} + Ni^{0}2Fe^{2+} + Ni^{2+}\ \Delta G^{0} = -\ 196.6\ \text{kL/mol} \qquad (2.12)$$

(b) Two-step bioleaching

The amalgamation of various toxic constituents in e-waste limits the contact of e-waste during the growth of microorganisms. Hence, two-step bioleaching processes have been proposed to promote the microorganism’s growth and lixiviant production without confronting any hindrance of toxic constituents of e-waste [129, 130]. In two-step bioleaching, potential microbial culture is first grown in a suitable nutrient medium under the influence of suitable growth parameters in the absence of e-waste till the exponential growth period. However, the second step is intended to supplement e-waste for the subsequent dissolution of various metals in the presence of efficient lixiviant. Due to the faster and greater metal solubilization rate, even at higher pulp density, this technique is anticipated to be more efficient and acceptable [131].

(ii) Indirect metal leaching

In an indirect leaching mechanism, the lixiviant produced by bacteria enables the complete dissolution of the metal without subjecting the bacteria to the waste [132]. The lixiviant is accountable for the oxidation of metals as per the phenomenon mentioned in Equations 2.13 and 2.14. Spent medium leaching is one strategy that accounts for the indirect leaching process [133].

$$MeS + Fe_2(SO_4)_3 \longrightarrow MeFeSO_4 + 2FeSO_4 + S^0 \qquad (2.13)$$

$$2S^0 + 3O_2 + 3O_2 + 2H_2O^{\text{ bacteria}} \longrightarrow 2H_2SO_4 \qquad (2.14)$$

(a) Spent medium indirect leaching

This mechanism employs a two-step bioleaching process to facilitate microbial growth in conjunction with lixiviant production, which is succeeded by separating culture from lixiviant through centrifugation [134]. Afterward, the spent medium of lixiviant is supplemented with an e-waste sample for metal solubilization. This method is intended to eliminate the toxicity of PCBs on microbial growth and metabolic activity, which was a significant constraint in the one-step process and prioritized over another method for efficient recovery of gold. In 2018, Wu et al. demonstrated the proficiency of spent medium bioleaching. They reported 93.4% recovery of Cu from 100 g/L PCB pulp density in only 9 days using spent medium leachate of oxidizing bacteria [135]. Various studies have suggested the exploitation of cyanogenic microorganisms in a spent medium leaching approach due to the HCN production ability of these microorganisms to reduce the toxicity of HCN during the bioleaching process [135].

2.6.3.5 Factors that affect the bioleaching potential

Assessing the scientific requirements for maximizing metal recovery involves considering the potential effects of various physical (e.g., temperature, the particle size of e-waste, composition, and potential difference), chemical (e.g., pH, medium), and biological factors (e.g., potential strain, interactions with waste, growth medium). These factors also impact bacterial growth and bioleaching potential [130, 136].

The temperature is a highly pertinent parameter that significantly influences the growth of microorganisms. Numerous studies have found that the ideal temperature range for bacterial growth and bioleaching activity is mesophilic (i.e., 25-40°C). However, the natural inhabitant of the microorganism specifies the ideal temperature for their growth and metabolic activity [137, 138]. Based on the climatic conditions and seasonal fluctuations in India, temperatures typically range from 0°C to 45°C, and these conditions are conducive to the growth of mesophilic and moderate thermophilic microorganisms that corroborate the feasibility of various potential bacterial strains for laboratory-scale studies. Some studies demonstrate efficient bioleaching from moderate to 80°C [139, 140].

The term pulp density (PD) refers to the finely powdered e-waste comprising various metallic and non-metallic constituents for metal dissolution operation. Pulp density is another crucial factor that affects bacterial growth and, therefore, triggers lixiviant production. Numerous studies have demonstrated pulp density optimization and observed that a range within 10 to 15 mg/L is optimal for the efficient recovery of metals. Furthermore, the extraction of metals positively correlates with pulp density, indicating that an increase in pulp density enhances the metal dissolution. However, at a specific threshold, the pulp density is the accomplished suppressive impact on the microbial growth, hindering the nutrient flow and restraining the mass transfer. Therefore, non-contact bioleaching, including the spent medium approach, is favored to address specific challenges caused by higher pulp density [54, 78, 141-143]. Therefore, it's crucial to have an extensive understanding of the ideal relationship between desired metal recovery and pulp density to establish an efficient and proficient bioreactor for large-scale bioleaching operations. In addition, particle size, type, and concentration of metallic constituents in the e-waste sample also significantly affect the bioleaching efficiency of potential bacterial isolates. The findings of Zhu et al. suggested that waste components with small-sized particles are better for efficient metal dissolution [144]; however, Adhapure et al. asserted that large particle size waste is preferable to avoid precipitate contamination and simplify the metals dissolution step [139]. Therefore, the solid-to-liquid ratio (e.g., e-waste sample and growth medium) should be considered when optimizing influential factors.

pH is another crucial element that plays a significant role in the bioleaching process. Since microorganisms exhibit different metabolic activities at different pH, selecting the suitable microbial strain based on the targeted metal is advisable. According to reported studies, acidic pH ranges between 1.0 and 4.0 provide high-efficiency base metal solubilization by various chemolithoautotrophic bacteria such as *Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans, Sulphobacillus sp.* [145]. In addition, fungi such as *Aspergillus niger* forms numerous organic acids at various pH extents. For example, citric acid production is highly favored at lower pH values (≤2), while gluconic acid and oxalic acid are produced at pH values (≥4). Cyanogenic bacteria are most active at alkaline pH, unlike the microorganisms mentioned above. Cyanogens such as *P. aeruginosa, C. violacium, and B. megaterium* produce HCN lixiviant with a pKa value of 9.21; therefore, pH greater than 9 is preferable for significant availability of CN^- ion; lixiviant that is responsible for leaching of various precious metals [78, 142].

The toxic nature of heavy metals and other organic constituents also hinders the efficiency of the bioleaching process. The elevated metal levels, especially Ni, Hg, and Cd metal toxicity, hinder bacterial growth and enzyme function. Thus, achieving a balanced trace element environment is crucial for bioleaching bacteria.[19]. Chen et al. [146]investigated the effect of Ni concentration and found that approximately 600 mM hindered bacterial growth and sulfur reduction ability in *A. thiooxidanss*. While metal toxicity doesn't entirely block acidophilic bacteria growth, it markedly decreases growth at elevated concentrations, as demonstrated by Ramos-Zúñiga et al. [147]. Cadmium's impact was assessed at concentrations ranging from 0 to 200 mM on *A. ferrooxidans* strains at 75, 100, and 200 mM. A notable reduction in growth was observed compared to cells without Cd. These findings align with the investigation from Sampson and Phillips [148], who reported that increasing concentrations of heavy metals inhibited the oxidation ability of mesophilic cultures of *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*. Thus, effectively managing metal toxicity in biomining requires understanding the tolerance threshold, implementing suitable measures, and understanding the evolutionary adaptations of bioleaching microorganisms, which is essential for addressing metal toxicity challenges. Adeleke suggests that gradual sub-culturing of microorganisms before bioleaching enhance their leaching potential. Additionally, bioaugmentation involves supplying essential nutrients support microbial leaching [149].

2.7 Industrialization of biohydrometallurgy technique

Years of research and practice have effectively implemented bioleaching at commercial biomining to recover numerous metals from sulfidic ores[150]. Metal retrieval from low-grade Cu ores and refractory Au ores are the two leading commercial implementations of biohydrometallurgy that account for approximately 20% and 5% of world Cu and Au production, respectively [151, 152]. Although the exploitation of microbial leaching in metal recovery has found successful application in biomining, its application in e-waste processing is still in its infant stages and limited to laboratory-scale investigation, as various challenges need to be addressed to boost its industrial-scale applications [150].

Despite the challenges, several groups have taken the initiative to demonstrate the e-waste bioleaching process at a large scale and commercialize their biohydrometallurgy technique. Some of the examples are mentioned below:

1) Mintek (www.mintek.co.za) is a conventional mining company with extensive experience in gold leaching from ores. “BacoxTM,” the commercial refractory gold bioleaching firm, is now collaborating with BacTech to demonstrate the bioleaching process at the “Beaconsfield Gold Mine” (Tasmania) and the “BioGold treatment facility” (Laizhou) in China [153]. They utilized thermophilic iron-oxidizing bacteria to leach off Au from refractory gold concentrate. The process is accomplished in a massive aerated stirred tank reactor to treat 70 to 100 tonnes of refractory gold concentrates daily [151]. The company is now establishing and refining e-waste treatment procedures for large-scale operations based on its experience in employing a bioleaching process for gold extraction from refractory ores [154].

2) Mint Innovation (www.mint.bio), a metallurgy firm in Auckland, New Zealand, is another example of implementing the bioleaching process at an industrial scale. Mint Innovation utilizes chemo-biohydrometallurgy procedures for the proficient processing of e-waste. E-waste is first processed through chemical lixiviants such as acids and oxidants to leach out the base metals such as Cu, Sn, Al, Fe, and other less valuable metals that are present in higher concentrations than other valuable metals and cause hindrance to their recovery in bioleaching process[155]. The solid e-waste is then subjected to another cycle of bioleaching to obtain gold ions by *Cupriavidus metallidurans*, which absorb the gold ions and solubilize the precious metals. The firm has processed approximately 1 Mt of PCBs per week, which has led to the recovery of about 150 g of Au [151]. The successful deployment of the chemo-biohydrometallurgy approach for PCB treatment prompted the firm to establish a commercial operation with 10 Mt PCB treatment per day [155].

3) N2S (www.n2s.co.uk), an IT asset lifecycle management organization, in 2018, the firm had endeavored to team up with a research group at Coventry University, UK. Collectively, they took the initiative to construct a bioleaching plant for large-scale e-waste treatment. They demonstrated the implementation of bioleaching and direct application electrowinning process for recovery of Cu metal from PCBs bioleachate. The shredded dust of PCBs was treated through the bioleaching potential of *A. ferrooxidans* under optimized conditions, followed by an electrowinning procedure to recover Cu from the bioleachate solution. The recovered Cu foil with a purity (>99%) confirmed the selectivity of the bioleaching and electrowinning method [156].

2.8 The obstacles in biohydrometallurgy technique

Biohydrometallurgy has demonstrated significant advantages over conventional metallurgical processes that prompt its continuous application in the biomining of metals from different ores [157]. However, several disadvantages mentioned in Table 2.5 limit its potential for industrial mining of low-grade ores such as e-waste. Thus, constraints mentioned in the subsequent sections must be addressed at different levels before using this efficient technique on a large scale [158].

2.8.1 Heterogenicity of e-waste constituents

E-waste comprises diverse toxic elements, including hazardous organic compounds such as PBD, PBDE, and BFRs and heavy metals like Pb, Hg, Cr, As, and Cd [159]. The concentration of these components varies from batch to batch, contingent upon the type of WEEE and the specific mechanical processing employed during the initial stages of e-waste processing [160]. In contrast to laboratory-scale investigations, where the toxicity of components is precisely defined and bioleaching parameters are optimized for a particular e-waste source, transitioning from lab scale to industrial scale presents challenges for achieving consistent bioleaching. This challenge arises from the significant impact of different batches of e-waste sources on microbial agents' growth and leaching activity [56, 161, 162]. Consequently, the toxicity of various constituents imposes limitations on the practical application of bioleaching in commercial-scale e-waste recycling.

2.8.2 More-time requirement in comparison to another process

Although biohydrometallurgy has numerous advantages, its prolonged processing time precludes it from competing with other established techniques, such as pyrometallurgy and hydrometallurgy[163]. Hence, the significant advancement in biohydrometallurgy necessitates the comprehensive investigation of the mechanism involved in the bioleaching process. Many researchers substantiated that the bioleaching process may face hindrances due to diverse competing metals forming complexes with the available lixiviant. Such interactions can decelerate the leaching rate of PCBs [158]. Hence, this constraint of e-waste toxicity must be tackled before implementation at an industrial scale [164].

2.8.3 Limitations of molecular toolkits

The limited molecular toolkit for genomic and proteomic approaches to *C. violacium*, *A. ferrooxidans*, and other reported bacteria precludes the engineering of these microbes from increasing their bioleaching capacity. This restriction makes it more difficult for the bioleaching method to be commercialized. In addition to the lack of molecular approaches, preserving some of these bacteria is difficult since they have limited vitality after preservation. The failure of conservation using conventional methods is often attributed to the differences in cell structure and sensitivity to ultra-low temperatures and desiccation processes observed in acidophilic thermophilic microorganisms [165-167]. Thus, these microorganisms need to be kept alive and therefore necessitate constant sub-culturing, storage at temperatures below optimal growth conditions, and application of slow-release substrates to extend the viability of the microbe. So, in addition to discovering new microbes for bioremediation, efforts must be made to establish strategies for the long-term survival of these microbes in the presence of toxic e-waste components [151].

2.8.4 Challenges to up-scale the recycling process

The progression of the bioleaching process, from successful lab-scale invention to pilot-scale demonstration and then to economically viable commercialization, is marked with various challenges. Studies suggest that, during the bioleaching scale-up, laboratory-scale kinetics may be more effective than field-scale kinetics, and the time requirement is more predictable than in industrial-scale operation [168, 169]. In addition to the unanticipated, complex nature of microorganisms, various physical and chemical conditions of the reaction setup, such as pH, humidity, and nutrition, also affect the bioleaching potential of microorganisms, and these conditions are frequently altered during the transition from laboratory-scale systems to field-scale bioleaching designs[170]. Therefore, heterogeneity in the e-waste, additional biomass and nutritional supplementation, and changes in the operational conditions make the scale-up of bioleaching processes challenging [171].

2.9 Future-road map of biohydrometallurgy technique in e-waste management

Despite the considerable difficulties involved in e-waste recycling, efforts toward attaining a zero-waste circular economy necessitate the feasible implementation of e-waste bioremediation and resource extraction at industrial scale operation[172]. Hence, it is crucial to identify and address the limitations in our current knowledge and methodologies to overcome the obstacles associated with biological approaches to e-waste treatment. A primary area requiring extensive research is the augmentation of the biological repertoire capable of facilitating the enzymatic remediation of e-waste. High-throughput screening of sizable gene pools responsible for proficient functional activities has been a possible way to propel the bioleaching process in a core automation prospect. Although it requires substantial investment in specific assets and skills, automation facilitates collaboration between companies and scientific communities equipped with high-throughput bio-foundries, thus accelerating the screening process. In addition, the urgency lies in improving the effectiveness and speed of the bioremediation process. Enzyme activity and toxin tolerance are critical factors that influence the efficacy of the e-waste bioremediation process. In the future, the use of biologically engineered microorganisms is likely necessary to achieve significant enhancements in enzymatic performance [173]. In this instance, the "Chinese-European MIX-UP" project is intended to develop a unique approach to use enzyme cocktails of engineered microbial strains to recycle plastic constituents of e-waste.

Moreover, by applying the "design, build, test, and learn" approach, synthetic biology allows the systematic and iterative creation of engineered microbes with feedback loop analysis [174]. Developing *in-vitro* mutant libraries with desired phenotypes relies on toolkits composed of standard genetic components for constructing transcriptional units and operons [175-177]. Molecular toolkits can improve the bioremediation capabilities of unconventional bacteria, such as using the well-established “BioBrick” system for model organisms in the remediation of metals like Au, Co, Hg, and Ni [178, 179]. However, screening extensive mutant libraries, where bio-foundries play a vital role, is crucial. Allonnia, a spin-off of the biotech company “Ginkgo Bioworks,” has initiated synthetic biology to develop microbes that effectively eliminate organic toxins from e-waste [180]. Additionally, advancements in computer-aided protein engineering and droplet-based microfluidics can enhance the production of valuable enzymes, further expediting the efforts in engineering bacterial bioremediation capacities [181].

Furthermore, recent research has highlighted the need to move away from relying solely on a single biological approach for remediating complex e-waste, regardless of its efficacy. Therefore, new investigations have redirected their focus toward addressing the heterogeneous nature of e-waste (metals or plastics) by harnessing the power of microbial communities [182]. A viable strategy for improving the consistency of bioremediation efficacy for e-waste treatment entails segregating waste according to its composition to promote its homogeneity and uniformity of material and minimize the impact of interfering components on bioremediation efficacy [183]. In addition, a pre-treatment step before the bioremediation through a microbial system can augment the biodegradation rate. Moreover, another potential strategy is utilizing a two-step bioleaching process, reported by many researchers, wherein biomass is allowed to grow initially, followed by the introduction PCBs. This approach facilitates the recovery of these valuable metals from PCBs [130].

It is imperative to consider the various factors involved in implementing advanced technology for e-waste recycling.

(1) Achieving optimal bioremediation efficacy and resource recovery rates should be balanced with sustainability in terms of cost and ecological impact. It is crucial to develop bioremediation workflows that are both efficient and environmentally friendly.

(2) To facilitate widespread adoption and commercialization, the technology employed should be feasible for implementation by small and medium-sized enterprises in terms of investment and infrastructure requirements. The scientific community should conduct pilot-scale tests to evaluate operational procedures and costs.

(3) Recognizing the complexity of e-waste, it is unlikely that a single bioremediation technology can accomplish complete recycling. Therefore, stakeholders should remain open to integrating multiple recycling technologies, including physicochemical treatments, to achieve comprehensive remediation of e-waste.

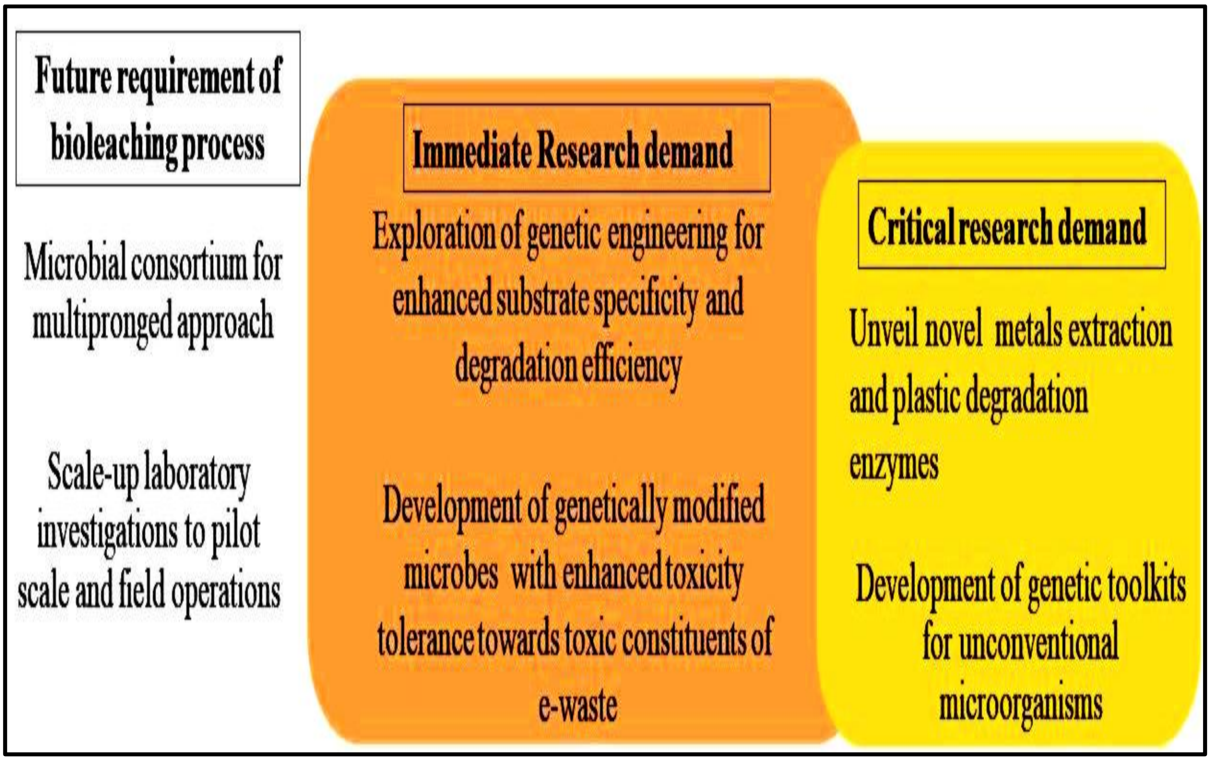


Figure 2.6 A comprehensive roadmap to overcome the existing limitations of the biohydrometallurgical technique to enhance its efficiency for the proficient management of e-waste through the bioleaching process.

2.10 An overview of e-waste management in India

India faces significant challenges in managing the entire value chain of e-waste, primarily due to the lack of suitable treatment methods. The country is confronted with 95% of e-waste being informally recycled in backyard operations [184-187]. As a result, the remaining 5% of e-waste is outsourced by formal recyclers in collaboration with IT companies such as Tata Consultancy Services (TCS), WIPRO, HP, Infosys, and others. The cost of outsourcing this e-waste ranges from USD 0.13 to USD 0.20/kg (INR. 10–15/kg). Once processed at the recycling plants, the e-waste is sold to traders or manufacturers at prices approximately ranging from 0.10 to 0.13 USD/kg (INR. 8–10/kg) for shredded plastics, 0.18 to 0.29 USD/kg (INR. 14–22/kg) for scrap metallic parts, 0.91 to 1.04 USD/kg (INR. 70–80/kg) for aluminum parts, and 0.013 to 0.26 USD/kg (INR. 1–2/kg) for Cathode Ray Tube (CRT) glass. The reported profit margin in this business is approximately 25-30%. Therefore, there is considerable potential for enhancing effective management and treatment capacities [50, 188].

The entire e-waste value chain, including collection, storage, transportation, and recycling, lacks proper regulation in India. In response to the escalating e-waste problem, the E-waste (Management & Handling) Rules were introduced in 2011, and subsequent modifications were made in 2016 and 2018 by The Ministry of Environment Forestry and Climate Change (MoEf&CC) [40, 46]. These modifications focused on strengthening the rules and expanding the roles of electronic goods producers, manufacturers, and consumers to include e-waste management components. The primary objectives of these rules are to facilitate e-waste treatment through sustainable and eco-friendly practices, circular resource management, applying the Polluter's Pays Principle (PPP), Extended Producer Responsibility (EPR), and creating public awareness for active participation. Several NGOs, including Toxic Link, Center for Science and Environment (CSE), Chintan, Environment Action Research Program, and ULBs, are collaborating with the public to develop systematic plans and layouts for effective and sustainable e-waste management [20, 46, 71, 189]. However, progress in this regard has been slow and requires immediate attention.

Due to the informal recycling of a significant portion of e-waste in India, valuable resources are lost, and the crude recycling methods employed expose heavy metal contamination that leads to adverse effects on health and environmental footprints. Moreover, bioleaching of e-waste, particularly computer waste, is limited and in its early stages [43, 71, 189, 190]. Therefore, urgent attention is needed to improve the bioleaching potential for maximum resource recovery from computer waste, which contains significant amounts of Cu, Au, and Ag [191]. Thus, exploration and implementation of the abovementioned road map for the bioleaching process at a larger scale operation can provide opportunities for significant resource recovery, contributing to the nation's economic growth

CHAPTER 3

MATERIALS
AND
METHODS

MATERIALS AND METHODS

3.1Materials

3.1.1 Glassware

The glass wares utilized, such as beakers, conical flasks, measuring cylinders, and standard volumetric flasks, were acquired from Borosil (India). Pipettes, tips, and Eppendorf tubes were attained from Eppendorf (Germany) and Tarsons (India). To avoid contamination, we thoroughly cleaned and sterilized each piece of glassware through an autoclave before using it for experimentation

3.1.2 Chemicals

All the cultural growth media, such as Nutrient Broth (NB) and Nutrient Agar (NA), were procured from Hi-media (India). Analytical grade chemicals, reagents, and standards used for experimentations were supplied from Sigma-Aldrich (USA), Merck (Germany), and Fluka (Switzerland). Molecular grade chemicals and products like the Bogar Bio Bee Pvt DNA extraction kit. Ltd (India), PCR master mix, primers, nuclease-free water, and DNA ladder were procured from Promega (USA) and Thermo-Scientific (USA). The chemicals were appropriately sterilized using autoclave and filter sterilization procedures.

3.1.3 Microbial cultures

Microorganisms were isolated from metal-contaminated soil samples using the enrichment culture method. In addition, *Pseudomonas balearica* SAE1 was obtained from “The Environmental Biotechnology Laboratory” of ‘The Department of Biotechnology and Bioinformatics,’ “The Jaypee University of Information Technology,” Waknaghat, Solan. Bacterial cultures were kept in glycerol stocks (final concentration 25%) at -80^{o}C for extended periods, whereas, for routine usage, nutrient agar slants were prepared and kept at 4°C. All microbiological processes, such as inoculation, sampling, and spreading, were performed under aseptic conditions in a laminar airflow (LAF) chamber.

3.1.4 E-waste procurement

The e-waste sample utilized in the current study is shredded dust of waste computer printed circuit boards (CPCBs) generated during the physio-mechanical processing of WEEEs. The model was obtained from the Exigo Recycling Pvt. Ltd stockroom. Panipat, Haryana, India (ExigoRecycling.com) and stored in zipper storage bags. The firm collects abandoned electronic equipment from various states/regions of India and subjects it to several physico-mechanical processes such as segregation, disassembly, shredding, and pulverization before metal extraction. The procured e-waste sample was utilized to determine its mineralogical and morphological features and extraction of various metals by potent bacterial strain at "The Jaypee University of Information Technology," Waknaghat, Solan, India. Before analysis, the powdered CPCBs were sterilized in autoclavable bags (HiMedia) at 121°C and 15 psi for 25 mins and dried at room temperature. After the bioleaching process, the leftover residue was further sterilized and kept in the storage bag to be transferred to the recycling unit for subsequent disposal processing at their treatment, storage, and disposal facility (TSDF).

3.2 Characterization of shredded dust waste CPCBs

PCB is the core component of all electronic devices, comprising various metallic and non-metallic elements, including 305 different polymers, 30% refractory oxides, and 40% metals [112]. The distribution of these components in PCB e-waste is influenced by WEEEs physico-mechanical processing, which renders the heterogeneity and complexity of e-waste. Therefore, determining the constituents, form, and toxicity of e-waste is vital for its management and processing. The present study aims to assess the mineralogical and morphological characterization of shredded dust CPCB e-waste, which is described in the following section:

3.2.1 Metallic content analysis

The standard aqua regia digestion method was employed to evaluate the metallic constituents of shredded dust CPCBs. A 100ml aqua regia ($HCl: HNO_3 = 3:1$) solution containing 1% w/v PCBs powder was heated at 100^oC for 1 hour. The solution was allowed to cool down, followed by filtration through Whatman filter paper before 0.45µm glass fiber filters (PALL-GF-A/E-I) [78, 136, 192]. The volume of the solution lost during digestion was further attained with sterile distilled water. The metals dissolved in particle-free leachate were determined using an "atomic absorption spectrophotometer" ("AAS, AAnalyst400, Perkin Elmer") at subsequent wavelengths (nm): 324.8 (Cu), 248.3(Fe), 232.0 (Ni), 213.9(Zn), Ag 328.1 (Ag), 242.8 (Au), 240.7 (Co).

3.2.2 Morphological and mineralogical analysis

Scanning Electron Microscopy and Energy Dispersive X-ray spectroscopy (SEM-EDS) were utilized to assess the morphology and mineralogy of CPCB e-waste samples. The sample was sterilized at 121°C and 15 psi for 25 mins, followed by heat drying at 50°C [113, 193]. The SEM-EDX (JEOL-SEM IT 300) analysis was done at the Indian Institute of Science (IISc) Bengaluru. The sample was further dried and mounted on an adhesive carbon tube, followed by coating it with a thin gold film to strengthen the high-resolution images. The sample was scanned at various magnification powers to elucidate the surface topography of CPCB e-waste. Subsequently, EDS was performed through X-ray illumination to reveal the elemental concentration of PCBs.

3.2.3 Elemental core analysis

X-ray diffraction (XRD) investigation at Himachal Pradesh University (HPU), Shimla, assessed the elemental intensity and core structure of CPCBs. The desiccated fine CPCB sample was placed in the interior core of the matrix and scanned along a peak range of 10^o-90^o at 2θ value through an X'PRT-PR Diffractometer. The peaks obtained through the diffractometer were interpreted using the "Joint Committee Powder Diffraction Standards" (JCPDS) statistical technique.

3.3 Exploration of potent bacterial strains

Rational of the objective

Several microorganisms have been reported for their bioleaching application. However, some limitations include time consumption, low toxicity tolerance, leaching rate, and selectivity [2, 194]. Thus, researchers are prompted to search for potential microorganisms that reside in various metal-inhabited sites that enable them to withstand elevated levels of toxic constituents found in CPCBs and are anticipated to exhibit higher leaching efficacy. Therefore, the current study intended to unveil potential autochthonous microbial strains from the metals contaminated site that can reveal more toxicity tolerance and overcome the specificity constraints of previously described microorganisms.

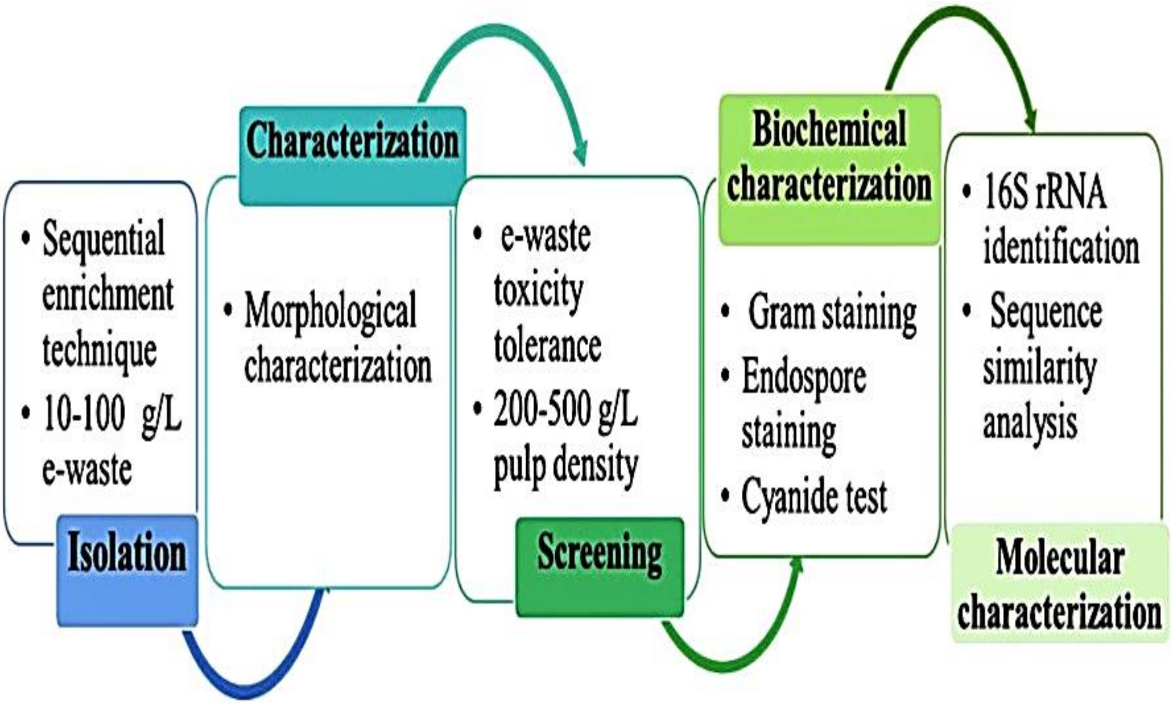


Figure 3.1 Diagrammatic representation of strategy for isolating potential bacterial isolates from metal-contaminated soil.

3.3.1 Site selection and soil sample collection

The autochthonous microorganism that resides in metal existence sites possesses higher adaptability towards the toxic constituents of waste CPCBs as they have the innate metabolism towards toxicity of various metals. Therefore, in the present study, we explored the microbial community from metal-enriched areas owned to landfilling numerous automobiles, which serve as a source of innumerable metals such as Cu, Al, Fe, Cd, and Zn. The site was located in the Tara Devi region (31o04’53.9” N and 77o10’49.0” E), 5 km from Shimla city of Himachal Pradesh (illustrated in Figure 3.2). The topsoil (i.e., 0-15cm layer) was collected in sterile zipper storage bags and kept in the laboratory at a temperature of 4°C until further processing was conducted.

Before using the soil sample to isolate bacterial communities, metal contamination was assessed through the aqua-regia digestion method per the protocol mentioned in section 3.2.1.

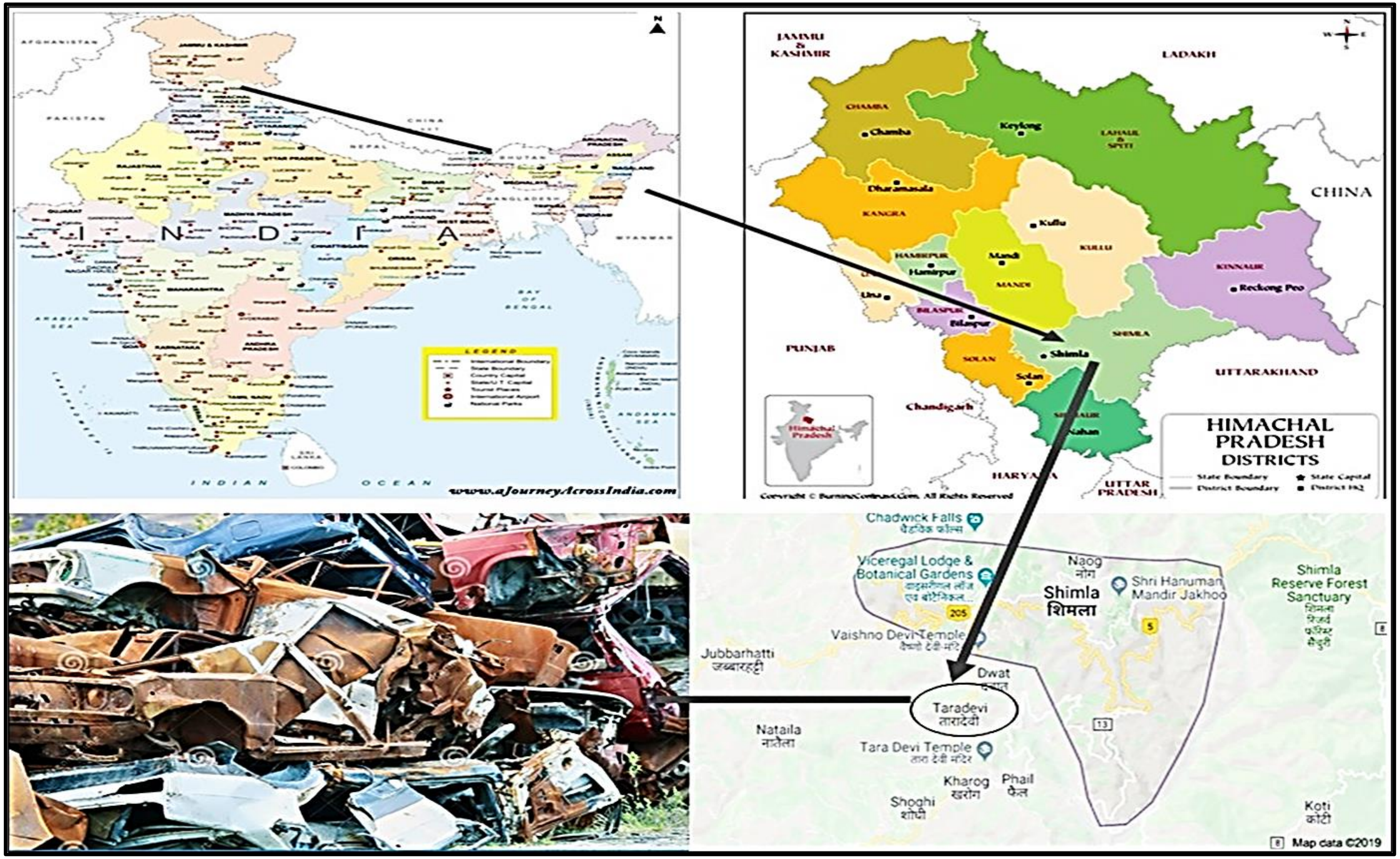


Figure 3.2 Location of metal-enriched soil sample source: a Shimla, Himachal Pradesh vehicle dump yard.

3.3.2 Sequential enrichment technique to obtain potential bacterial isolates

A progressive enrichment strategy was implemented to isolate potential bacterial isolates from the metal-abundant site. The experiment was conducted in a 250 mL non-baffled canonical flask. One gram of unsterile soil sample was incorporated into 100mL of NB medium and incubated for three days at 37°C and 150 rpm in a shaking incubator (Thermo Scientific MAXQ800). Following 3 days, 1mL sample was inoculated into fresh sterile 100mL NB medium enriched with 10g/L sterile waste CPCBs e-waste sample as shown in Figure 3.3 and proceeded for 3 days incubation under similar incubation conditions. Subsequently, 1mL culture was transferred to successively e-waste (i.e., 25, 50,75, 100 g/L) enriched nutrient medium and kept at similar incubation conditions (i.e., temperature-37°C, rpm-150). This persistent sequential enrichment screening of potential bacterial isolates was demonstrated up to 100g/L P.D. for 15 days. After that, a 1ml sample was obtained from a 100 g/L P.D. flask and serially diluted to enumerate the bacterial colonies on the NA medium via the spread plate method. The isolated bacterial strains were sub-cultured repeatedly to obtain a pure culture. Another flask supplemented with 1% w/v sterilized CPCBs without soil sample was kept as a control [13, 195].

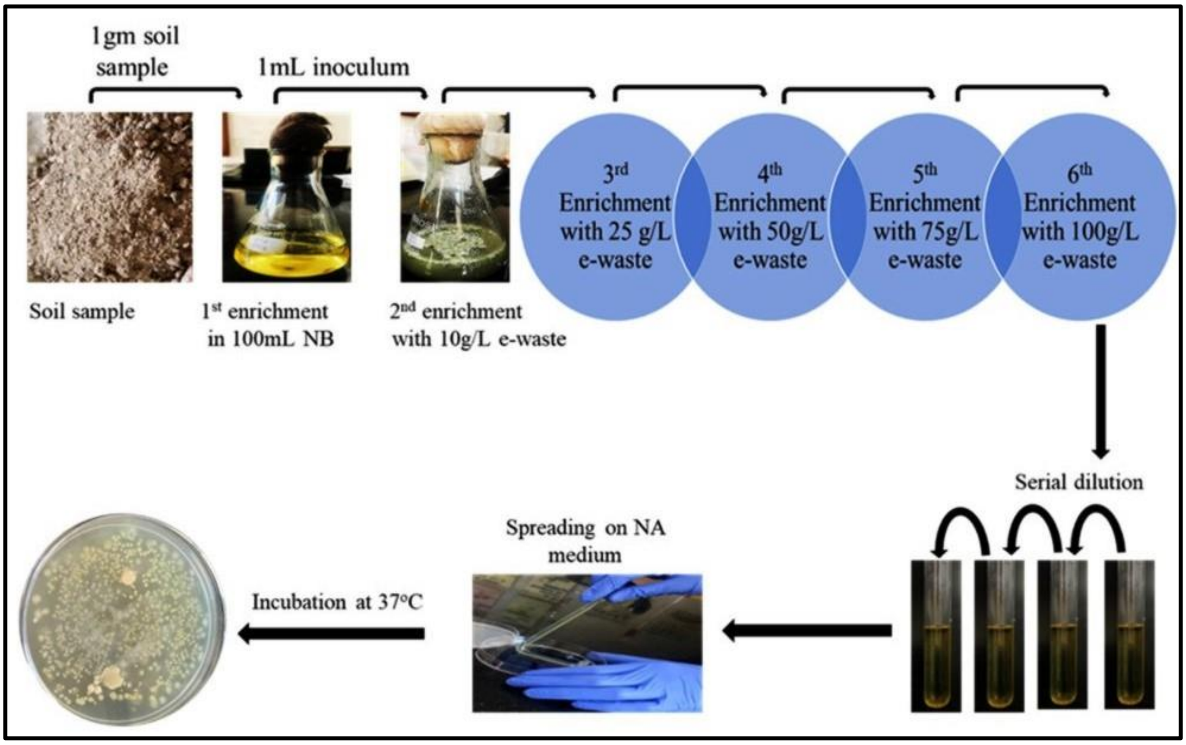


Figure 3.3 Illustration of sequential enrichment approach to isolate potent bacterial isolates from a metal-enriched soil sample.

3.4 Evaluation of toxicity tolerance toward shredded dust waste CPCBs

To ensure the sustainability and effectiveness of the bioleaching process, the investigation of e-waste toxicity tolerance of isolated bacterial strains is a critical parameter, as this reveals the ability of bacterial strains to thrive and produce lixiviants in the presence of various toxic metals. Hence, the study was intended to evaluate the toxicity tolerance of the isolated bacterial strain and "*P. balearica*SAE1" toward the shredded dust of PCB e-waste.

3.4.1 Assessment of toxicity tolerance

In analyzing the toxicity tolerance of potential bacterial strains, we adhered to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The plate count approach was utilized to evaluate the toxicity tolerance towards CPCBs at PD ranging from 200-500g/L at the difference of 50g/L (shown in Figure 3.4). The investigation was carried out in duplicates in 250 mL "non-baffled conical" flasks. Each strain's 1% v/v bacterial inoculum (approximately 2×10^6 CFU/mL) was incorporated into respective sterilized P.D. in 100mL of NB media. A separate medium flask inoculated with a similar volume of each inoculum without a CPCBs sample was run as a control set. The experimental flasks were incubated at 37°C for 24 hours, followed by the enumeration of cells counting as per the formula mentioned in equation 3.1, through serial dilution of each sample and spreading 10µL each sample on NA medium [196].

$$\mathrm{CFU/ml} = \frac{\text{No.of Colonies x Dilution Factor}}{\text{Culture volume spread on nutrient media}} \qquad (3.1)$$

3.4.2 Dose-response analysis

A dose-response analysis divulges the 50% effective concentration (EC_{50}) of e-waste permissible for bacterial growth and metals bioleaching. The EC50 is a statistically derived estimation representing the concentration of e-waste sample required to inhibit 50% of the microbial population during a specified time interval. Thus, cells enumeration (CFU/mL) obtained through toxicity assessment was plotted to create a dose-response curve to evaluate the percentage inhibition response (%IR) with respective pulp density as per the following formula:

$$\%\,\mathrm{IR} = \frac{\text{control} - \text{test}}{\text{control}} \times 100 \qquad (3.2)$$

*'%*IR' means the percentage 'inhibition response'

'Control' refers to bacterial growth without a CPCB e-waste sample.
'Test' means bacterial feasible growth in the presence of CPCBs e-waste sample.

3.5 Characterization and identification of the potential bacterial isolate

3.5.1 Assessment of morphological characteristics

The potential bacterial isolate screened based on toxicity tolerances (ISO1) was initially assessed for its physical features on NA medium, such as distinctive shape, size, margin, elevation, consistency, opacity, and pigmentation. Later, bacterial strains were morphologically distinguished through staining using standard Gram staining and Schaeffer-Fulton's endospore staining procedure (mentioned in Appendix B). Furthermore, the biochemical characterization was done using the KOH string test, methyl red assay, Simonson citrate agar test, hemolytic assay, cyanide assays, and hydrogen peroxide catalase assay (procedure mentioned in Appendix B).

3.5.2 Genomic DNA Extraction

The potential bacterial isolate 'ISO1' was grown in an NB medium for 24 h, followed by cell harvesting for genomic DNA extraction. The Microbial DNA isolation kit was employed to perform the DNA extraction (Bogar Bio Bee Pvt Ltd.) per the manufacturer's protocol. The DNA concentration was measured by spectrophotometer through Qubit fluorometer 3.0 and 1% agarose gel electrophoresis.

3.5.3 16S rRNA Gene Amplification and Sequencing

The universal primers 27 F (5' AGAGTTTGATCTGGCTCAG 3') and 1492 R (5' TACGGTACCTTGTTACGACTT 3') were utilized to amplify the 16S rRNA gene. 25 µL of the PCR reaction solution was prepared by adding 5 µL of isolated DNA to1.5 µL volume of each primer (i.e., forward and reverse), deionized water (5 µL), and Taq master mix (12 µL). The PCR machine (Thermocycler) was employed to amplify the gene under the thermal cycling conditions mentioned in Table 3.1.

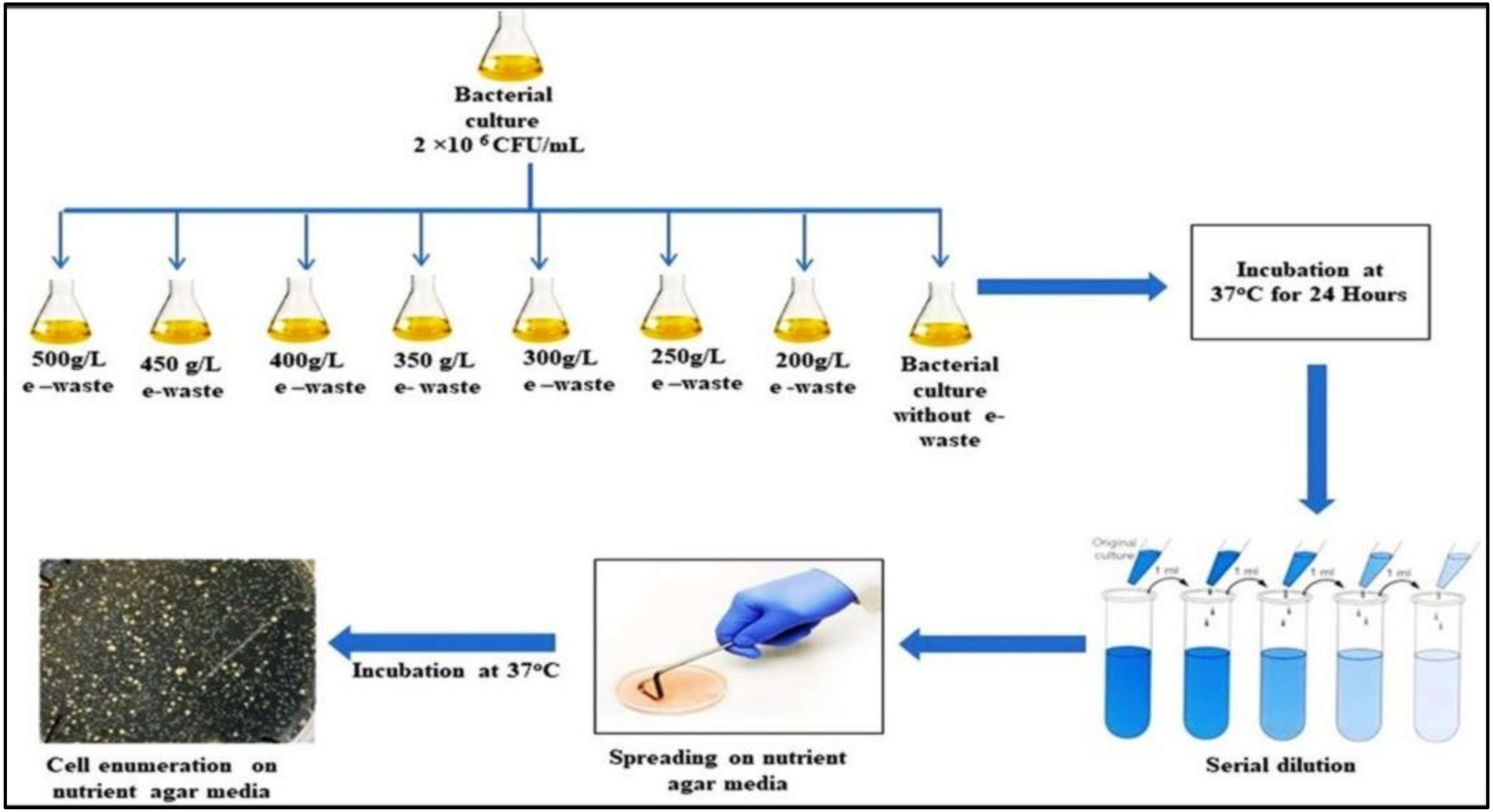


Figure 3.4 Illustration of methodology opted for assessment of bacterial toxicity tolerance towards constituents present in shredded dust CPCBs - waste.

Afterward, the single pass sequencing process was employed to attain the base pair sequence of PCR amplified 16S rRNA gene through ABI3730xl sequencer (Applied Biosystems) at "Yaazhxenomic Pvt. Ltd.", Coimbatore, Tamil Nadu, India. Furthermore, the approximately 1354 bp sequence obtained was subjected to comparison with the "GenBank" database via the NCBI BLAST sequence similarity search tool. Subsequently, the multiple sequence alignment and phylogenetic analysis were done through the MUSCLE 3.7 PhyML 3.0 program tool, respectively.

Table 3.1 PCR condition for 16S rRNA gene amplification.

Stages	**Temperature**	**Time**	25 cycles
Initial Denaturation	95^{o}C	2 min	
Denaturation	95^{o}C	30 sec	
Annealing	50^{o}C	30 sec	
Extension	72^{o}C	2 min	
Final Extension	72^{o}C	10 min	
Hold	4^{o}C	∞	

3.6 Factors that influence bacterial growth and lixiviant production for maximum metals solubilization

Rational of the Objective

Numerous factors influence bacterial growth and their metal's leaching efficiency. The optimization of these factors is a critical step in achieving maximum biocyanidation and metal solubilization in any industrial production process because a minor difference in these factors may have a significant impact on bioleaching efficiency [138, 197]. Hence, following earlier reported studies, vital factors such as temperature, pH, pulp density, selection of suitable precursor and its concentration, and incubation period were selected to optimize for maximum lixiviant production by potential bacterial strain. The conventional OFAT and statistical RSM approaches were used to evaluate the optimization process for various factors in metals solubilization via a two-step bioleaching method. In addition to factors optimization, the effect of various precursors (amino acids) was also assessed for the maximum biocyanidation process.

3.6.1 Selection of suitable precursor for the biocyanidation process

Glycine has formerly been recognized as the primary precursor for cyanide production by various cyanogenic bacteria [97, 109]. The current study investigated the impact of different amino acids on the formation of biogenic cyanide and metal dissolution. The 5g/L concentration of various amino acids, such as histidine, tyrosine, asparagine, alanine, cysteine, phenylalanine, leucine, glycine, lysine, and methionine, were added to NB medium and adjusted to pH 9. Each sterilized medium was inoculated with 4% v/v (about 4×10^8 CFU/mL) culture inoculum and incubated at 37^oC for 48 hours before adding 1% w/v pulp density into each flask for Cu solubilization. Every day, 2 mL of leaching solution from each flask was retrieved to analyze pH change and bacterial growth. Another flask with comparable nutritional conditions except amino acid supplementation was run as a control.

3.6.2 One-Factor-at-a-Time (OFAT) approach to assess the effect of various parameters

This approach considers one variable at a time, and the maximum value is employed in subsequent phases. The investigation was started with the growth of 4% (v/v) cultural inoculum at 37^oC, 150 rpm for 48 hours. The temperature was the first variable to be optimized, ranging from 25^oC to 40^oC with a difference of 5^oC (i.e., 25, 30, 35, 40). Afterward, the optimized temperature was kept constant while the medium's pH was changed from 6-9 (i.e., 7, 8, 9). Following that, the glycine precursor concentration was considered in the 1-10g/L range. Subsequently, pulp density was considered from 10g/L to 200g/L in the interval of 50, such as 10, 50, 100,10 and 200 g/L. The incubation period required for metals solubilization was assessed from 1-8 days in a two-step bioleaching method [195, 198]. Each optimization study was carried out in duplicate, with nutritional media at similar conditions, excluding culture inoculum as a controlled set. In addition, an NB medium was run as a control set for precursor optimization without supplementing the glycine precursor.

3.6.3 Response Surface Methodology (RSM): a statistical approach for parameters optimization

The significant parameters that exhibited maximum influence on bioleaching activity were selected from OFAT and subjected to the RSM statistical approach to evaluate the synergetic effect of these variables on the biocyanidation and metals' solubilization potential of bacteria at different levels. Three distinct levels -α, 0, +α. (i.e., lower, mid, and higher level); each factor (enlisted in Table 3.2) was chosen to design the experimental sets in the central composite design (CCD) system through the design of experiment (DOE) in Minitab®18 software. The program developed 31 experimental sets through two-level factorial design. One experiment set from the 31 was generated six times. Therefore, 25 sets were comprehensively evaluated experimentally with an inoculum size of 4% v/v in 100 mL of NB media.

3.6.4 Model validation

The regression equation with coefficient of determination (R2) and correlation coefficient (altered R2) was applied to evaluate the model's lack of fit and sufficiency. Moreover, Analysis of Variance (ANOVA) was utilized to assess the individual, square, and interaction n effects of the various experimental sets, with a P-value <0.005 at the 95% confidence level. After that, a 3D surface plot was formed to illustrate the bioleaching capacity of bacterial strains in terms of Cu and Ag solubilization rates.

Table 3.2 Levels of specified parameters for constructing Central Composite Design (CCD).

Variables	**Units**	**Range and Level**		
		Low Level (-1)	**Mid-Level (0)**	**High Level (+1)**
A: Temperature	ºC	34	37	40
B: pH	-	8	9	10
C: Glycine concentration	g/L	2.5	5	7.5
D: Pulp density	g/L	10	55	100

3.7 Development of methods to enhance the biocyanidation and metals solubilization potential of bacterial isolates

3.7.1 Chemo-biohydrometallurgy approach to enhance the leaching efficiency

The inadequate leaching rate and selectivity towards specific metals limitations impend the utilization of the bioleaching process for industrial scale implementation. Thus, the objective was designed to unveil approaches that can increase the leaching efficiency of the reported bacterial strain to elevate the bioleaching process for industrial-scale operation.

Rational of the Objective

A single bioleaching process is inadequate for effective metal recovery in large-scale operations**.** The higher concentration of base metals causes impediments to recovering other precious metals since most of the cyanide is consumed during the dissolution of base metals, particularly Cu, the most prominent metal in CPCBs. The lower standard electrode potential of these base metals (E^0Au = 1.83 V, E^0 Ni = 0.67 V, E^0 Fe = 0.44 V, E^0 Cu = 0.34 V) facilitates significantly faster and more stable cyanide complexes than Au [43, 50]. Thus, pre-treatment of e-waste before the bioleaching process can mitigate the specificity limitation and facilitate the leaching of metals other than Cu. The chemo-biohydrometallurgy process is a manifested alternative that employs chemical lixiviant to extract Cu before biological treatment for other metals [199]. The sequential leaching method used in the current study is described in the following section.

3.7.1.1 Assessment of ferric chloride lixiviant and incubation time for maximum recovery of Cu metal

Before the bioleaching process, ferric chloride ($FeCl_3$), a chemical lixiviant, was utilized for the pretreatment of CPCBs for Cu extraction. The experiment was carried out in triplicates in a 250 mL non-baffled canonical flask containing 100 mL $FeCl_3$ solution per the protocol illustrated in Figure 3.5. A sterilized pulp density of 10g/L was added to various concentrations of $FeCl_3$ solution (i.e., 5, 10, and 20g/L). The flasks were incubated in an orbital shaker at a temperature of 50°C and an agitation speed of 150 rpm for different time intervals (1, 2, and 3 hours). After incubation, the leachate was filtered using Whatman filter paper and 0.45μm glass fiber filters to ensure particle-free leachate and examined for Cu extraction. In addition, the e-waste pellet was washed with distilled water to eliminate chemical lixiviant, followed by sterilization at 121°C and 15psi for 15 mins.

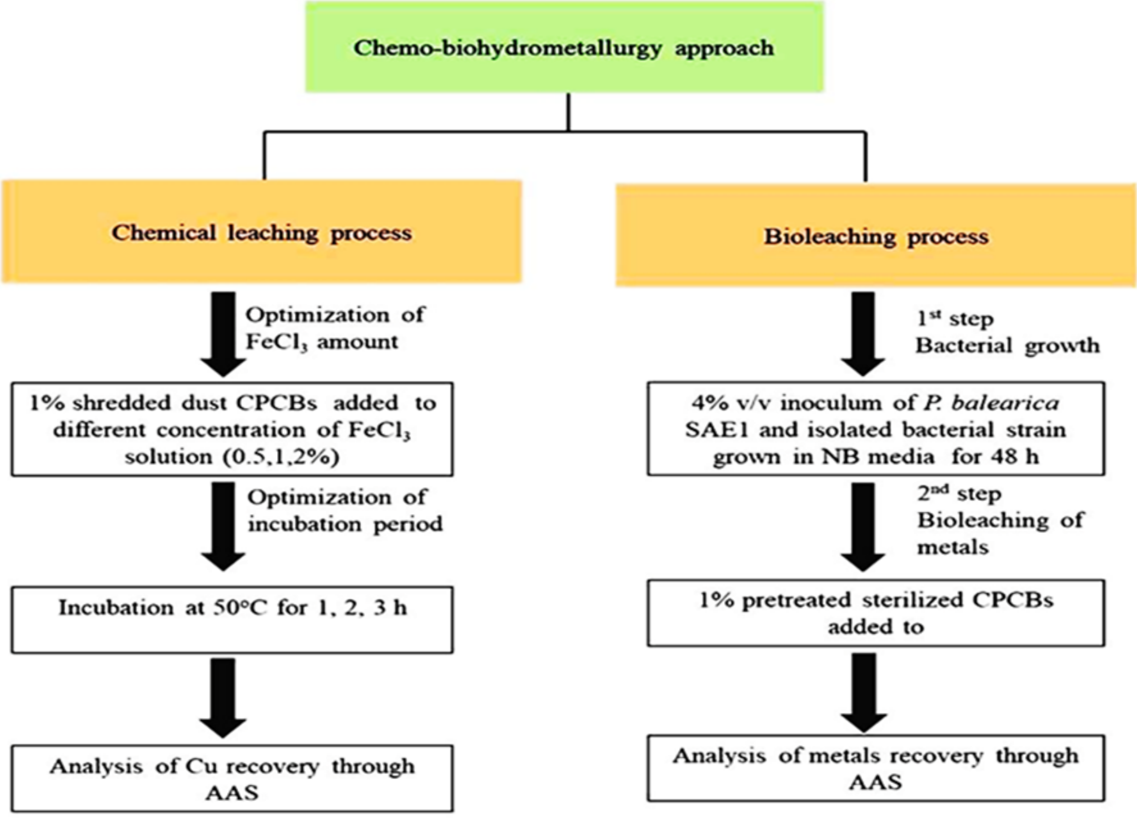


Figure 3.5 Illustrative representation of the steps involved in the chemical and biological treatment of CPCBs.

3.7.1.2 Two-step bioleaching for extraction of metals other than Cu

The sterilized e-waste was subjected to a two-step bioleaching process by *P. balearica*SAE1 and isolated bacterial strain for further metal extraction. In a two-step bioleaching process, 4% v/v (4×10^8 CFU/mL) inoculum of isolated bacterial strain and *P. balearica*SAE1 were grown in 100mL of NB media for 48 hours (OD_{600}= >0.8) at temperature 40°C and 37°C respectively, in the absence of CPCBs pulp density. Then, 1% w/v pre-treated sterilized shredded dust CPCBs was added to each flask and kept at a similar incubation condition for 8 days to allow metals dissolution. After the bioleaching process, waste CPCBs particles and cell biomass were removed from the bioleaching solution using Whatman filter paper and centrifugation (Eppendorf Centrifuge 5804 R) at 7000 rpm for 10 mins. The supernatant was filtered using 0.45m glass fiber filters to ensure particle-free leachate. Then, the solution was investigated for the dissolution of precious metals using AAS. The bioleaching capacity of two cyanogenic strains was compared to select the dominant one to implement the biocyanidation process at an increased volume of bioleaching solution. The bioleaching control set was run with equal volumes of nutrient medium and P.D. without inoculating the bacterial culture.

3.7.2 Effect of methionine stimulant on bioleaching potential of isolated bacterial strain

Amino acid is an essential nutritional component vital to bacteria's growth and physiological functions [200]. Hence, the influence of methionine amino acid on bacterial growth and biocyanide synthesis was assessed. With glycine as a primary precursor, methionine concentration was optimized to stimulate the cyanogenesis process and metals solubilization potential of potent bacterial strain through a two-step bioleaching process.

The different amount of methionine additive (i.e., 0.5, 1, 5 g/L) was added to NB medium, which was supplemented with an optimized concentration of glycine precursor (i.e., 5g/L) followed by pH adjustment to 9 and sterilization at 121^{o}C for 15 mins. Subsequently, 4% v/v cultural inoculum was introduced to each flask and incubated at optimal growth conditions such as 40^{o}C and 150 rpm until the OD value reached >0.8 (approximately 40 hours). Then, 1% w/v sterilized e-waste was added to each flask and kept at a similar incubation condition for 8 days to allow metals dissolution. After the bioleaching process, waste particles and cell biomass were removed from the solution using Whatman filter paper and centrifugation (Eppendorf Centrifuge 5804 R) at 7000 rpm for 10 mins. The supernatant was filtered using 0.45m glass fiber filters to ensure particle-free leachate. Then, the solution was investigated for Cu and Au metal dissolution using AAS.

3.8 Implementation of bioleaching process at 3L working volume

Most of the investigations on e-waste bioleaching have been conducted on a laboratory scale with limited pulp density. In contrast to small-scale media, microorganism kinetics may vary with increased leaching volume as flow, nutrient availability, and other variables alter in large-scale media [201]. Therefore, the sustainability of biocyanidation and metal solubility propensities of potent bacterial strain was assessed in a large volume (3Lworking volume) bioleaching solution with adequate (i.e., 30 grams) PD.

The experiment was conducted in triplicates in a “non-baffled” canonical flask (5L capacity). The two-step bioleaching approach was implemented with an optimized concentration of cyanogenesis stimulant and precursor for maximum metals solubilization. The 3L NB media, supplemented with methionine additive and glycine precursor, was prepared into a 5L flask. The media pH was adjusted to 9 using NaOH pellets and sterilized for 15 minutes at 121°C and 15 psi. The 4% v/v culture inoculum (14×10^4 CFU/mL) was added into a sterile medium and incubated in a shaking incubator at 40°C and 180 rpm for 48 hours without shredded dust CPCBs. After attaining the maximum bacterial growth (OD_{600}=1.234) and cyanide production, 10g/L sterilized pulp density was added to the medium and kept for 20 days of the incubation period for additional biocyanidation and metals dissolution reaction. In addition, a 5ml sample was taken daily to examine bacterial growth, pH change, and metal solubilization.

3.9 Characterization of e-waste after bioleaching of metals

3.9.1 Mass balance analysis

In addition to metals solubilization evaluation using AAS, mass balance calculations were also done to validate the bioleaching ability of an isolated bacterial strain. The weight of e-waste utilized for bioleaching at 3L working volume (i.e., 30g/L) was compared to the weight of e-waste collected after 20 days of the bioleaching process. The solution was filtered using Whatman filter paper and repeatedly washed with distilled water. The bioleached e-waste sample was dried in an oven at 50°C, and the sample was weighed using an analytical weighing scale (Citizen CTG 602) to determine the percentage weight loss owing to the dissolution of certain metals.

3.9.2 Morphological and mineralogical analysis

Following the two-step bioleaching process, the collected e-waste sample was examined for its morphological and mineralogical changes. The sample was evaluated through aqua regia digestion, SEM-EDX, XRD, and following the procedure outlined in sub-sections of 3.2

3.9.3 Determination of metals recovery

Metals solubilization by potential bacterial strain through biocyanidation processes was calculated by applying the following formula

$$m = c\ (\text{mg L}^{-1}) \times v\ (\text{mL}) / 1000 \tag{3.3}$$

m= metal concentration, c = concentration of metal analyzed through AAS after aqua regia digestion, v= volume of leaching solution

mg/L to mg/g

$$M/w = \text{Metal content} \tag{3.4}$$

w = amount of e-waste added to the leaching solution

$$\text{Percentage metal recovery} = \frac{\text{Concentration of metal in leachate solution after bioleaching process}}{\text{Concentration of metal in CPCB before bioleaching process}} \times 100 \tag{3.5}$$

CHAPTER 4

RESULTS AND DISCUSSION

RESULTS AND DISCUSSIONS

4.1 Characterization of waste computer printed circuit boards (CPCBs)

4.1.1 Metal content analysis

The typical composition of a printed circuit board (PCB) includes 0.3 to 0.4% precious metals, such as Au, Ag, and Pt, in concentrations ranging from 20 to 500 ppm, 200 to 3000 ppm, and 10 to 200 ppm, respectively. Additionally, ferrous and non-ferrous metals contribute 28%, with Cu being the predominant metal at 10-20% and Ni accounting for 1-3%. Various quantities of Al and steel are present, along with hazardous metals like Pb, Hg, As, Cr, and Cd [102, 202].

The metal composition of shredded dust of waste CPCBs used in the current study was determined using the aqua regia digestion method, as described in section 3.2.1. In this study, aqua regia digestion was employed to determine the metal composition of shredded dust from waste Circuit Printed Circuit Boards (CPCBs). Cu, Fe, Al, Ni, and Co were the most common base metals, and precious metals such as Au, Ag, and Pt were identified (Table 4.1). Cu was found to be the most abundant base metal, while Au exhibited the lowest abundance, categorizing the shredded dust as low-grade 'e-scrap.' The prevalence of Cu is attributed to its use in laminating non-conductive substrates for creating electric signal pathways.

In contrast, precious metals like Au and Ag, used in thin films over PCBs, contribute to chemical stability and oxidation resistance, ensuring the longevity of electrical contacts [48, 80, 203]. The Ag concentration was higher than Au in the shredded dust CPCBs because Au is primarily used for plating and in ICs only, whereas Ag is utilized in plated pins, ICs, solder junctions with Sn, thin layer on CPCBs, and alloy fragments [19, 204, 205]. Furthermore, the results of the current investigation are consistent with a previous study from Sun et al. they reported that Cu is the predominant constituent of PCBs with large-size particles ranging from 1 to 8mm, whereas precious metals display elevated levels within particle sizes of ≤ 0.2mm. Thus, the disparity between base metals and precious metals' concentrations may be ascribed to the ductile properties of Cu, which leads to its dispersal in more significant fractions compared to precious metals like Au [115].

Comparing results with previous studies of Kumar et al. [13], metal concentrations, except for Ag, were notably lower. The variation in the metallic constitution may be attributed to e-waste provenance and particle sizes resulting from the mechanical dismantling of WEEEs. The current investigation employed shredded dust from physio-mechanical procedures, while previous studies used unprocessed CPCB samples. Size reduction in the current sample likely liberated impeded valuable metals, leading to increased solubility during aqua regia digestion and yielding a significant concentration of Ag in small-sized shredded dust CPCBs (≤150µm) [13].

Furthermore, our current findings align with previous studies conducted by Chauhan and colleagues, indicating a relatively low concentration of precious metals in shredded e-waste dust. They found organic matter constitutes the highest proportion of 73.1%, with lower amounts of certain base metals—4.65% Al, 4.55% Fe, and 2.67% Cu. Variations in the total metal content of Circuit Printed Circuit Boards (CPCBs) across studies can be attributed to differences in the source and type of e-waste analyzed, as well as variations in industrial pre-treatment procedures such as sorting, manual disassembly, pulverization, and shredding[19, 43, 76, 206]. This heterogeneity in the metal composition of waste CPCBs poses a significant challenge in characterizing the material and designing recycling processes for industrial-scale application. However, the metal content analysis of shredded dust samples underscores the importance of employing an effective dust collection system to prevent the loss of valuable waste during the physical dismantling processes of any WEEE.

Table 4.1 The metallic constituents of shredded dust of computer-printed circuit boards (CPCBs).

S. No	Metals	Concentration (mg/g)
1	Cu	12.75±3.78
2	Ag	10.34±2.46
3	Fe	2.46±0.85
4	Al	0.39±0.02
5	Ni	0.24±0.02
6	Pt	0.08±0.06
7	Au	0.04±0.01
8	Co	0.03±0.07

$$Cu + 6HCl + 3HNO_3 \longrightarrow Cu^{2+} + 6Cl^{-} + 2NO + 4H_2O \quad (4.1)$$

$$2Au + 9HCl + 3HNO_3 \longrightarrow 2AuCl_3 + 3\ NOCl + 6H_2O \quad (4.2)$$

4.1.2 Morphological and mineralogical analysis

PCBs are also known to encompass diverse non-metallic constituents, including 49% glass and ceramics and 19% plastics, that are inextricably linked to metallic constituents to form a complex structure and design [206] and were difficult to detect through atomic absorption spectrophotometer. So, the lattice of the complex constituents of waste CPCBs was assessed through Scanning Electron Microscopy (SEM). The surface morphology that is attributed to the meshwork of various constituents is depicted in Figure 4.1. It is inferred from Figure 4.1 that there was a variety of textures and an uneven structure with smooth, rod-like, polygonal-shaped particles depicting the presence of non-metallic components such as plastic, silica, and ceramic. In addition, small flakes that covered the smooth surface of the non-metallic components revealed the metallic constituents of waste CPCBs. The morphological feature of the present waste CPCBs was corroborated by the analysis of virgin waste of CPCBs, investigated by Natrajan and Ting. They assessed the morphology of PCBs and found the meshwork of fibrous particles with different textures of smooth, uneven rod-like structures of non-metallic constituents [113]. Another study also revealed the granular structures of metallic constituents of PCB that exhibit a spherical and flakes-like morphology with an irregular surface texture. [193]. In addition, the investigation by Garg et al. also supported the morphological feature of shredded dust CPCBs. They also confirmed the smooth and uneven distribution of small flakes-like structures in mobile phone PCBs, which were disrupted and trenched after the bioleaching by *A. thiooxidans* [201].

After that, energy dispersive spectroscopy (EDS) validated the presence of various base and precious metals (shown in Figure 4.1b) detected through AAS and other constituents such as Si, Ti, Ca, Mg, and O. A comparable distribution of O, Al, and Si in the form of aluminum oxide, silicate minerals, and aluminosilicates in waste mobile phone PCBs was reported by various investigations. The EDX mapping investigation in 2019 by Garg et al. revealed a notable presence of Cu in the form of copper iron sulfide, Cu element along with other base metals such as Ni, Fe, and Al, where non-metallic content such as Si was the major contributor (i.e., 21.7%), followed by Ca 14.7%, Br 9.6%, and Sn 3.1% [201]. One of the researchers asserted the presence of various non-metallic fractions in waste PCB fragments. Their findings proclaimed the meshwork of glass or ceramic fibers, made up of Al, Si, and Ca, to the smooth surface morphology of PCBs [207].

Furthermore, the existence of the constituents, as mentioned above, was validated through XRD analysis. XRD spectrum depicted in Figure 4.2 inferred the structure regularity and molecular arrangements that contribute to the different properties of the materials present in waste CPCB fine particles. Figure 4.2 illustrates that most of the material in shredded dust PCBs was amorphous, primarily contributed by non-metallic components such as ceramics, gels, and polymers since no long order or well-defined diffraction planes and sharp peaks were obtained. In addition, the occurrence of four prominent peaks at 2θ values of (31.75), (45.47), (56.51), and (66.22) unveil various metals constitutions like Cd, Ag, Cu, and Au, respectively, of waste CPCBs that were scrutinized using "joint committee powder diffraction" (JCPDS) standard data file at JCPDS number 85-1328, 03-0931, 86-1868 and 04-0784 respectively.

The XRD pattern of current findings favors the outcome of earlier studies. In one of the investigations, there is a discrete pattern of material distribution during XRD investigation of spend PCBs e-waste and suggested that in mixed spent PCBs, non-metallic constituents hindered the presence of metallic components that were revealed after bioleaching by the *Frankia* consortium; therefore, four peaks at 2θ value of 44, 32, 21, 38 was reported for Au, Ag, Cu, and Zn in bioleachate solution [208]. Hence, the current findings of the mineralogical analysis are comparable to the many earlier reports, including Naryanasamy and colleagues, who proclaimed the irregularity in the spectra of XRD analysis that contributed by the non-metallic fraction of PCBs along with specific peaks for various metallic constituents at different 2θ values [58, 193]. So, the presence of all the metallic and non-metallic components in CPCBs contributes to the toxicity of e-waste and harms the environment and human health during informal treatment. Additionally, the toxicity of this complex solid waste stream challenges designing the process for metal recovery through a bioleaching approach [44, 207, 209].

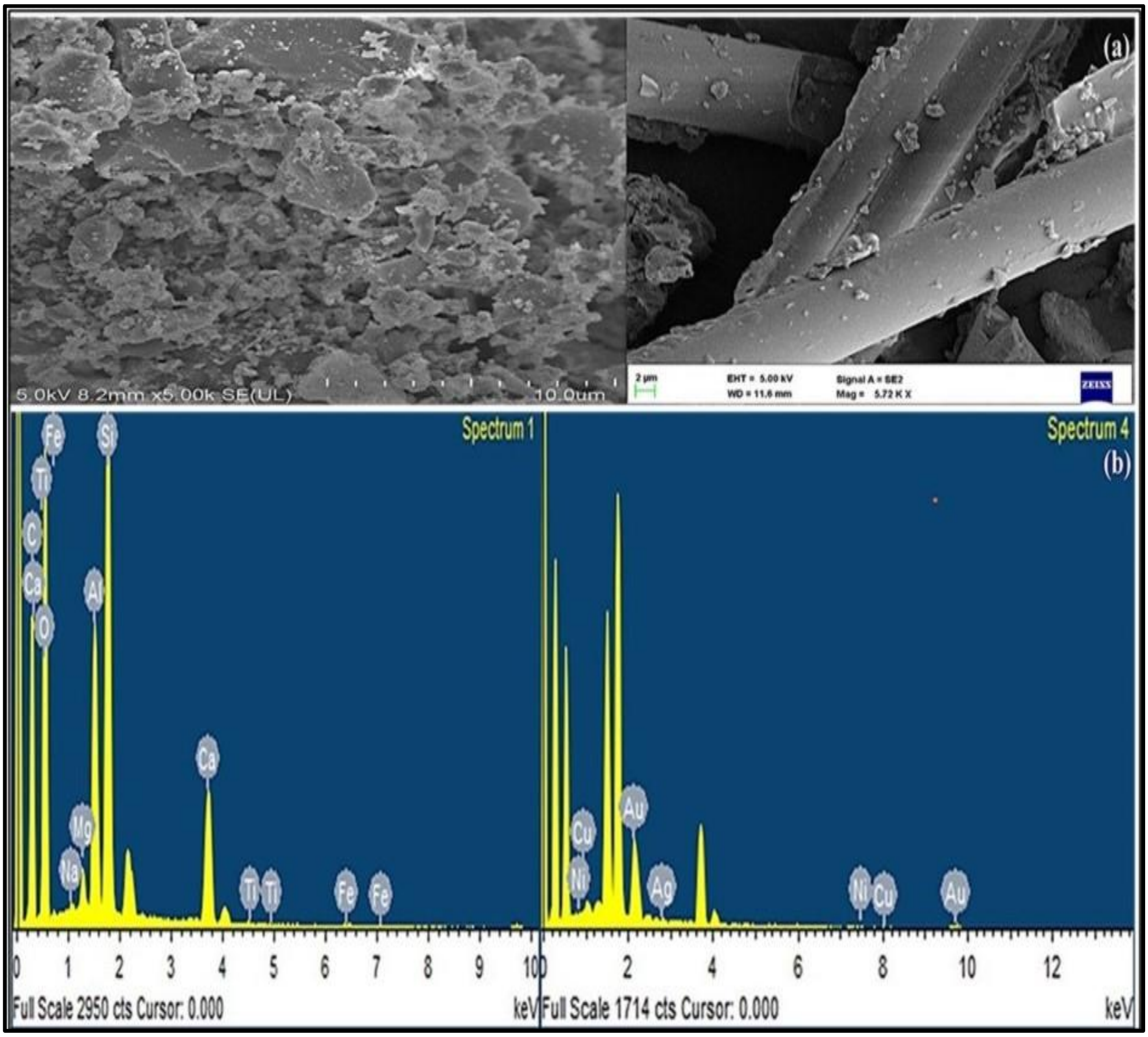


Figure 4.1 Scanning Electron Microscopy- Energy Dispersive Spectroscopy (SEM-EDS) analysis of shredded dust PCBs e-waste (a) Morphological description of e-waste with various distinct features at 5.00KX magnification **(b)** EDS spectrum depicted the presence of different constituents of shredded dust CPCBs.

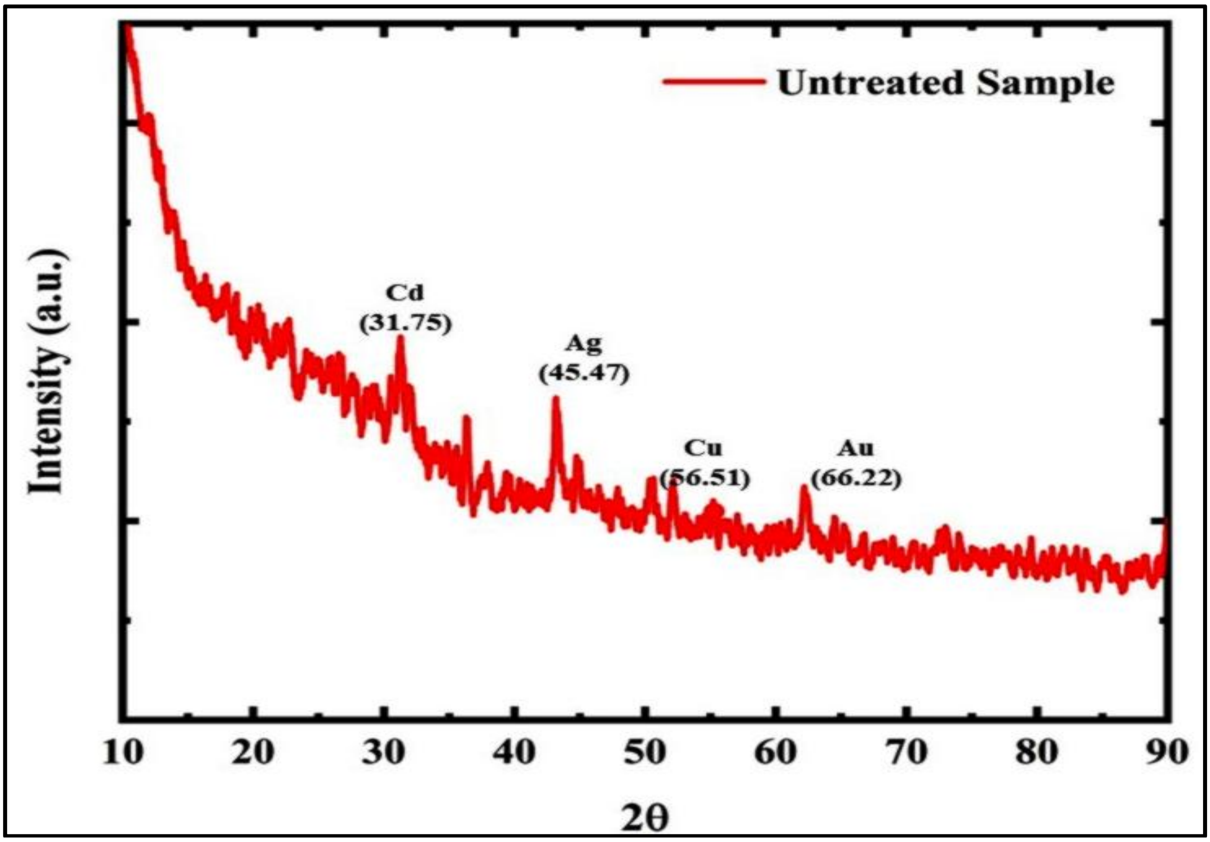


Figure 4.2: XRD spectrum depicted the amorphous character of e-waste contributed by the non-metallic constituents.

4.2 Exploration of potent bacterial strain from metal existence soil source

4.2.1 Metal content analysis of soil sample

The evaluation of the shredded dust CPCBs showed the presence of numerous toxic elements that may impede the bioleaching of metals from this valuable waste. Therefore, the current study intended to explore the bacterial community that dwells in a natural environment of metal contamination and the soil sample collection site was selected considering toxic CPCB constituents.

The soil sample was obtained from the landfill area of numerous waste automobiles, a source of diverging metallic elements (location mentioned in section 3.2.1). The prolonged existence (approximately > 25 years) of the dump yard resulted in metal contamination in the vicinity that may leach into the soil source, thus expected to sustain the bacterial community that may possess metabolic machinery towards various metals. Metals in the soil source were ascertained before use to isolate the bacterial community. The aqua regia digestion of the soil sample revealed the presence of several base metals in significant amounts, as shown in Table 4.2. Fe (46.56mg/g) was the most abundant metal in the soil, followed by Al (i.e., 23.54mg/g), along with other heavy metals such as Cu, Ni, Zn, Co, and Cr in a significant amount. The presence of these metals in soil samples ensures that the bacterial community habituated to this site exhibits innate metabolism towards these metals. However, precious metals, such as Ag, Au, and Pt, were absent in the soil. Still, the bacterial isolate obtained from the soil sample could be expected to exhibit potential leaching of these precious metals present in the CPCBs e-waste because most of the reported cyanogenic microorganism indicates similar mechanisms of bioleaching towards base metals and precious metals. Furthermore, many researchers have explored the bacterial strains from the metal-contaminated site, but the low leaching efficiency compels the continuous search of the bacterial community and optimization of the condition that favors the maximum leaching potential. An earlier study by Arab et al. in 2019 explored 5 (S22, N13, N37, N23, and N41) cyanogenic bacteria isolated from the e-waste landfilling site, but they did not identify the genus of these isolates [138].

Table 4.2: Presence of various base metals present in a soil sample from metal habituated site.

S. No.	Metals	Concentration (mg/g)
1	Fe	46.56± 0.18
2	Al	23.54± 0.13
3	Cu	2.88± 0.02
4	Zn	2.14± 0.07
5	Ni	1.22± 0.01
6	Co	0. 91± 0.06
7	Cr	0.85±0.09

4.2.2 Isolation of bacterial strains

Adopting progressive amelioration of e-waste (i.e., CPCBs) in the nutrient medium resulted in exploring potent microbial isolates capable of tolerating substantial amounts of toxic constituents in waste CPCB dust samples. The two bacterial isolates obtained at 100g/L pulp density were initially named hypothetically ISO1 and ISO2. The further streaking of these isolates on nutrient agar media provided the pure culture of these two bacterial isolates. Figure 4.3(b) illustrates the morphologically distinct features of microbial strains ISO1 and ISO2. The isolate ISO1 was Gram +ve with white, entire, raised, mucoid, and circular colonies on nutrient agar plates, whereas ISO2 was found to be Gram -ve with yellow pigmented colonies with whole, flat, circular edges. The control medium containing sterilized e-waste displayed no microbial growth. To select the isolated strain for metal solubilization from waste CPCBs, we further screened both isolates for their toxicity tolerance

4.3 E-waste toxicity tolerance assessment

The toxicity tolerance assessment is an important aspect that ensures microbial strains' feasible growth and efficient bioleaching potential. According to the authors' knowledge, the earlier reported studies have investigated the toxicity effect of e-waste in terms of metal solubilization efficiency, where they determine the impact of pulp density directly on the rate of metal solubilization rather than assessing bacterial growth and dose-response analysis such as in 2019, Garg et al. evaluated the effect of increased pulp density (i.e.,7%, 10%, and 15%) directly on base metals solubilization using sulfur-oxidizing microorganisms. They observed a significant decline in viable cell count, the prolonged time requirement for lixiviant production, and less bioleaching of Ni metals with 15% PD due to a high solid-to-liquid ratio that resulted in less availability of lixiviants in 15% PD [201]. However, the toxicity tolerance assessment in bacterial feasible growth is essential before assessing the bioleaching potential of the respective bacterial strain. The toxicity tolerance assessment estimates the maximum permissible PD at which bacteria can exhibit feasible growth and bioleaching potential. This concept gives an idea to increase the PD to its highest amount for pilot-scale operation. Hence, in 2018, Kumar and colleagues investigated the toxicity tolerance assessment in the aspect of dose-response analysis. They assess the toxicity tolerance of *P. balearica*SAE1 towards the toxic constituents of WPCBs [196]. The dose-response method of toxicity assessment is a statistically measurable analysis employed to ascertain the tolerance level to toxicity at the maximum treatment concentration and the 50% effective concentration (EC_{50}), specifically applicable to the metals bioleaching process [13]. The toxicity tolerance of ISO1 and ISO2 was compared to another indigenous bacterial isolate, *P. balearica*SAE1, at various e-waste pulp densities (i.e., 200, 250, 300, 350, 400, 450, 500 g/L). The strain *P. balearica*SAE1 was included for toxicity tolerance assessment alongside isolates ISO1 and ISO2 to compare the potential of two different bacterial strains obtained from two other sources and to select a better one for further experimentation up to pilot scale investigation so that the current study can provide the most proficient bacterial strain that can contribute to the field of biohydrometallurgy. The growth of isolated bacteria was measured regarding cell enumeration (CFU/mL) at each pulp density, along with a control set. Figure 4.4 illustrates the toxicity tolerance of these two bacterial strains in terms of percent inhibition response (%IR). Figure 4.4 shows that the bacterial isolates ISO1 and ISO2 had greater toxicity tolerance, which can be accredited to their natural environment of metal contamination that might have prompted the specific metabolic machinery and enabled them to resist the detrimental effect of toxic e-waste.

Furthermore, the EC_{50} value was found to be approximately 425g/L, 400g/L, and 300g/L for ISO1, ISO2, and *P. balearica*SAE1, respectively, which was comparatively higher than the research outcomes of Kumar et al. [13]. They reported 300, 128.9, 98.7, 90.8, and 83.70 g/L toxicity tolerance for *P. balearica*SAE1, *Bacillus* sp. SAG3, *B. megaterium* SAG1, *L. sphaericus* SAG2, and *C. violacium,* respectively. The variation in the outcomes may be attributed to the type of e-waste considered for the investigation, as in the current analysis, dust of CPCBs was employed, which exhibits lower metal concentration than earlier reported studies. The comparison of the tolerance potential of the respective strain was evaluated statistically at $p < 0.05$, which showed the significant difference in EC_{50} value of ISO1 and ISO2 (i.e., 400 and 425g/L, respectively). Therefore, ISO1 was considered the potential bacterial strain that exhibited higher toxicity tolerance than ISO2 and was further exploited for the metal’s retrieval process from the dust of waste CPCBs.

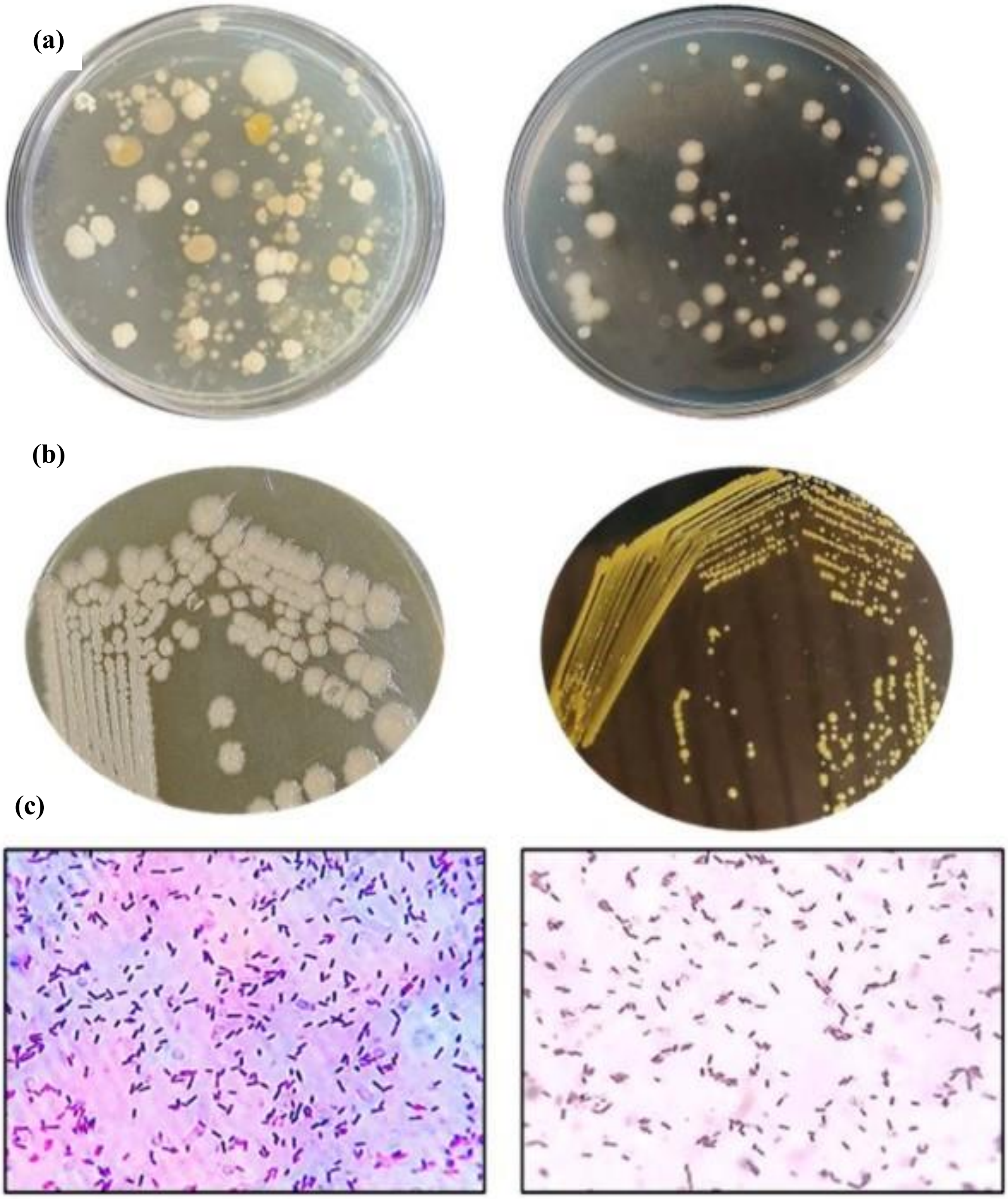


Figure 4.3 (a) Bacterial isolates obtained through sequential enrichment technique on nutrient agar media **(b)** Morphology of bacterial isolates ISO1 and ISO2 on NA media **(c)** Gram nature of ISO1 and ISO2 bacterial isolates at 1000X magnification.

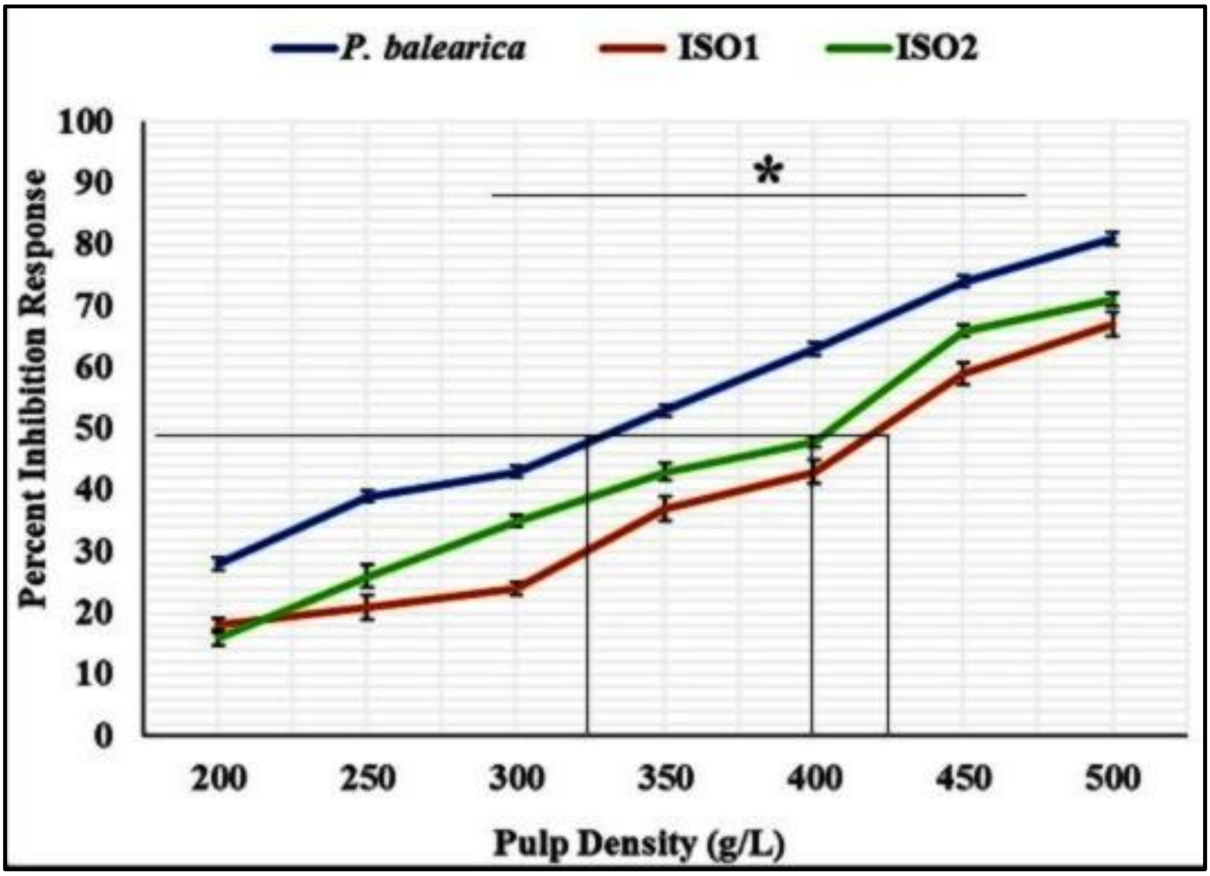


Figure 4.4 Toxicity tolerance assessment of bacterial isolates (ISO1 and ISO2) obtained from the metal habituated site and *Pseudomonas balearica* SAE1. Data are represented as mean with ± SD of triplicates. The significant variations ($p < 0.05$) between the tolerance of ISO1 with ISO2 and *P. balearica*SAE1 is denoted as*.

4.4 Characterization of the indigenous bacterial isolate

4.4.1 Biochemical characterization of potential bacterial isolate

The biochemical characterization of the isolated strain is enlisted in Table 4.2. Figure 4.5 depicts the cyanide production ability to isolate ISO1 as there was a change in the Whatman filter paper strip and the medium when the bacterial culture was grown on King’s B medium. Moreover, Figure 4.5a shows that isolate ISO1 can use citrate as the only carbon source since it responded favorably to the Simonson citrate agar media. Additionally, the isolate demonstrated the ability to produce endospores (Figure 4.5g). Likewise, the isolate exhibited hemolysis and catalytic activity.

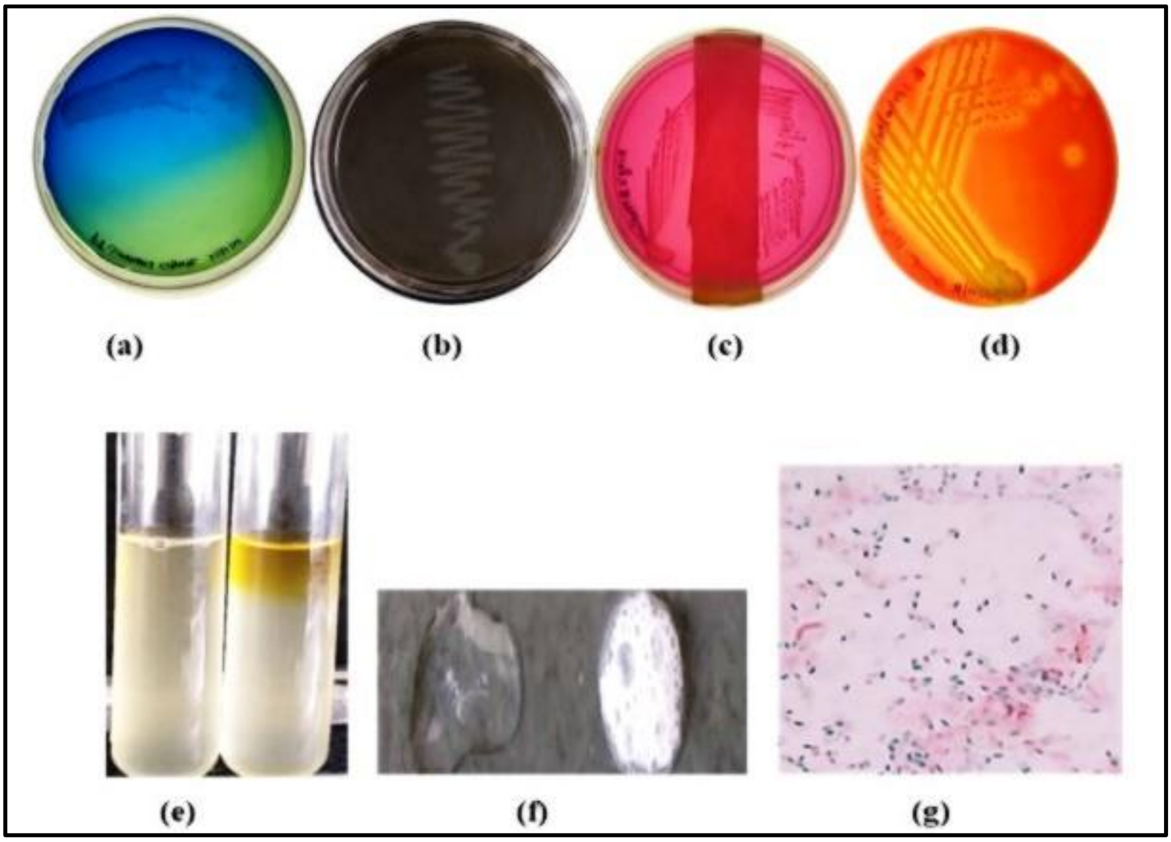


Figure 4.5: Biochemical characteristics of bacterial isolate ISO1 **(a)** Simmons Citrate Agar **(b)** Starch Hydrolysis **(c)** Cyanide Production **(d)** Hemolysis **(e)** Methyl Red **(f)** Catalase **(g)** Endospores at 1000X magnification.

Table 4.3 Biochemical features of a bacterial isolate ISO1 obtained from metals habituated site.

Test	**Result**
Gram staining	+ve
Endospore staining	+ve
Methyl red	-ve
Hemolytic	+ve
Catalase	+ve
Starch hydrolysis	-ve
Cyanide production	+ve

4.4.2 Molecular identification of potential bacterial isolate ISO1

A high-quality DNA sample was isolated for molecular identification of isolated bacterial strain (i.e., ISO1). The concentration of DNA was obtained at 100 ng/L and determined spectrophotometrically (Thermo Fisher Scientific, Inc., Massachusetts, USA). The acquired DNA was utilized to amplify the 16S rRNA gene through 27F and 1492R universal primers. The amplified 16S rRNA gene with an approximate size of 1500bp (Figure 4.6) was sequenced at Yaazhxenomic, Coimbatore, Tamil Nadu, India.

Furthermore, the acquired 16S rRNA gene sequence was BLAST against NCBI GenBank database, and based on the utmost sequence similarity with "GenBank" database, the bacterial isolate 'ISO1', isolated from the metal habituated site was identified as *Bacillus sporothermodurans* (99.85% sequence similarity). A 1309-base pair sequence of 16S rRNA gene has been successfully submitted to the GenBank database that can be accessed and retrieved using the unique accession number "OQ373005". The phylogenetic analysis using PhyML 3.0 aLRT revealed the evolution of isolated strains from group 'Firmicutes.' Figure 4.7 illustrates the evolutionary correlation between the current *Bacillus* strain ISO1 and another *Bacillus* sp. As per the author's knowledge, this isolate ISO1 is a new *Bacillus* strain that has been investigated for the first time to assess its bioleaching potential. Earlier, numerous studies reported the bioleaching potential of *Bacillus megaterium* strains. Arshadi and Mousavi (2015a) imitated the utilization of *B. megaterium* PTCC1656 for Au solubilization from mobile phone PCBs and successfully solubilized 64g/ton Au [210]. They further endeavored to enhance the bioleaching efficiency of *B. megaterium* PTCC1656 by optimizing the different factors that had affected its growth and leaching efficiency [211, 212].

4.5 OFAT approach to evaluate the effect of various parameters on the bioleaching

4.5.1. Selection of suitable precursor for cyanide production

Bacillus sporothermodurans ISO1 is a cyanogenic bacterium producing cyanide, a primary lixiviant for metal solubilization. Various cyanogenic microorganisms, such as *C. violacium, P. aeruginosa, P. fluorescence, and B. megaterium,* have been explored for biocyanide production and utilization for metals recovery potential from e-waste [47, 50, 149, 202]. In earlier reported studies, investigation of amino acid's effect on the biocyanidation process revealed the usage of amino acids as a precursor for biogenic cyanide production and suggested using glycine as a primary precursor for cyanide generation [119, 209]. However, it has not been established that all bacteria comparably respond to amino acids because distinct microbial species may favor different amino acids as a precursor for the physiological process. Therefore, investigating various amino acids as a vital nutrient source for bacterial growth and a precursor for biocyanidation is a significant aspect of the bioleaching process. An *In-silico* study by the researcher assessed the influence of 13 different amino acids on genes involved in HCN generation by *B. megaterium* DSM319, and it was found that Cys, Glu, and His had the most pronounced impact on HCN production, as their exclusion led to 50% decrease in production of CN. They also found that Ile, Pro, Thr, and Val amino acids did not affect biomass production significantly.[200]

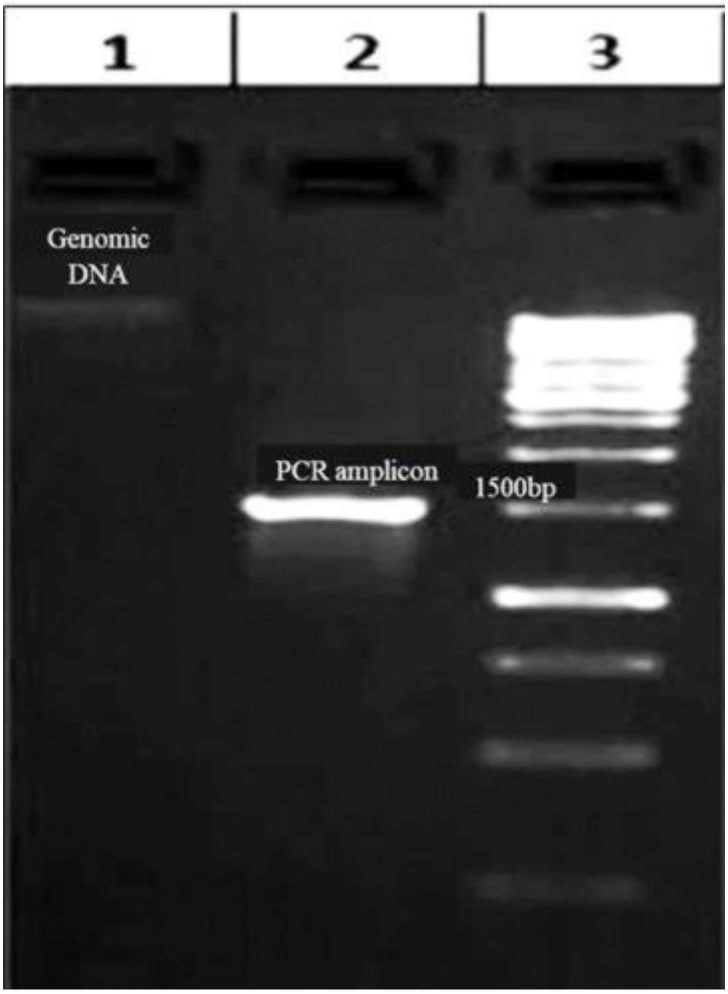


Figure 4.6 PCR amplified 16S rRNA gene on 1.2% agarose gel.

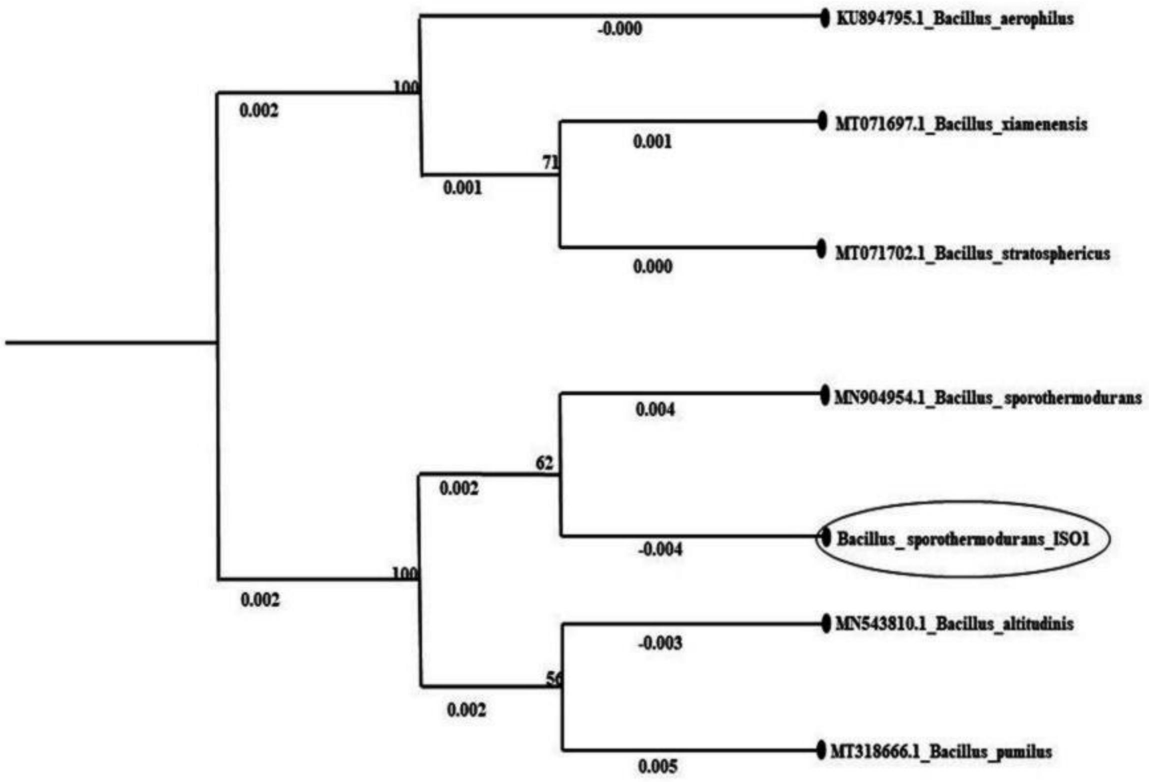


Figure 4.7 Phylogenetic tree to depict the evolutionary relationship between *B. sporothermodurans* ISO1 with other *Bacillus* species.

Therefore, considering the earlier studies, the current study intended to assess the impact of different amino acids on the growth of *B. sporothermodurans* ISO1 and its potential for Cu solubilization that indirectly suggested the influence of various amino acids on the production of CN lixiviant as well. Figure 4.8a illustrates the effect of different amino acids on the growth of *B. sporothermodurans* ISO1; it is inferred that methionine supported maximum bacterial growth after 24 hours of incubation, a suitable growth phase for efficient biocyanide production. In contrast, phenylalanine supplementation reduced bacterial growth significantly during the late log phase. In addition, Figure 4.8b depicts the trend of Cu solubilization by *B. sporothermodurans* ISO1. Hence, in comparison to other amino acids, *B. sporothermodurans* ISO1 preferred glycine as the primary precursor for cyanide lixiviant production that forms complexes with Cu metal present in waste CPCBs. The supplementation of 5g/L glycine led to 63% Cu solubilization (i.e., 8.03 mg/g).

Furthermore, both Lys and His demonstrated considerable Cu solubilization but did not significantly affect bacterial growth. Conversely, methionine did not substantially impact Cu solubilization but greatly influenced biomass production. So, this implies a noteworthy role of methionine in the growth of *B. sporothermodurans* ISO1; however, it cannot be directly utilized as a precursor for cyanide production. The outcomes of the current study diverge significantly from earlier reported investigations by Faraji et al. They also evaluated the effect of Thr and Glu on *B. megaterium* cyanide production for Au recovery and found that Thr had a more substantial impact on bacterial growth stimulation than Glu and Gly. Still, biocyanidation was influenced equally by Glu and Thr, which is higher than glycine, so Thr and Glu are a considerable precursor for biogenic CN lixiviant production by *B. megaterium* [119].

Moreover, an *in-silico* study by Aminian-Dehkordi and co-workers also suggested using other amino acids than glycine for biocyanidation mechanisms, although their investigation did not give any experimental proof for the *in-silico* proclaimed [200]. In the current study, only glycine significantly impacted the lixiviant production capacity among the tested amino acids. However, the complete and precise mechanism underlying this effect remains unexplained. Since glycine utilization for cyanide production is endothermic and non-spontaneous, it must be accomplished through an indirect pathway, such as regulation of the glycine pool glycine transportability[108, 200, 213].

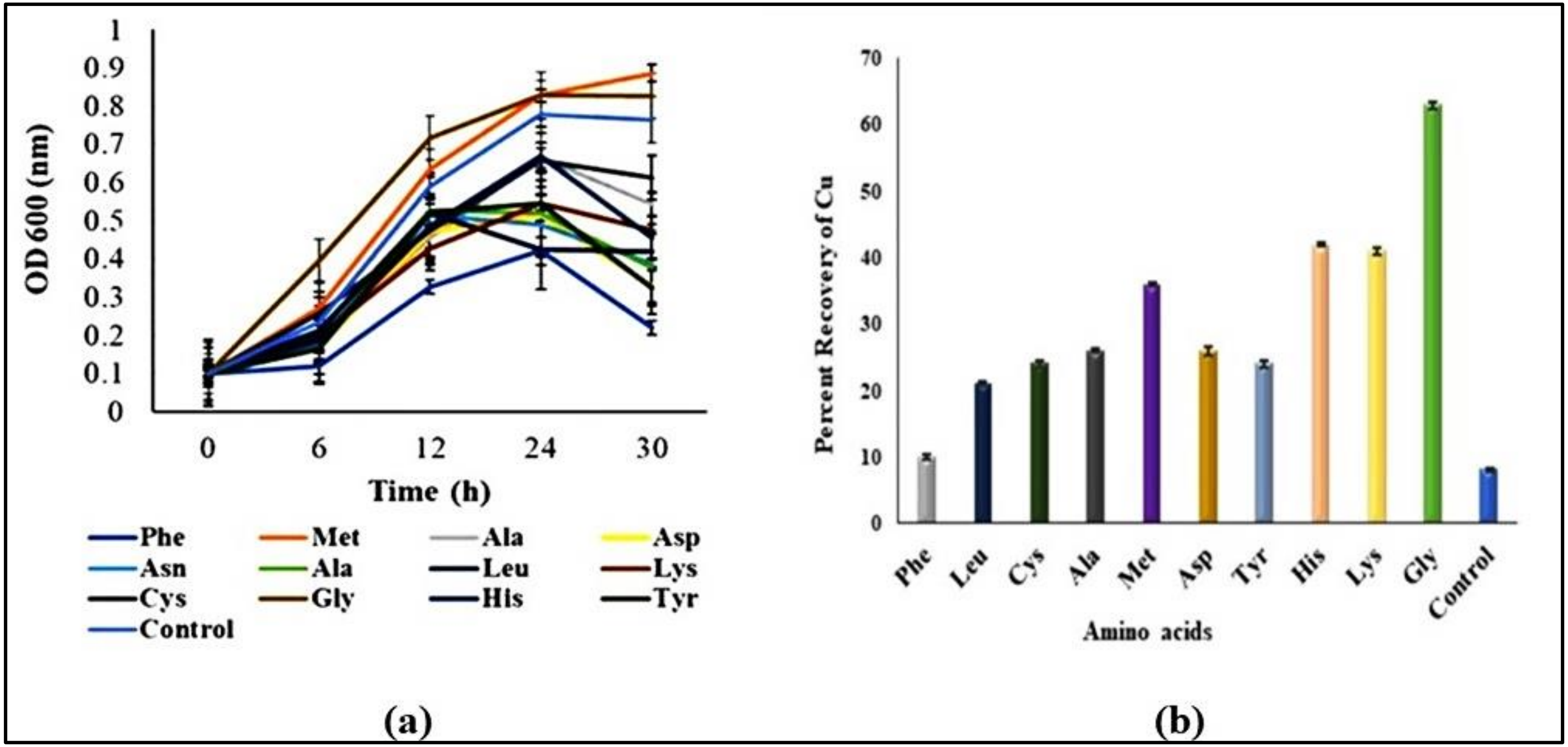
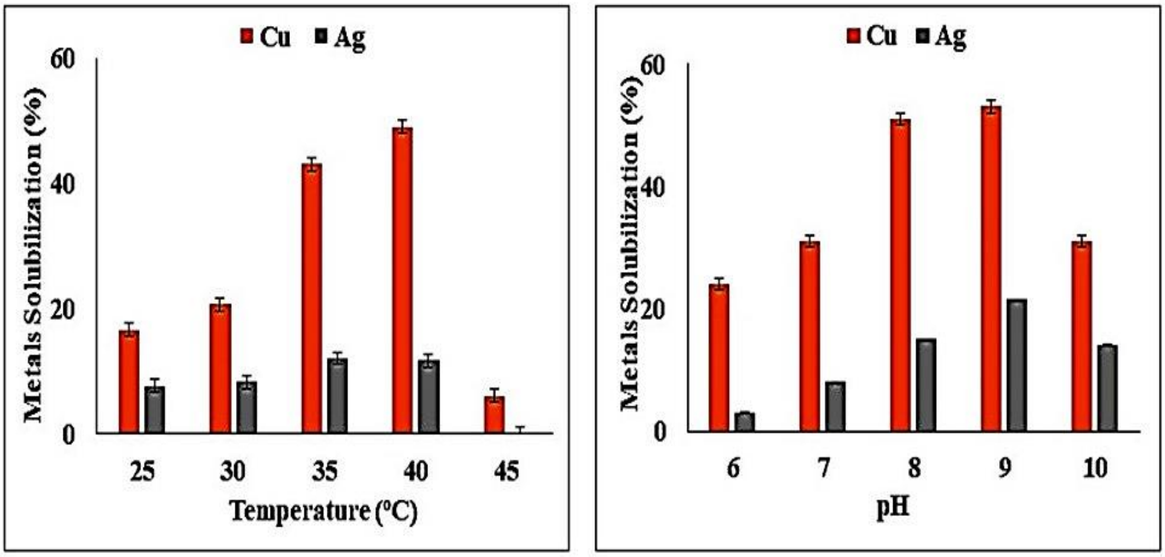


Figure 4.8 Effect of various amino acids (a) Growth rate of *B. sporothermodurans* ISO1 at 37ºC utilizing various amino acids **(b)** Copper recovery rate using different amino acids as precursors for cyanide production by *B. sporothermodurans* ISO1. The data presents the mean of triplicates with ±SD.

4.5.2 Effect of temperature

Wide temperature ranges can affect bacterial growth and activity. Numerous investigations have suggested the moderate range (i.e., 20°C–40°C) as a suitable temperature condition for growth, lixiviant synthesis, and efficient metals solubilization activity by cyanogenic bacteria, including *C. violaceum, B. megaterium,* and *Pseudomonas sp.* [198]. Therefore, the growth and bioleaching capacity of the current bacterial isolate (*B. sporothermodurans* ISO1) was evaluated at a mesophilic temperature range of 20-40ºC. According to past studies, the ideal temperature range for *C. violaceum* was 25–30 °C [138, 195, 211]; for *Pseudomonas* sp. and *Bacillus megaterium*, it was 35–37°C [106]. The solubilization of Cu and Ag metal by *B. sporothermodurans* ISO1 varied widely at different temperature ranges. The trend in metals solubilization by this potential isolate is depicted in Figure 4.9a. It is shown in the Figure that with an increase in the temperature from 20ºC to 40ºC, there was an increase in the metal dissolution potential of isolate ISO1. The earlier investigations reported optimum temperatures of 37ºC and 30ºC for *P. balearica*SAE1 and *C. violacium*, respectively [196] [214]. In the current study, *B. sporothermodurans* ISO1 exhibited notable bioleaching activity between 35-40°C. While there was no substantial difference in Ag solubilization at 35 and 40°C, Cu solubilization showed significant variation between these temperatures.

Furthermore, a rise in the temperature beyond the threshold, i.e., 45ºC, significantly decreased the bioleaching activity; bacterial growth ceased above 40ºC. However, at each temperature value, the rate of Cu solubilization dominated over Ag solubilization due to the abundance of Cu content in e-waste, which may have consumed most cyanide lixiviant and caused hindrance to other metals solubilization [104, 116]. However, the current finding conflicts with the results of Arab et al. investigations as they reported the maximum cyanide production (1.6mg/L) at 20ºC by cyanogenic bacterial strain NS23[138]. They claimed that low temperatures support the low oxygen demand for bacterial growth and lessen the HCN volatilization so that maximum CN^- is available for metal complex formation and lowers the oxygen needed for bacterial growth [209]. However, no firm evidence has been found to support the low-temperature effect on HCN volatilization. However, the present bacterial isolate exhibited the optimum growth at 40ºC; therefore, maximum lixiviant availability and metals' dissolution capacity were attained at the respective incubation temperature (i.e., 40ºC). As per the author's knowledge, unlike earlier reported cyanogenic strains, the *B. sporothermodurans* ISO1 has emerged as a noteworthy cyanogenic bacterial isolate, displaying remarkable bioleaching capabilities, specifically at a temperature of 40°C. This investigation holds significant promise for developing a pilot-scale bioleaching system, offering a cost-effective and energy-efficient approach.



(a) (b)

Figure 4.9 Parameters optimization that influences the solubilization of metals (a) Percent solubilization of Cu and Ag at different temperature ranges **(b)** Rate of Cu and Ag solubilization at various pH ranges. Data are presented as the mean of triplicates along with ±SD value.

4.5.3 Effect of pH

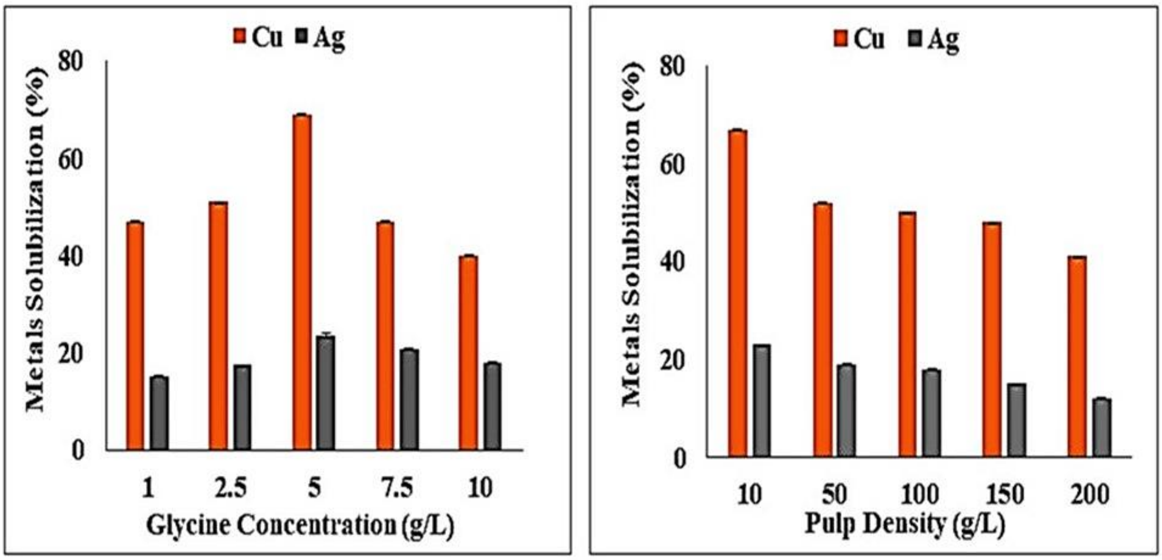
The pH of the medium plays a significant role in the bioleaching activity of bacterial strains as pH exerts a significant impact on the generation of CN lixiviant in the form of cyanide ions (CN^-) and the volatilization of hydrogen cyanide (HCN) [212]. The earlier reported studies claimed alkaline conditions favor higher biogenic yields than acidic environments. The HCN exhibits a pKa value of 9.3. Therefore, a high pH medium upsurges the conversion of HCN to CN^- as per equation 4.3, a main lixiviant that can make complex with various metals present in waste CPCBs [78, 108, 136, 209, 215]. Although the pH range 7-8 is most often favorable for the growth of microorganisms, leaching potential is divergent from the respective pH range. Hence, the effect of the initial pH of the medium on the growth and metals' solubilization potential of *B. sporothermodurans* ISO1 was assessed from neutral to alkaline pH ranges of 7, 8, 9, and 10. Figure 4.9b inferred the Cu and Ag leaching capacity of *B. sporothermodurans* ISO1 at different pH values. It is depicted from the Figure that pH 8-9 inclined maximum leaching capacity (i.e., Cu 53% and Ag 21%) because an alkaline pH changes equilibrium towards the release of more cyanide ions (CN^-), as shown in equation 4.3. Which form metals-cyanide complexes, whereas, at lower pH levels, there is a tendency for the presence of more HCN rather than CN^- , a volatile gas with less solubility in water [202, 216]. The present investigation assessed pH variation in the two-step bioleaching process. The initial pH of the medium was 9, which provided optimal conditions for growth and metabolite production that led to a reduction in the pH of the medium up to 8.3 within the first 2-3 days of incubation in the absence of waste CPCBs. Afterward, pH was raised to 9.1 after adding the shredded dust e-waste sample, attributed to the alkaline nature of e-waste that favors the condition for maximum CN availability and metals solubility since pH is maintained near pKa of HCN. However, an additional surge in the medium pH (i.e., 10) caused a decline in bacterial growth (maximum OD: 0.427) as well as the rate of metal solubilization. The outcomes of various reported studies corroborate the findings of the current investigation. Shin et al. disclosed that a pH above 9 ceases bacterial growth, reducing the metals' solubilization rate [217]. Another investigation by Arshadi and Mousavi also supports the change in the trend of medium pH. They explained the pH disparity caused by *B. megaterium* while mobilizing the Cu and Au metals in a two-step bioleaching process. They found an initial pH fall from 10 to 8.8 during the growth of *B. megaterium,* followed by a rise to 9.4 with 5g/L glycine concentration [197].

Moreover, some studies also claimed a significant metal efficiency above pH 9, such as Natarajan and Ting, which found the highest Au mobilization at pH 9.5 by transforming the *C. violaceum* strain. Meanwhile, other findings also stated 30% Cu and 95.7% Au dissolution at pH 10 using a spent medium leaching process [216] [116]. The outcomes of the various studies showed that the medium's pH diversely impacts the metal leaching efficiency of bacterial strains, but alkaline pH was associated with increased metal solubilization potential in all the investigations. The applicability of alkalophilic cyanogenic microorganisms has been explored substantially at an industrial scale for leaching Au and Ag from mine tailings [3, 49, 151, 207]. However, very few reports have suggested using alkaline pH conditions for Ag leaching by microbial strains capable of biogenic CN^- -production.

$$HCN \longleftrightarrow H^+ + CN^- \qquad (4.3)$$

4.5.4 Effect of suitable precursor concentration

The biocyanidation process, mediated by cyanogenic microorganisms, is impacted by carbon sources and amino acid precursors. [218]. As elucidated in section 4.5.1, *B. sporothermodurans* ISO1 preferred glycine as the primary precursor over other amino acids to produce HCN lixiviant. Therefore, optimizing glycine concentration is essential for this potential bacterial isolate's efficient biocyanidation and metal solubilization capacity [138]. The earlier studies suggested that glycine concentration below 10g/L is sufficient for efficient cyanide production by various cyanogenic microorganisms such as *C. violacium, P. aeruginosa, and P. fluorescence.* Thus, glycine concentration was ascertained by supplementing the glycine in the nutrient medium at pH 9 from 1-10 g/L in intervals of 2.5 g/L. The dissolution rate of Cu and Ag metals by ISO1 at optimized temperature (i.e., 40°C) determined the precursor's impact on the respective isolate's bioleaching efficiency. The trend of Cu and Ag solubilization in the presence of various glycine concentrations is illustrated in Figure 4.10a. There was a continuous inclination in the Cu and Ag retrieval from 2.5 to 5g/L glycine supplementation and peaked at 5g/L (Cu 69% and Ag 23%). However, further glycine increases adversely impacted the metal solubilization capacity of *B. sporothermodurans* ISO1. At 7.5g/L, the Cu and Ag leaching rates were reduced to 47 % and 20%, respectively. This analysis confirms that 5g/L glycine is sufficient for efficient bioleaching activity of *B. sporothermodurans* ISO1 by using it as a precursor for cyanide production. Whereas, above 5g/L, there may be degradation of excessive cyanide and formation of β-cyanoalanine compound. The current investigation's outcome supports earlier reported outcomes of Shani et al. and Arshadi and Mousavi [117] [211]. They also obtain maximum leaching of Cu and Au with 5g/L glycine precursor by *P. fluorescence* and *B. megaterium,* respectively. Unlike other studies, Faramarzi et al. reported divergent outcomes. They obtained the highest cyanide production and Ni recovery as tertracyanonickelate using *C. violacium* at a range of 8-10g/L glycine supplementation; however, above 10g/L' they obtained reduced cyanide production and Ni recovery from nickel powder and Au recovery from waste PCBs [110]. The higher need for glycine for recovery of Ni by *C. violacium* has not been addressed, but it might be the kinetics of the cyanide synthase enzyme, which uses glycine as a main precursor to produce cyanide.



(a) (b)

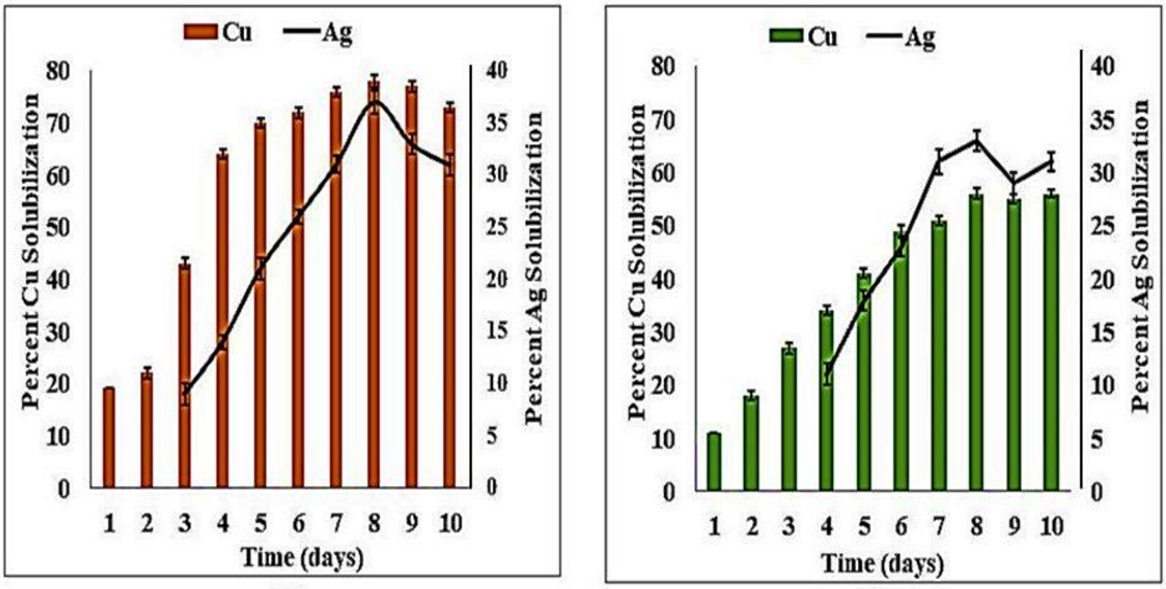
Figure 4.10 Parameters optimization that influence the solubilization of metals (a) Glycine concentration and metals solubilization rate **(b)** Rate of metal solubilization at various pulp densities. Data are presented as the mean of triplicates along with±SD value.

4.5.5 Effect of pulp density

The toxic effect of various hazardous constituents in WEEEs increases with an upsurge in the P.D. The coexistence of numerous metallic and non-metallic elements harms microbial growth, thus reducing the CN-lixiviant synthesis and metal extraction potential [136, 202]. The assessment of toxicity tolerance of *B. sporothermodurans* ISO1 in section 4.3 revealed the highest amount of shredded dust of CPCBs that can be considered for feasible bacterial growth (e.g., EC_{50}= 425g/L). The recovery of the metals from e-waste depends on the availability of the CN lixiviant released by the potential bacterial isolates. Therefore, the pulp density suitable for the efficient bioleaching capability of a *B. sporothermodurans* ISO1 may differ from the amount that can be considered for its feasible growth. Thus, the amount of shredded dust CPCBs was determined from the 10-200 g/L range to assess this valuable waste's efficient metal retrieval potential. Figure 4.10b shows the Cu and Ag solubilization trend at various pulp densities. Figure 4.10b displays that the rate of metal solubilization declined as pulp density increased. *B. sporothermodurans* ISO1 showed maximum leaching of Cu (67%) and Ag (23%) at 10g/L pulp density that was decreased adversely at 200 g/L (i.e., 40% Cu and 12% Ag). Although *B. sporothermodurans* ISO1 showed significant recovery of Cu (55%) at 50g/L, Ag recovery reduced to 19% at this pulp density. Henceforth, 10g/L pulp density was considered the optimum amount of pulp density that can be considered for efficient leaching of metals even at a substantial volume of bioleaching operation. However, the trend of metal leaching changed with the volume of the bioleaching solution, as there was a change in the kinetics of mass flow, energy flow, bacterial growth, and metabolic activities. The investigation by Hubau et al. in 2020 reported a decline in the rate of Cu, Zn, Ni, and Co solubilization while demonstrating the bioleaching at 1L leaching volume under optimized conditions [207]. Thus, parameter optimization needs to be done at different volumes of bioleaching operation. In addition, in the present study, Cu leaching efficiency by *B. sporothermodurans* ISO1 was greater than that of another native bacterial isolate of Arab et al. [138]. They reported 41.72% and 31.52% solubilization of Cu at only 1g/L pulp density by indigenous strains N23 and N41, respectively, isolated from soil sources from e-waste landfilling areas. Moreover, our results are substantiated by the outcomes of Pradhan and Kumar [31] and Mara et al. [45], who also reported 10g/L pulp density as the optimum amount for efficient recovery of Cu, Au, and A g by various cyanogenic microorganisms. Additionally, Natarajan and Ting suggested less than 10g/L pulp density as the optimum amount for efficient leaching of Cu and Au by *C. violacium* [113]. Though the bioleaching efficiency of cyanogenic microorganisms can be satisfactory at low pulp density, it may not be economical for industrial-scale applications, as there is leaching of specific metals by cyanogens due to limited production of lixiviant that may not be proficient at large-scale operations. To the best of the author's knowledge, no studies have been reported yet that claim an optimum concentration of more than 10g/L pulp density for efficient recovery of metals by using various potential microorganisms at laboratory scale experimentation. However, the pilot scale bioleaching was demonstrated by Garg et al. at 7%, 10%, and 15% pulp density in a stirred tank bioreactor and found more than 90% Cu solubilization with each pulp density but due to selectivity towards Cu metal limits its industrial scale implementation [201]. Henceforth, in the present study, significant recovery of Cu (i.e., 55%) and Ag (i.e., 18%) above 10g/L pulp density and higher toxicity tolerance of *B. sporothermodurans* ISO1 suggested the further optimization of parameters at pilot scale operation for its the industrial-scale application.

4.5.6 Bioleaching of Cu and Ag under optimized conditions

The metal leaching capacity exhibited by *B. sporothermodurans* ISO1 was assessed under optimized conditions that validated the extent of various factors affecting this potential bacterial isolate's growth and leaching capacity. In addition, the leaching capacity of this indigenous isolate was compared to *P. balearica*SAE1, another autochthonous bacterial strain isolated from the site surrounding the e-waste recycling firm. The comparative analysis was done through two-step bioleaching for 10 days at 40°C for *B. sporothermodurans* ISO1 and 37°C for *P. balearica* SAE1. The medium was supplemented with 5g/L glycine, pH 9, and pulp density 10g/L. Figure 4.11 illustrates the bioleaching capacity of Cu and Ag of these two indigenous isolates. It is depicted from the Figure that the rate of Cu solubilization was higher than Ag with both bacterial isolates. Moreover, Cu solubilization was initiated on the 1st day of the leaching process after obtaining the maximum growth of bacteria, whereas Ag solubilization was obtained on the 3rd and 4th days after sufficient solubilization of Cu. This trend was attributed to the higher abundance of Cu than Ag, which initiated the utilization of lixiviant. In addition, *B. sporothermodurans* ISO1 surpassed the leaching efficiency of *P. balearica* SAE1. The 78% and 36% leaching of Cu and Ag, respectively, were obtained by *B. sporothermodurans* ISO1 on 8 days of the leaching process, whereas *P. balearica*SAE1 leached only 49% Cu and 34% Ag on the 8th day of the incubation period. Utilizing a two-step bioleaching process agrees with several earlier reported studies. Farmaji et al. obtained only 14.9% recovery of Au as dicyanoaurate in one-step bioleaching [108], whereas Brandl et al. achieved 68.9% Au solubilization through a two-step bioleaching process using *C. violacium* [209]. Moreover, in 2020, Karim and Ting attained recovery of Pt, Pd, and Rh up to the range of 35, 41, and 82% by *B. megaterium* from 1% pre-treated expended automotive catalyst through a two-step bioleaching process [219]. These outcomes attributed to mitigating the e-waste toxicity through a two-step bioleaching approach that achieved sufficient lixiviant production in the exclusion of PCBs sample and, therefore, attained higher metals leaching than the one-step bioleaching process. In addition, Pradhan and Kumar suggested the utilization of microbial strains consortium for enhanced leaching of Cu, Au, and Ag. They reported 83, 73, and 8% solubilization of respective metals using a consortium of *C. violacium* and *P. aeruginosa* [106]. These findings enlightened the bioleaching potential of cyanogenic microorganisms for metal recovery from low-grade metal sources such as e-waste through different approaches to the ‘biocyanidation’ process. However, the current study unveils the niche indigenous bacterial strain that exhibited high toxicity tolerance and efficient bioleaching potential in the “two-step bioleaching” process. Therefore, this niche *Bacillus* sp. might take attention for an industrial-scale bioleaching operation.



[a] [b]

Figure 4.11 Solubilization of metals through biocyanidation process (a) Percentage recovery of Cu and Ag through *B. sporothermodurans* ISO1at 40°C temperature, pH 9, glycine 7.5g/L, 10g/L pulp density **(b)** Percentage recovery of Cu and Ag through *P. balearica*SAE1 at 37°C temperature, pH 9, 5g/L glycine, pulp density 10g/L. The data are presented as a mean value of triplicate experiments along with ±SD value.

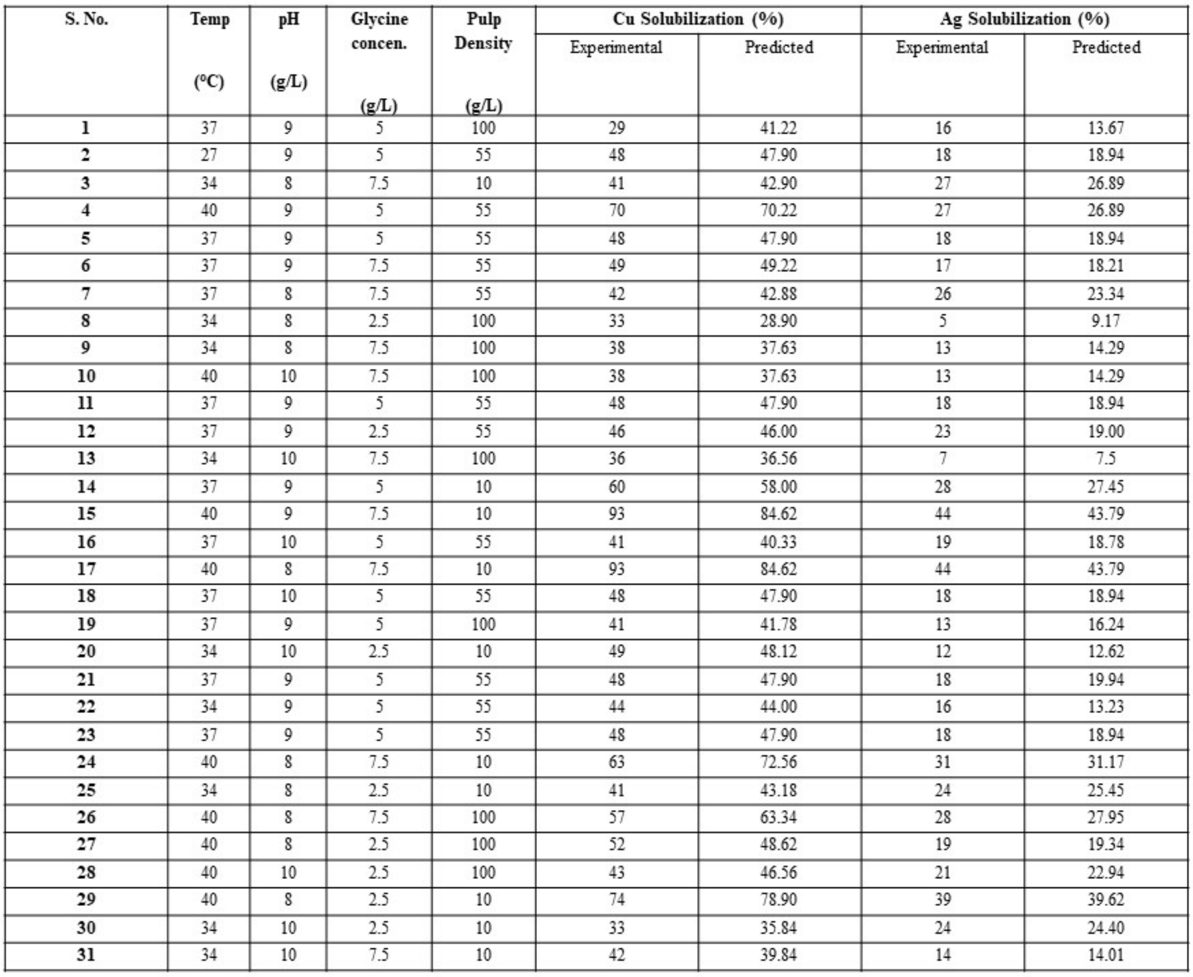
4.6 Response Surface Methodology (RSM) approach for parameters optimization

The variables mentioned above, such as incubation temperature, medium pH, precursor concentration, and pulp density, were crucial for the bioleaching activity of *B. sporothermodurans* ISO1, hence playing a significant role in bioleaching activity. The one factor at a time (OFAT) approach revealed that pH and pulp density were the two predominant factors that adversely affect the metals' leaching potential of *B. sporothermodurans* ISO1. Numerous reported studies support the outcomes of the current optimization objective. Arshadi and Mousavi documented that the generation of biogenic CN^- is exclusively influenced by the pH level of the medium. Since HCN possesses a pKa value of 9.3, an alkaline pH promotes the formation of a more significant amount of CN^- and facilitates the formation of complexes with metal ions. Unlike under acidic conditions, most CN^- is lost as HCN gas due to volatilization [19, 197, 198]. In addition, pulp density is another challenging factor that significantly affects the metal's leaching ability. As the concentration of e-waste increases, the amalgamation of hazardous metals and inorganic pollutants becomes toxic to microorganisms, ceasing the growth during the bioleaching process and significantly reducing the lixiviant production and metals solubilization potential [46, 151]. The analysis is based on the OFAT approach, although they may change in the interactive effect of each other where a favorable factor may outweigh the impact of the unfavorable factor [198, 210]. Therefore, a statistical approach, RSM, was employed to comprehend the interactive effect between the several physiochemical parameters and increase the metal leaching potential of *B. sporothermodurans* ISO1 because multiple interacting parameters cannot be studied simultaneously using the OFAT approach. The use of the OFAT approach before the implementation of the RSM approach provides an idea to prioritize the factors and their extent that affect the bioleaching potential of any microorganism and abandon the factors that are not significant. As a result, the optimization process may become more cost-effective when parameters are selected through the OFAT approach.

4.6.1 Bioleaching of Cu and Ag under different parameters conditions

The CCD-RSM approach was used to determine the interactive and cumulative effect of the earlier parameters that would improve the leaching rate of Cu and *B. sporothermodurans* ISO1. CCD depicted 31 test conditions, including 16 factorials, 8 axial, and 6 center points, that were demonstrated experimentally to determine their effect on Cu and Ag solubilization. Table 4.4 displays the experimentally obtained and software-anticipated Cu and Ag solubilization rates at various test conditions. The actual responses corresponded to the experimental obtained metals leaching rate, while the predicted responses were statistically estimated metal leaching responses by using CCD experimental design software. It is depicted in Table 4.4 that the 13th test set condition, comprising a temperature of 40°C, pH of 8, glycine concentration of 7.5 g/L, and pulp density of 10 g/L, resulted in the maximum leaching efficiency. Under these specific parameter conditions, *B. sporothermodurans* ISO1 achieved a remarkable recovery rate of 93% for Cu and 44% for Ag.

Table 4.4 The percentage solubilization of Cu and Ag at different sets of conditions designed by CCD- RSM in Minitab18 software.



S. No.	Temp (°C)	pH (g/L)	Glycine concen. (g/L)	Pulp Density (g/L)	Cu Solubilization (%)		Ag Solubilization (%)	
					Experimental	Predicted	Experimental	Predicted
1	37	9	5	100	29	41.22	16	13.67
2	27	9	5	55	48	47.90	18	18.94
3	34	8	7.5	10	41	42.90	27	26.89
4	40	9	5	55	70	70.22	27	26.89
5	37	9	5	55	48	47.90	18	18.94
6	37	9	7.5	55	49	49.22	17	18.21
7	37	8	7.5	55	42	42.88	26	23.34
8	34	8	2.5	100	33	28.90	5	9.17
9	34	8	7.5	100	38	37.63	13	14.29
10	40	10	7.5	100	38	37.63	13	14.29
11	37	9	5	55	48	47.90	18	18.94
12	37	9	2.5	55	46	46.00	23	19.00
13	34	10	7.5	100	36	36.56	7	7.5
14	37	9	5	10	60	58.00	28	27.45
15	40	9	7.5	10	93	84.62	44	43.79
16	37	10	5	55	41	40.33	19	18.78
17	40	8	7.5	10	93	84.62	44	43.79
18	37	10	5	55	48	47.90	18	18.94
19	37	9	5	100	41	41.78	13	16.24
20	34	10	2.5	10	49	48.12	12	12.62
21	37	9	5	55	48	47.90	18	19.94
22	34	9	5	55	44	44.00	16	13.23
23	37	9	5	55	48	47.90	18	18.94
24	40	8	7.5	10	63	72.56	31	31.17
25	34	8	2.5	10	41	43.18	24	25.45
26	40	8	7.5	100	57	63.34	28	27.95
27	40	8	2.5	100	52	48.62	19	19.34
28	40	10	2.5	100	43	46.56	21	22.94
29	40	8	2.5	10	74	78.90	39	39.62
30	34	10	2.5	10	33	35.84	24	24.40
31	34	10	7.5	10	42	39.84	14	14.01

4.6.2 Statistical analysis through ANOVA

The significance of the parameters that affect the solubilization of Cu and Ag was determined through analysis of variance (ANOVA) and evaluated at $p<0.05$ with a 95% confidence interval. In 2011, Rastegar et al. stated that lower *p*-values ($p < 0.05$) indicate the statistical significance of the corresponding coefficient with a high confidence level*,* where *p*-values represent the probability of errors and thus validate the model [220]. Table 4.5 shows the ANOVA analysis to reveal the factors with significant *p*-values influencing Cu and Ag bioleaching at the linear, quadratic, and interactive levels. The correlation between the experimentally obtained and software-predicted bioleaching responses was determined using R2 values to assess the model fit of the obtained data. A value close to one for R2 indicates a better fit of the data to the regression line of the model. In the current investigation, Cu and Ag leaching responses corroborated the R^2 values 0.93 and 0.97, respectively. These high R^2 values suggested that the models are reliable and reproducible under experimental conditions. Therefore, the R^2 value for the obtained data confirms a strong correlation between the software-predicted and experimentally obtained bioleaching responses.

Table 4.5 ANOVA analysis to depict the significance of factor that affected Cu and Ag solubilization at $p<0.05$

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cu Solubilization					
Model	14	5317.94	379.85	15.76	0.000
Linear	4	4437.06	1109.26	46.03	0.000
Temperature	1	3094.22	3094.22	128.39	0.000
pH	1	29.39	29.39	1.22	0.286
Glycine	1	46.72	46.72	1.94	0.183
Pulp density	1	1266.72	1266.72	52.56	0.000
Square	4	358.88	89.72	3.72	0.025
Temperature*Temperature	1	220.04	220.04	9.13	0.008
pH*pH	1	102.74	102.74	4.26	0.056
Glycine*Glycine	1	0.22	0.22	0.01	0.925

Pulp density*Pulp density	1	7.57	7.57	0.31	0.583
2-Way Interaction	6	522.00	87.00	3.61	0.019
Temperature*pH	1	81.00	81.00	3.36	0.085
Temperature*Glycine	1	36.00	36.00	1.49	0.239
Temperature*Pulp density	1	256.00	256.00	10.62	0.005
pH*Glycine	1	64.00	64.00	2.66	0.123
pH*Pulp density	1	4.00	4.00	0.17	0.689
Glycine*Pulp density	1	81.00	81.00	3.36	0.085
Error	16	385.61	24.10		
Lack-of-Fit	10	385.61	38.56	*	*
Pure Error	6	0.00	0.00		
Total	30	5703.55			
R^2	93.24				
R^2 (Adj)	87.32				
Ag Solubilization					

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Model	14	2110.35	150.739	39.33	0.000
Linear	4	1791.67	447.917	116.86	0.000
Temperature	1	840.50	840.500	219.28	0.000
pH	1	93.39	93.389	24.36	0.000
Glycine	1	3.56	3.556	0.93	0.350
Pulp density	1	854.22	854.222	222.86	0.000
Square	4	128.68	32.171	8.39	0.001
Temperature * Temperature	1	3.26	3.263	0.85	0.370
pH*pH	1	11.68	11.678	3.05	0.100
Glycine*Glycine	1	0.37	0.372	0.10	0.759
Pulp Density*Pulp Density	1	6.82	6.822	1.78	0.201
2-Way Interaction	6	190.00	31.667	8.26	0.000
Temperature*pH	1	0.25	0.250	0.07	0.802
Temperature*Glycine	1	12.25	12.250	3.20	0.093

Temperature*Pulp density	1	16.00	16.000	4.17	0.058
pH*Glycine	1	121.00	121.000	31.57	0.000
pH*Pulp density	1	20.25	20.250	5.28	0.035
glycine*Pulp density	1	20.25	20.250	5.28	0.035
Error	16	61.33	3.833	*	*
Lack-of-Fit	10	57.90	5.790	10.13	0.005
Pure Error	6	3.43	0.571		
Total	30	2171.68			
R^2	97.18				
R^2 (Adj)	94.71				

4.6.3 Regression analysis

The relationship between the various parameters of the model and the bioleaching response of Cu and Ag metals was determined using non-linear regression kinetics. This analysis involved expressing the fitted data model as a mathematical function, wherein non-significant parameters were eliminated. The study revealed the second-order kinetics governing the solubilization of Cu and Ag by *B. sporothermodurans* ISO1. The final coded equation represents the correlation between the bioleaching factors' linear, quadratic, and interactive effects and the corresponding response obtained. Equation 4.4 represents the percentage recovery of Cu metal, while Equation 4.5 represents the percentage recovery of Ag metal. These equations incorporate variables such as temperature, pH, glycine concentration, and pulp density in their linear, squared, and interactive forms.

Cu recovery = $238 - 38.2A + 116.2B - 13.3C - 1.237D + 0.859A^2 - 0.77B^2 + 0.277C^2 + 0.00406D^2 - 2.687AB + 0.508AC - 0.0069AD - 1.325BC + 0.0764BD + 0.0450CD$ (4.4)

Ag recovery = $82 - 0.4A - 19.3B + 4.35C - 0.119D + 0.023A^2 + 1.20B^2 - 0.128C^2 + 0.000347D^2 + 0.021AB + 0.1917AC - 0.00602AD - 1.175BC + 0.0125BD + 0.00833CD$ (4.5)

Where A, B, C, and D represent the value of temperature (ºC), pH, Glycine Concentration (g/L), and Pulp density (g/L), respectively.

4.6.4 Response surface plot

The 3D surface model was utilized to illustrate the value of the response variable (i.e., percent solubilization of Cu and Ag), which is influenced by two independent predictor parameters, such as incubation temperature, pH, pulp density, and precursor amount.

(i) Cu Recovery

Table 4.5 enlisted the various parameters significantly affecting Cu recovery in their linear, square, and quadrant way at $P<0.05$. Figure 4.12 illustrates the percentage solubilization of Cu by *B. sporothermodurans* ISO1 according to various independent variables. Figure 4.12a illustrated that the glycine concentration and pH as two independent variables that exerted a significant influence on the solubilization of Cu in their cumulative manner, and *B. sporothermodurans* ISO1 showed maximum solubilization of Cu at a pH value of 8.5 and glycine concentration of approximately 7.5g/L. In addition, 40ºC incubation temperature and pulp density of less than 20g/L attained maximum recovery of Cu metal. Figure 4.12d depicts the extent of precursor and PD that significantly affected the Cu solubilization. 7.5g/L glycine supplementation achieved maximum solubilization of Cu at 10 g/L pulp density.

(ii) Ag Recovery

The effect of several significant variables on the Ag solubilization capacity of *B. sporothermodurans* ISO1 is illustrated in Figure 4.13. Table 4.5 shows that temperature, pH, and pulp density were the independent variables analyzed significantly through ANOVA. Figure 4.13a depicts those higher temperatures (i.e., 40ºC) and pH greater than 8 favor the simultaneous solubilization of Ag metals in the presence of Cu. The effect of glycine precursor and pulp density in Figure 4.13d shows that solubilization of Ag at pulp density at higher pulp density requires glycine concentration greater than 5g/L.

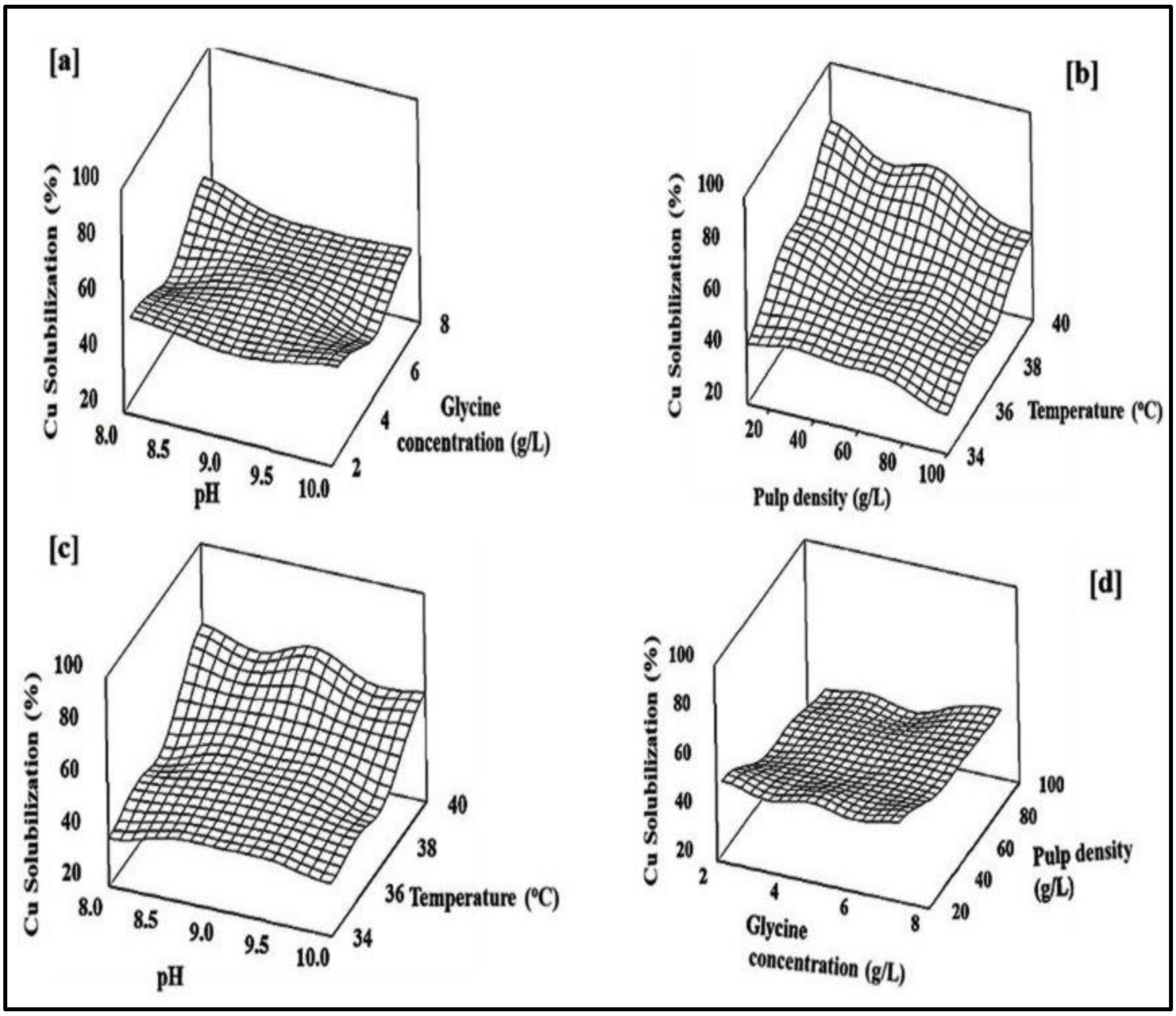


Figure 4.12 3D surface structure to illustrate the cumulative effect of two independent variables on Cu solubilization (a) Effect of pH and glycine concentration on Cu solubilization **(b)** Pulp density and temperature **(c)** pH and temperature **(d)** Glycine concentration and pulp density.

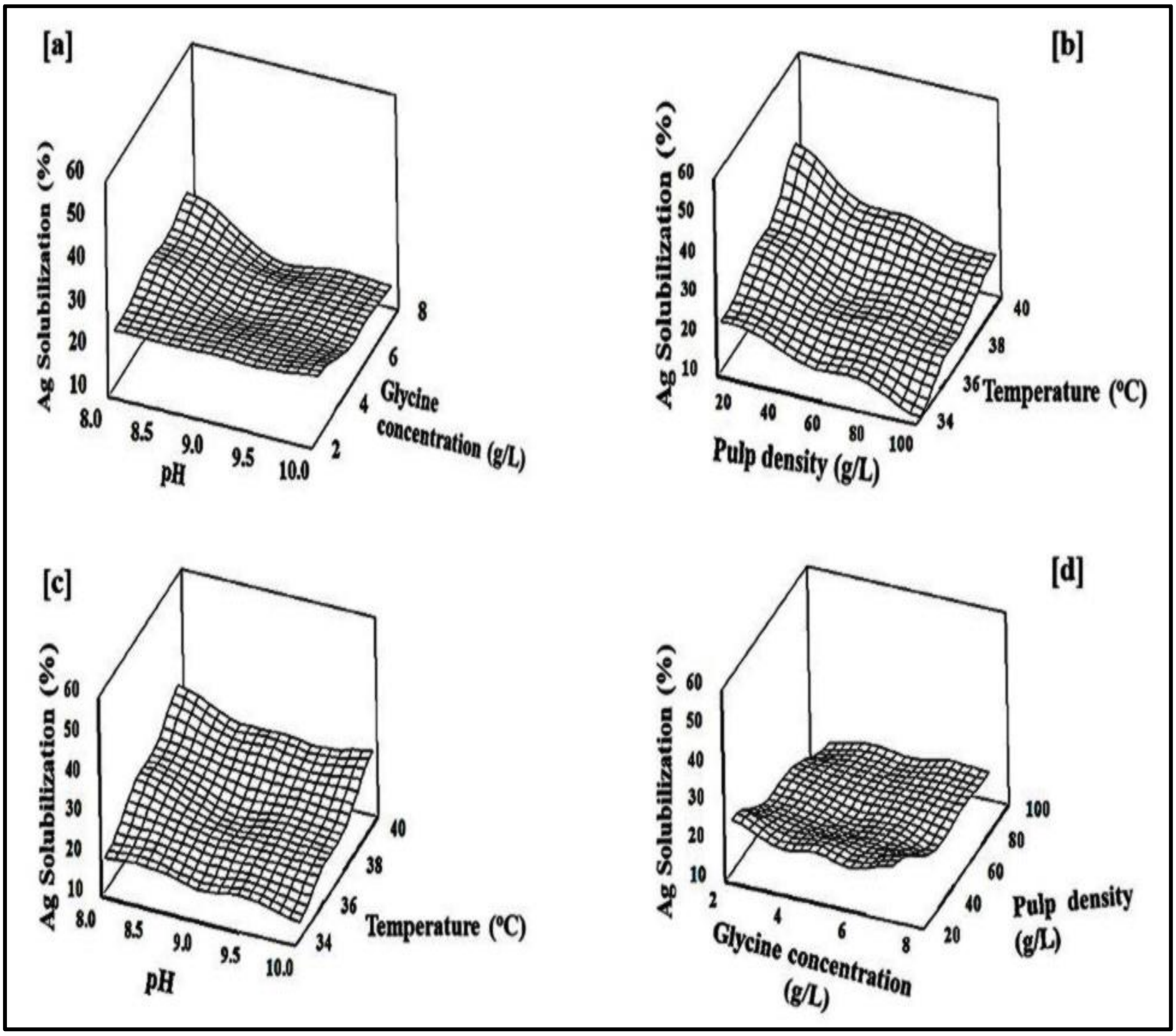


Figure 4.13 3D surface plot to illustrate the interactive effect of two independent variables on Ag solubilization **(a)** Effect of glycine and pH on Ag solubilization **(b)** Effect of temperature and pulp density **(c)** Temperature and pH **(d)** Glycine and pulp density.

4.7Augmentation in metals leaching capacity of potential bacterial isolates

4.7.1 Metals leaching through chemo-biohydrometallurgy method for the base and precious metals solubilization

A single bioleaching process is insufficient for effective metals recovery at large-scale bioleaching because of a higher concentration of base metals than precious metals in e-waste, and base metals consume most of the biogenic lixiviant (i.e., CN^-) produced by microorganisms. Therefore, an abundance of Cu hinders the retrieval rate of precious metals and thus limits its implementation at industrial-scale operations. Consequently, researchers intended to develop new economical methods to enhance the metal leaching rate even at large-scale operations. In recent years, chemo-biohydrometallurgy methods have attracted considerable attention among researchers. These methods involve sequentially utilizing chemical and biological lixiviants to recover diverse metals. This approach employs a chemical lixiviant to recover Cu metal before initiating the bioleaching process. This approach has been substantiated as an effective method for enhancing the solubilization of precious metals through biocyanidation. Many chemical lixiviants have been studied to recover Cu metal before the bioleaching of precious metals from PCBs. Ferrous sulfate was used by Shah et al. for the recovery of base metals (Cu, Zn, Ni) from PCBs before the bioleaching of precious metals by the iron-oxidizing consortium. They obtained 84.16 % Cu recovery through 3g ferrous sulfate [221].

Moreover, Das and Ting reported 85.5% Cu recovery through strong sulfuric acid with hydrogen peroxide [222]. Furthermore, Khatri and his co-workers reported 99 % recovery of Cu within 5 days of incubation using ferric and ferrous sulfate (70:30) [223]. In addition, one researcher suggested the pretreatment of e-waste with nitric acid before the bioleaching process to improve the application of this economical method to manage this swiftly growing solid waste stream [117].

In the present study, ferric chloride ($FeCl_3$), a chemical lixiviant, was utilized for Cu solubilization before the bioleaching process to enhance metal recovery through biocyanidation. $FeCl_3$ reacts with shredded dust of waste CPCBs and leaches Cu metal into a liquid solution. Cu dissolution in $FeCl_3$ solution was accredited to Fe^{3+} ions as oxidizing agents and Cl^- ions as complexing agents. Additionally, this mild acid has long been employed in the Cu etching process during the fabrication of microelectronic components, according to Cakir et al. $FeCl_3$ is a practical, environmentally acceptable, cost-effective chemical lixiviant for Cu etching [224].

4.7.1.1 Assessment of ferric chloride concentration and incubation period

The study aimed to accomplish the Cu metal leaching before the biocyanidation leaching process because the abundance of Cu content hinders the other metals' leaching rate and impediments the utilization of the bioleaching process at a large scale. Therefore, the concentration of $FeCl_3$ was optimized at different incubation periods to target the recovery of Cu metal without altering the presence of precious metals. Figure 4.14a illustrates the trend of

Cu solubilization achieved at different concentrations of $FeCl_3$ lixiviant at a 50°C incubation period. It shows that 1% $FeCl_3$ lixiviant showed maximum recovery of Cu (95%) without significantly altering the concentration of other metals. Further, an increase in $FeCl_3$ concentration did not affect the Cu recovery. Therefore, a 1% FeCl3 solution was considered the optimum amount of chemical lixiviant that can lead to efficient recovery of Cu metal to make the bioleaching process more efficient.

Additionally, compared to earlier reported studies for chemical pretreatment of e-waste, $FeCl_3$ attained Cu leaching in the shortest period. The presence of two reactive components in $FeCl_3$ lixiviant contributes to this phenomenon. The ferric ions (Fe3+) act as potential oxidizing agents, while chloride ions (Cl^-) function as potent complexing agents that expedite the Cu solubilization rate. The $FeCl_3$ facilitated Cu solubilization $FeCl_3$ can be ascribed as per equations 4.6, 4.7, and 4.8. The optimal period for leaching out maximum Cu was a 3h incubation at 50°C (illustrated in Figure 4.14a). During the reaction of $FeCl_3$ with PCB e-waste, $FeCl_3$ initially dissociates into Fe^+ and Cl^- ions. Fe^{3+} oxidizes Cu metal while reducing itself to Fe^{2+}, while Cl^- ions form a complex with oxidized Cu metal, forming $CuCl_2$. $CuCl_2$ also acts as an oxidizer (as shown in equation 4.7) to further oxidize Cu once $FeCl_3$ is completely utilized. Therefore, $FeCl_3$ lixiviant provides two oxidizing agents that reduce the required concentration of the lixiviant compared to other reported chemical lixiviant [225]. The thermodynamic nature of the chemical reactions occurring during the chemical treatment with $FeCl_3$ lixiviant was evaluated regarding Standard Gibbs free energy (ΔG^0). The initial reactions between $FeCl_3$ and Cu were unfavorable, non-spontaneous, and highly endergonic ($\Delta G>0$), which require energy for complex formation. However, the overall reaction was found to be favorable, spontaneous, and exergonic ($\Delta G<0$) in nature.

$$FeCl_3 + CuCl \longrightarrow FeCl_2 + CuCl_2 \quad \Delta G^0 = +749.76 \qquad (4.6)$$

$$CuCl_2 + Cu \longrightarrow 2CuCl \quad \Delta G^0 = -10,011.82 \qquad (4.7)$$

Overall reaction: $$FeCl_3 + Cu \longrightarrow FeCl_2 + CuCl \quad \Delta G^0 = -9370.1 \qquad (4.8)$$

One-way ANOVA statistical analysis assessed the significance of variables (i.e., the concentration of $FeCl_3$ and incubation period) that affected the Cu recovery rate at $p \leq 0.05$ (Figure 4.14b). Figure 4.14b shows that the Cu solubilization rate at 0.5% $FeCl_3$ varied significantly from 1% and 2% $FeCl_3$ solution with time from 0 to 180 mins. At 120 and 180 mins of the incubation period, there was a non-significant difference in the Cu solubilization at $p < 0.05$. 1% $FeCl_3$ showed significant variation in Cu solubilization from 0 to 180 mins of the incubation period. The overall Cu recovery obtained with 1% $FeCl_3$ at 60, 120, and 180 mins was 78%, 94%, and 95%, respectively.

The non-linear regression analysis (shown in table 4.6) depicted the chemical reaction between $FeCl_3$ lixiviant and Cu metal present in shredded dust CPCBs as a second-order reaction as per the equation below:

$$\text{Overall reaction: } FeCl_3 + Cu \xrightarrow{\qquad} FeCl_2 + CuCl \quad \Delta G^0 = -9370.1 \tag{4.8}$$

The reaction follows a second-order reaction, and kinetics is as follows:

$$1/[A] = 1/[A]_o + kt \tag{4.9}$$

Here, $[A]$ and $[A]_o$ changes in the initial concentration of Cu with incubation time and initial concentration of Cu.

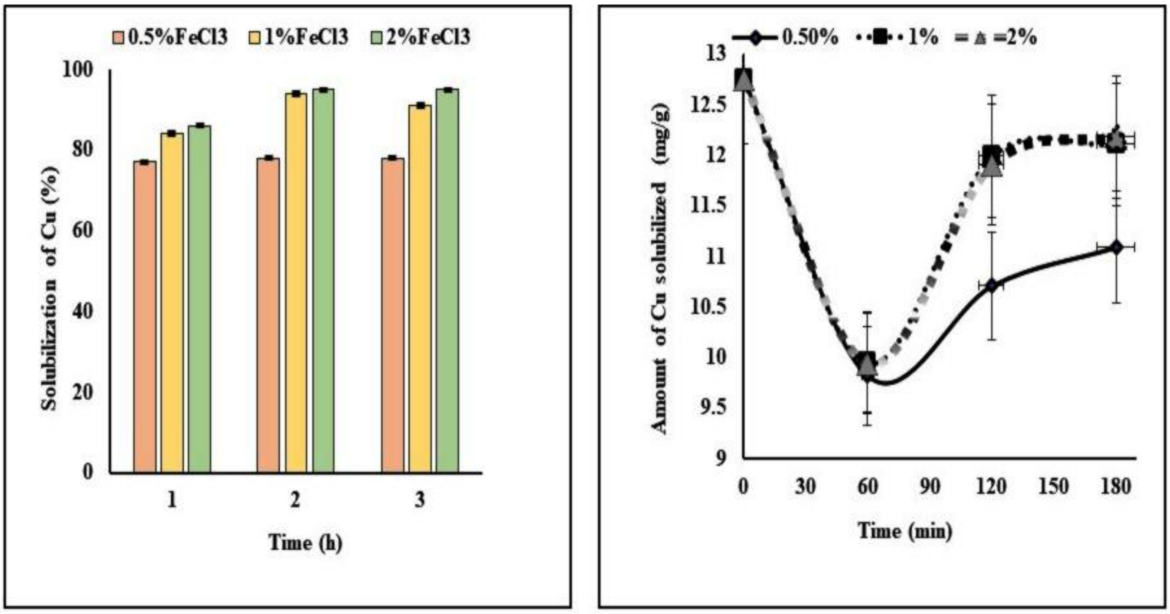
If we plot $1/[A]$ vs. t (time), it will provide a straight line where slope = k

$$t_{1/2}\,(M^{-1}S^{-1}) = 1/k[A]_o \tag{4.10}$$

In present experiments, $[A]$ is 12.75 mg/g.

Table 4.6 Statistical analysis ($p \leq 0.05$) of Cu recovery with different $FeCl_3$ concentrations and incubation time intervals.

Time (min)	**Cu recovery rate at different conc. of $FeCl_3$ (mg/g ± SD)**		
	0.5% $FeCl_3$	**1% $FeCl_3$**	**2% $FeCl_3$**
0	12.75 ± 3.78	12.75 ± 3.78	12.75 ± 3.78
60	9.82 ± 0.134	9.95 ± 0.378	9.94 ± 0.089
120	10.71 ± 0.186	11.99 ± 0.087	11.91 ± 0.216
180	11.09 ± 0.087	12.11 ± 0.056	12.18 ± 0.113
Equation of Line	Y = 0.00286806x + 0.11995841	Y = 0.00901809x + 0.01683805	Y = 0.00974744x - 0.03597942
R^2	0.96	0.94	0.97
$t_{1/2}$ ($M^{-1}S^{-1}$)	27.346 ± 0.207	8.697 ± 0.105	8.046 ± 0.107



(a) (b)

Figure 4.14 (a) Chemical leaching of Cu from shredded dust of waste CPCBs using 0.5, 1, and 2% $FeCl_3$ concentrations. **(b)** Statistical analysis of $FeCl_3$ concentration on Cu recovery at $p < 0.05$. The data represents the mean of triplicates at ±SD value.

4.7.1.2 Two-step bioleaching to extract metals other than cu

The pre-treated PCB e-waste was utilized to recover other metals, such as Ag and Au, through the biocyanidation process by *P. balearicaSAE1* and *B. sporothermodurans* ISO1. The bioleaching potential of these two bacterial strains was compared to implement the better one at a large scale. The two-step bioleaching process opted to solubilize the metals leaching through the biocyanidation process by these bacterial strains. According to reported studies, the one-step bioleaching process is not advantageous as bacterial growth ceases due to the toxic nature of e-waste [50, 106, 217]. Figure 4.15 elucidates a comparison of the solubilization rate of metals by *B. sporothermodurans* ISO1 and *P. balearica*SAE1 after the pretreatment of CPCBs with $FeCl_3$ chemical lixiviant. We aim to compare the metal solubilization rate of our potential bacterial isolate (*B. sporothermodurans* ISO1) with *P. balearica*SAE1 to evaluate the leaching efficiency of these two distinct cyanogenic strains. So, the strain with the higher bioleaching potential can be implemented for a pilot-scale operation through the chemo-biohydrometallurgical approach. *P. balearica* was preferred over *C. violacium* because it exhibits a higher toxicity tolerance and leaching efficiency. Researchers assessed the potential of *C. violacium* and obtained only a 22.5% recovery of Au through mutation with 100 mM

NNitroso-N-ethyl urea as a growth promoter mutagen [216]. In another investigation, in 2015, Natrajan and Ting achieved only 11.3% Au recovery at 0.5% electronic scrap medium (ESM) pulp density [113]. Additionally, Li and colleagues investigated the bioleaching capacity of *C. violacium* and attained only 20% Au solubilization from discarded PCBs [226]. Moreover, further experimentation to enhance the rate of Au solubilization through pretreatment of waste PCBs for Cu solubilization with iron-oxidizing bacteria led to 40% Au recovery. Thus, the specificity limitation of this strain was unveiled by all of these investigations that claimed exclusively Au recovery by *C. violacium*. In addition, Kumar and co-workers reported a lower toxicity tolerance of *C. violacium* (83.70g/L) than *P. balearica*SAE1 (300g/L) towards waste CPCBs with a particle size of 150μm [13].

The comparison of the present potential isolate with *P. balearica*SAE1 in Figure 4.15 inferred that *B. sporothermodurans* ISO1 exhibited higher bioleaching of metals than *P. balearica*SAE1. Prior treatment of CPCBs before the bioleaching process resulted in improved solubilization of many metals, such as a 44% to 57% increase in Ag solubilization, as well as substantial recovery rates for other metals, including Au at 67%, Pt at 60%, Fe at 49%, Ni at 32%, and Co at 27%. These findings suggested that this indigenous bacterial isolate exhibit more potential for biogenic lixiviant production than *P. balearica*SAE1. Additionally, the constraints related to the specificity of the bioleaching method were surpassed by the prior recovery of Cu, which facilitated the availability of biocyanide for these metals. As a result, the chemo-biohydrometallurgical hybrid method, as explained in section 4.7.1, emerged as a significant approach for recovering a wide range of metals from the shredded dust of CPCBs. From an environmental perspective, by employing $FeCl_3$ as a mild acid, the limitations associated with using strong acids such as HCl and HNO3 in conventional hydrometallurgy techniques were effectively eliminated [19]. In addition, the bioleaching efficiency of *B. sporothermodurans* ISO1 was improved for metals other than Cu, which can mitigate the selectivity constraint of the bioleaching process. So, this method may be considered as a potential way to recover metals from e-waste at industrial scale bioleaching operation; as such, hybrid methods have gained the attention of many biometallugry industries over conventional methods (i.e., Pyrometallurgy and hydrometallurgy) to recover metals for e-waste at large scale operation to sustain the urban mining strategies. A notable bio-metallurgy corporation, "Mint Innovation," located in Auckland, New Zealand, has emerged as a leader in the field, pioneering chemo-biohydrometallurgical processes to recover precious and base metals from the e-waste efficiently [151].

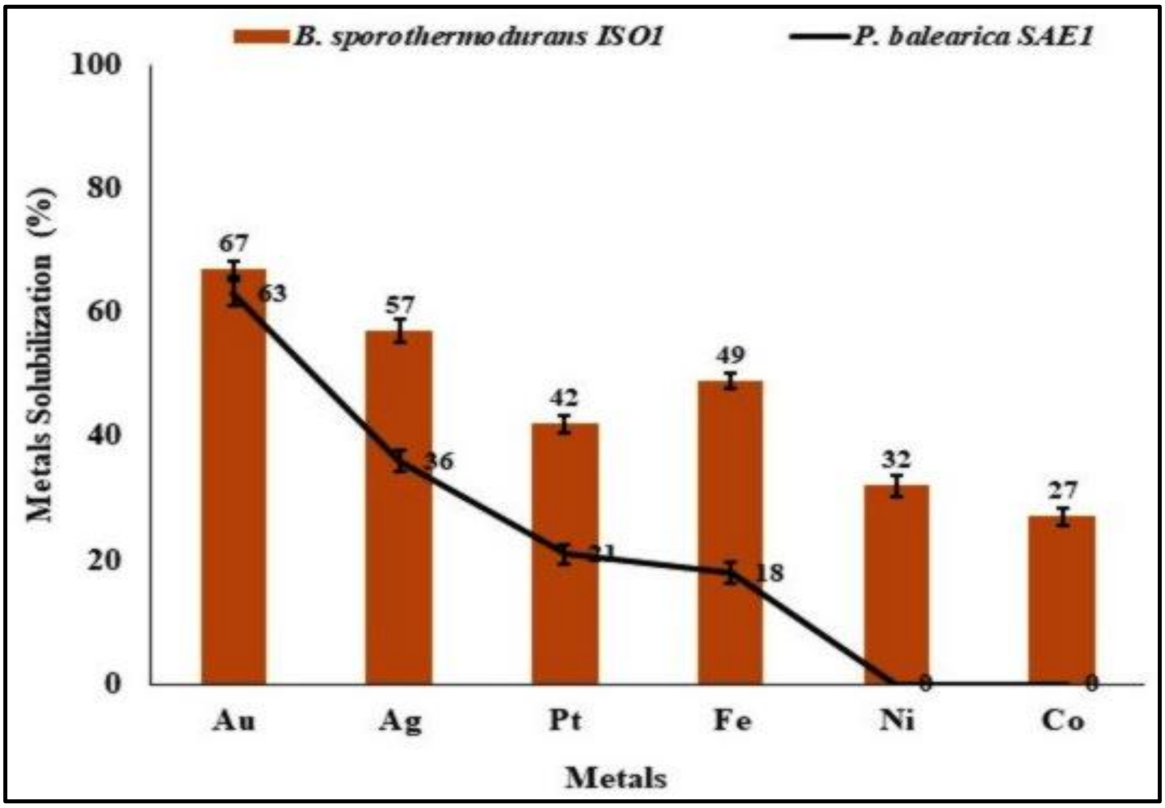


Figure 4.15: Metals recovery by *B. sporothermodurans* ISO1 at 40ºC and *P. balearica*SAE1 at 37ºC using 7.5g/L glycine precursor at pH9 from $FeCl_3$ pre-treated waste CPCBs.

4.7.2 Methionine stimulant for efficient bioleaching process

Section 4.7.1.2 elucidated the advantages of the chemo-biohydrometallurgy approach and $FeCl_3$ chemical lixiviant. But to make the process more economical and eco-friendlier, the present study further endeavored to explore an alternative that makes it feasible to limit the use of chemical lixiviant and make the biocyanidation process sufficient and economically viable for leaching of base and as well precious metals from shredded dust CPCBs**.** The current objective was to increase the production and availability of cyanide lixiviant by *B. sporothermodurans* ISO1 by using a methionine stimulant.

Methionine is a proteinogenic amino acid pivotal in initiating translation and S-adenosyl methionine cofactor, a leading cellular carrier of methyl groups [227]. In addition, the methionine significantly impacted the growth of *B. sporothermodurans* ISO1 (as mentioned in section 4.5.1); therefore, it was considered a suitable stimulant for cyanide production and metal solubilization. The methionine concentration was optimized to influence biomass production and the cyanogenesis process. Figure 4.16 depicts that 1g/L methionine and 5g/L of glycine precursor were optimum for stimulating significant biomass production and cyanogenesis, implying the maximum metal solubilization. 1g/L methionine led to provide the cyanide for solubilization of 87% Cu and 76% Au metal.

Meanwhile, more than 1g/L methionine decreased the solubilization of both metals (78% Cu and 43% Au). Therefore, methionine above 1g/L did not significantly impact cyanide production since it may even become toxic and cease the growth of *B. sporothermodurans* ISO1. Many studies also reported that methionine as a positive supplement to medium upsurge the biogenic cyanide production that may be attributed to methyl group donor to the cellular activities and due to induced expression of HCN synthase enzyme [228] [229]. During the initial investigation of the biocyanidation process of cyanogenic microorganisms, Michaels and Corpe proposed using methionine, betaine, dimethyl glycine, and choline for elevation in bio-CN yield [230]. The current study's finding, methionine for cyanogenesis stimulation, has been supported by Castric [231]. They evaluated the effect of methionine additive on *Pseudomonas aeruginosa* to enhance glycine-stimulated cyanogenesis at glycine concentration of between 1-20 mM and found that above these levels of glycine, methionine does not affect the cyanogenesis [231]. Without glycine, methionine concentrations between 5 and 30 mM do not stimulate cyanide production. The precise mechanism of methionine utilization in cyanogenesis is uncertain, but these studies implied that the methylation of homocysteine to generate methionine is a critical utilization of the C-1 tetrahydrofolate pool that influences cyanogenesis in *C. violaceum* [232]. However, not many studies related to this concept have been reported, and the chemo-biohydrometallurgy approach has been emphasized more to improve the role of the bioleaching process over the past two decades. But now, a recent study by Aminian-Dehkordi has investigated the role of other amino acids that can influence cyanide production. They suggested the role of cysteine in cyanide overproduction by *B. megaterium* for gold leaching from waste PCBs [200].

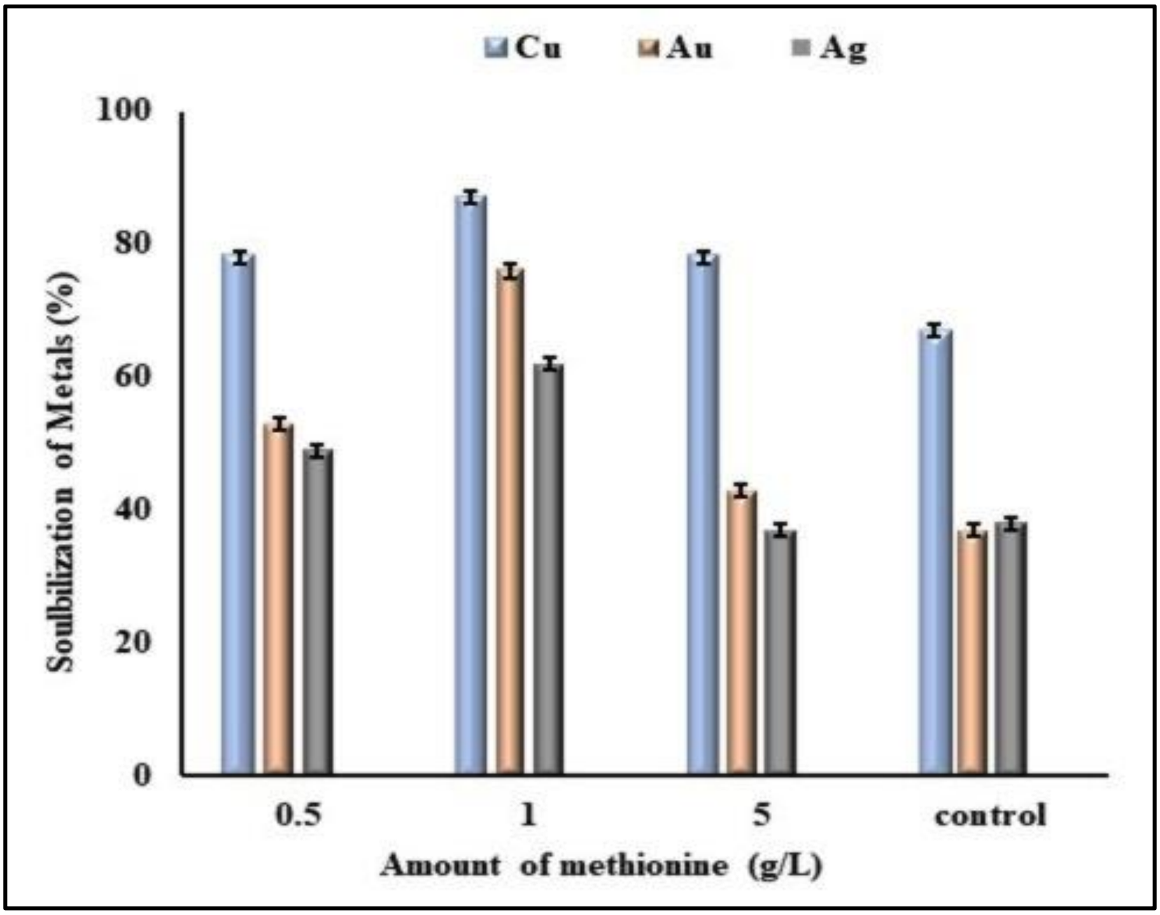


Figure 4.16: Percentage solubilization of metals by *B. sporothermodurans* ISO1 at 40°C temperature, pH 9, glycine concentration 7.5g/L supplemented with 1g/L methionine as a suitable stimulant.

4.7.3 Implementation of two-step bioleaching at optimized conditions

The two-step bioleaching with an optimized methionine stimulator was carried out under optimized conditions (i.e., 40°C temperature, pH 9, 10g/L PD, 1g/L methionine, and 7.5g/L glycine precursor) to assess the augmentation in the metal solubilization capability of *B. sporothermodurans* ISO1. 1g/L methionine supplementation showed a significant effect on glycine utilized biocyanidation (shown in Figure 4.16). However, it was not utilized as a direct precursor by *B. sporothermodurans* ISO1 for cyanide production (as mentioned in section 4.5.1). The methionine may contribute to stabilizing the hydrogen cyanide synthase, increasing the availability of more biogenic cyanide lixiviants, and increasing the solubilization of metals other than Cu metal. Figure 4.17 illustrates the metal solubilization rate of *B. sporothermodurans* in the presence of a 1g/L methionine stimulator. It determines that *B. sporothermodurans* ISO1 produced an adequate amount of lixiviant, accelerating the solubilization of metals other than Cu. The *B. sporothermodurans* ISO1 demonstrated effective Cu, Au, Ag, Fe, Ni, and Co leaching. According to the author, this strain can potentially overcome the earlier reported bacterial strain's specificity limitations.

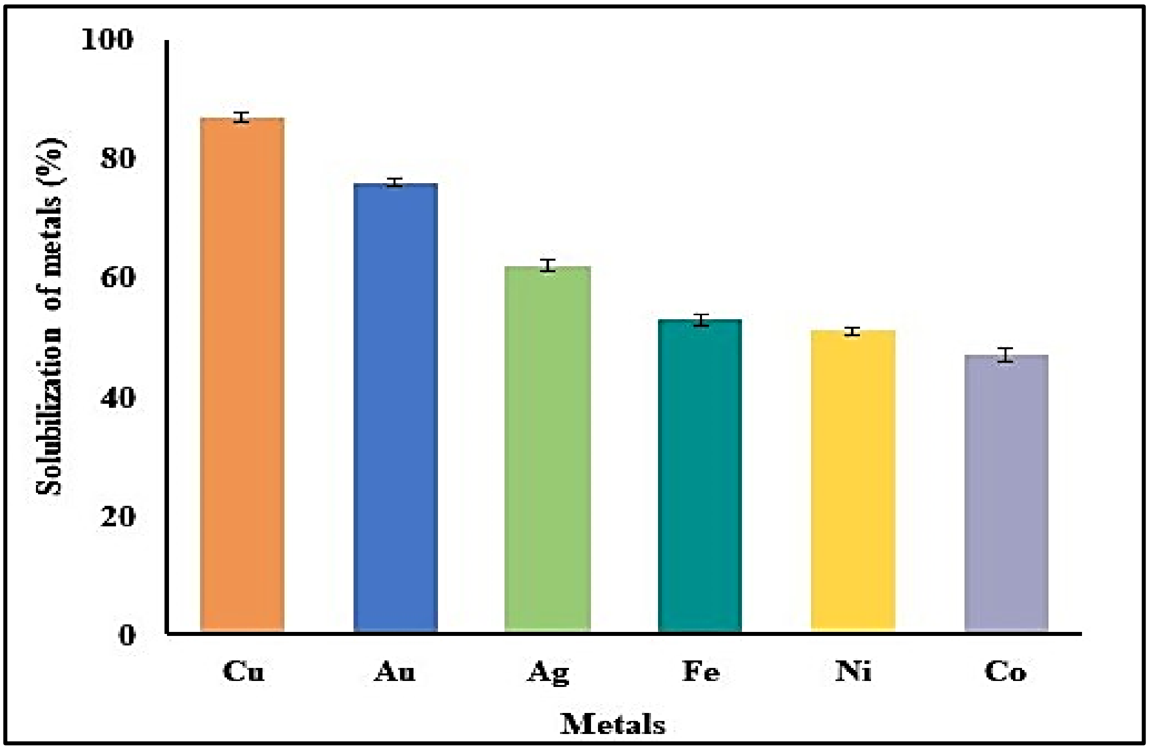


Figure 4.17: Recovery of metals using 1g/L methionine, 7.5g/L at 40°C temperature, pH9, and 7.5g/L glycine to enhance the biocyanidation and bioleaching of metals. The data represents the mean of triplicates along with the ±SD value.

4.8 Biocyanidation solubilization of metals at 3L working volume

Assessing leachate from a substantial bioleaching volume is vital to ensure consistent and representative outcomes of small-scale bioleaching. Initially, metal leaching was evaluated in shake flasks with optimized parameters, where pulp density was limited to a few grams, usually less than 10%, which is an optimal concentration for maximizing leaching. However, the heterogeneous nature of e-waste poses challenges in representing such a small sample in the initial bioleaching batch. The composition and solubilization of metals continually fluctuate, posing difficulties in evaluating the outcomes of respective operations [201]. Therefore, the bacterial growth and biocyanidation were assessed at a large volume (3L) with an optimal pulp density (10g/L). The volume of the bioleaching solution (i.e., 3L) was determined considering the flasks' availability to be operated in shaking incubators since bioleaching was carried out in 5000mL Erlenmeyer flasks under optimal conditions.

Current findings on various metals' solubilizations at an increased volume represent a repeated scenario of small-scale operations and unveil the potential for industrial-scale implementation of this promising economic approach. The rate of Cu solubilization and pH change of the medium at an increased volume of leachate solution is shown in Figure 4.18a. The figure depicts a progressive increase in the recovery of metals, which was facilitated by the increase in pH. The figure shows that metal solubilization was initiated on 2nd day of the bioleaching period, as 22% Cu was recovered on 2nd day of the incubation period. The leaching performance improved remarkably with the prolongation of leaching time. Cu and Au's concentrations and leaching ratios peaked at day 13 (i.e., 62 and 52%, respectively) and then plateaued.

Furthermore, the final alkaline pH aided in forming more CN^- and enhanced the solubilization of other metals, which is evident for increased metal solubilization at this substantial volume. The 52%, 40%, and 37% solubilization of Au, Co, and Ni were obtained after Cu leaching (Figure 4.18b). The initial pH of the medium decreased to 8.6, which is attributed to bacterial growth and HCN production during the early stages of bioleaching. The most plausible explanation for this initial decrease in the pH is the mild acidification process during the conversion of organic compounds into carboxylic acids such as glyoxylic, cyano-formic, and oxamic acids and HCN production [108, 213]. Furthermore, the release of metabolites in the early phase influences biomass production and HCN production. Subsequently, a progressive intensification in pH was observed after supplementation of the waste CPCB sample, which was contributed to by metal-cyanide complex formation. The trend of current findings is also supported by numerous studies that found a comparable pattern of pH variation during the process of metal extraction from discarded PCBs through the utilization of the cyanogenic bacterium *B. megaterium* and indigenous *P. balearica*SAE1, respectively [13, 211].

Moreover, in 2020, Arab et al. also claimed the pH variation with an initial pH of 7–10 converged to approximately 8.2 during the initial growth phase and then elevated to approximately 9.2 [138]. All these outcomes suggested that pH is a pivotal parameter for an efficient leaching process as it influences the availability of CN^- and affects the metal's solubilization. Hence, optimizing initial pH and maintaining an alkaline environment during bioleaching is essential for efficient metal recovery.

The current study's finding unveils the industrial-scale applicability of bioleaching with *B. sporothermodurans* ISO1. However, a notable difference in leaching rates emerged upon transitioning to a large-scale volume, as indicated in Table 4.7. The declined efficiency of bacterial leaching at this scale could be attributed to various factors within the system, including mass flow dynamics, dissolved gases, and nutrient depletion. These elements play a pivotal role and necessitate careful consideration for successful large-scale bioleaching. However, it is essential to note a limitation in the current study. The bioleaching experiments were conducted in 5L shaking flasks within an incubator. This experimental setup simultaneously posed challenges in maintaining precise control over all contributing factors. The inability to perform leaching in a reactor, where conditions could be more rigorously controlled, represents a constraint in the study. It is noteworthy that this limitation has been addressed by numerous researchers in their investigations, emphasizing the importance of conducting bioleaching experiments in reactors for a more comprehensive understanding of the process under controlled conditions. In 2019, Garg et al. investigated the bioleaching potential of iron-oxidizing bacteria in a 2 L baffled glass reactor with a working volume of 1 L. They attained 98–99% copper recovery and reasonable Zn, Al, and Ni extraction from 15% pulp density under lab-scale optimization conditions [201]. The study conducted by Erust and co-workers in 2020 aimed to impend the optimal values of crucial parameters at the shake-flask level and assess the practicability of these optimal conditions in bench-scale bioreactors. The optimized parameters were utilized in laboratory-scale experiments that led to attaining maximum recovery Cu (98.1%) for Ni (79.5%) and Al (55.9%) within 8 days. Meanwhile, in the bench-scale reactor, optimized conditions yield a recovery rate of 97.3% for Cu, 55.8% for Al, and 79.3% for Ni. [233]. Moreover, another study investigated the metals leaching potential of acidophilic consortium in a double-stage continuous bioreactor. It claimed that at large-scale volumes, the bioleaching efficiency of acidophilic microorganisms depends on the solid-liquid-gas mass transfer, thus designing a reactor to perform the bioleaching process in two stages. The first column of the reactor was utilized for the cellular activity of microbial consortium, whereas the second column of the reactor was utilized to leach out the metals contained in PCBs through ferric ions lixiviant via mechanical stirring. Utilizing a two-stage bioreactor approach enabled the bacteria to adapt progressively to the presence of PCB toxic constituents and achieve high dissolution yields. They attained the significant leaching of Cu, Ni, Zn, and Co (i.e., 96, 73%, 85%, 93%) from 1% w/v PCB scraps into the double-stage bioleaching reactor. They further claimed to increase the PD to 1.8% without compromising the bioleaching performance in a double-stage bioreactor [207]. Thus, the significant findings of the reported studies and current study impelled the industrial-scale applications of this economical and eco-friendly technique for recycling vulnerable e-waste.

Table 4.7: Recovery of metals obtained through biocyanidation by *B. sporothermodurans* ISO1 at different working volumes.

S.No.	**Metals**	**The initial concentration of metals in CPCBs (mg/g)**	**Chemical leaching (Ferric chloride lixiviant)**	**Bioleaching at 250mL working volume**		**Bioleaching 3L working volume**	
				Concentration (mg/g)	Percentage recovery	Concentration (mg/g)	Percentage recovery
1	Cu	12.75	12.11 (95%)	11.09	87	7.90	62
2	Ag	10.34	-	6.41	62	4.96	48
3	Fe	2.46	-	1.30	53	1.01	41
4	Al	0.39	-	-	-	-	-
5	Ni	0.24	-	0.12	51	0.09	40
6	Au	0.04	-	0.03	76	0.02	52
7	Co	0.03	-	0.01	47	0.01	37
8	Pt	0.08	-	-	-	-	-

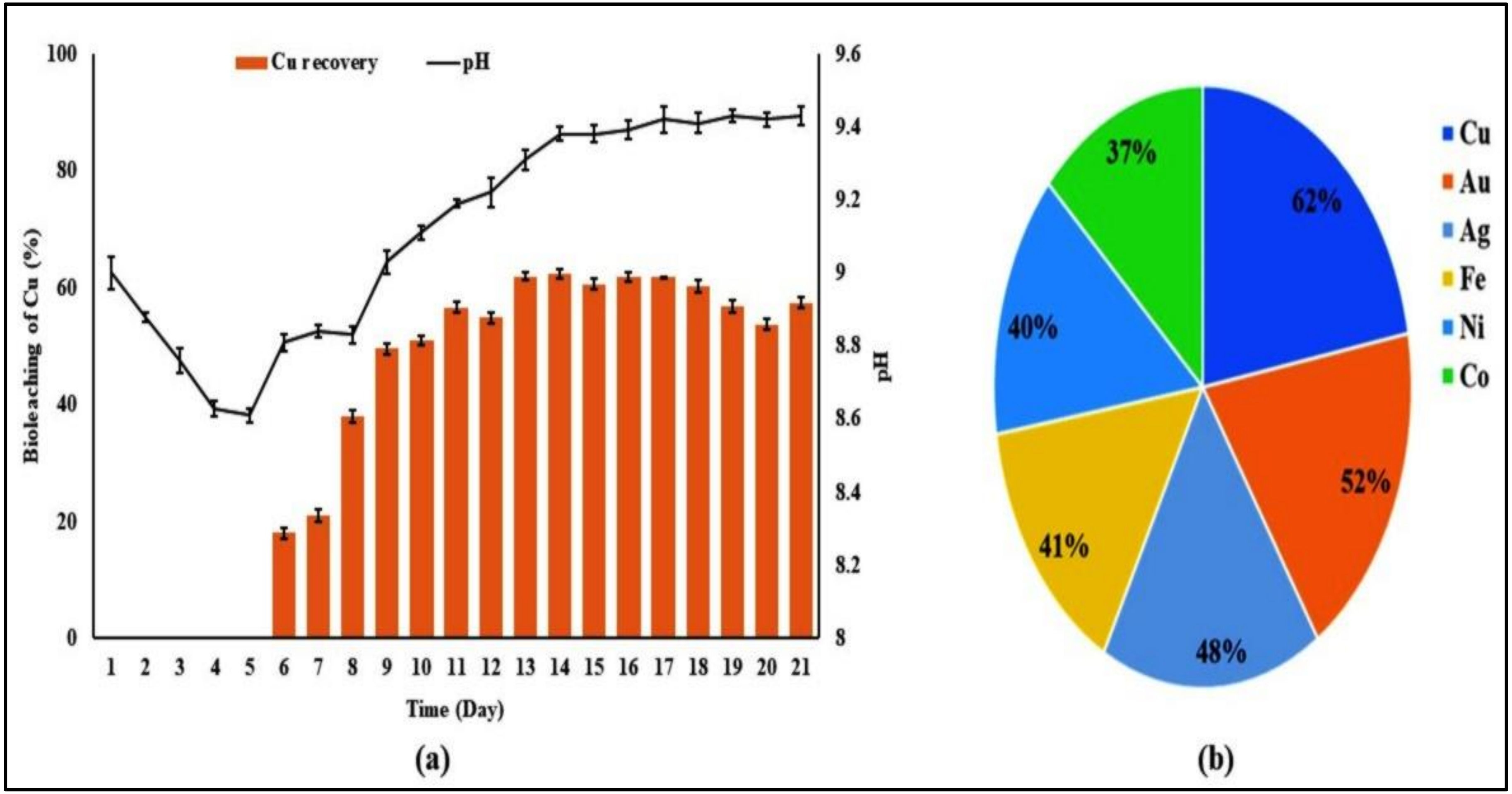


Figure 4.18 Metals leaching capacity of *B. sporothermodurans* ISO1 at 3L working volume (a) Cu solubilization and pH change during bioleaching process by *B. sporothermodurans* ISO1 at 40ºC temperature in NB medium supplemented with 1 g/L methionine inducer and 7.5g/L glycine precursor **(b)** Recovery of various metals on the 13th day of bioleaching process. Data represents the mean of triplicates with ±SD value.

4.9 Processing of residual e-waste after bioleaching of metals

The residual shredded dust CPCBs after bioleaching of various base and precious metals were characterized for metal content analysis. Table 4.7 indicates the metallic constituents left in the residual CPCBs after the bioleaching process. The concentration of the metals in residual CPCBs was lower than the feed-shredded dust CPCBs, which confirmed the leaching of these metals by *B. sporothermodurans* ISO1. In addition, a mass balance analysis via measuring the weight of the CPCB utilized in the experiment before and after bioleaching confirmed the leaching of metals. The weight loss of CPCB e-waste was up to 6.951 g after 20 days of *in vitro* bioleaching from 30 g of pulp density, which was initially supplemented. The bio-dissolution of different metals from shredded dust CPCB waste to leaching solution reduced the overall weight by 23.17%. Cu extraction was responsible for most weight loss (62% recovery), as the total weight of Cu was approximately 382.5 mg, whereas 62% Cu recovery consisted of 237.05 mg of Cu. Aside from Cu, Au, Co, and Ni, all contributed significantly to reducing CPCB weight. Other non-metallic compounds, such as plastic and Si, also contributed to the material's loss, although these were not estimated for mass analysis and spectroscopy detection but revealed through XRD analysis. Figure 4.19 revealed the change in the spectrum of various constituents in the leaching solution compared to the metallic constituents left in the residual waste. It is inferred from the Figure that most of the metals detected through AAS in bioleached solution (illustrated in Figure 4.18) showed maximum intensity in XRD analysis through JCPDS (detailed in Appendix D). The residual left after bioleaching did not show any significant peak, indicating that most of the material left in the residual waste is amorphous, mostly non-metallic. Bioleaching techniques utilizing cyanogenic microorganisms have demonstrated potential in extracting metals from waste CPCBs. However, achieving complete metal recovery remains always a challenge. Thus, residual waste left after the bioleaching process needs attention to safe and proper disposal. In the present case, the residual e-waste has been collected and responsibly handed over to our trusted industrial collaborator, Exigo Recycling Pvt. Ltd. They possess the necessary expertise and facilities, including a Treatment, Storage, and Disposal Facility (TSDF), to ensure the safe and proper disposal of the residual e-waste.

Table 4.8 Concentration of various metals found in shredded dust CPCBs after bioleaching through *B. sporothermodurans* ISO1 at 3L working volume.

S. No.	Metals	Concentration (mg/g)
1	Cu	4.32±0.89
2	Ag	4.21±1.02
3	Fe	1.21±0.68
4	Al	0.27±0.17
5	Ni	0.15±0.07
6	Au	0.01±0.002
7	Co	0.016±0.003
8	Pt	0.08 ± 0.06

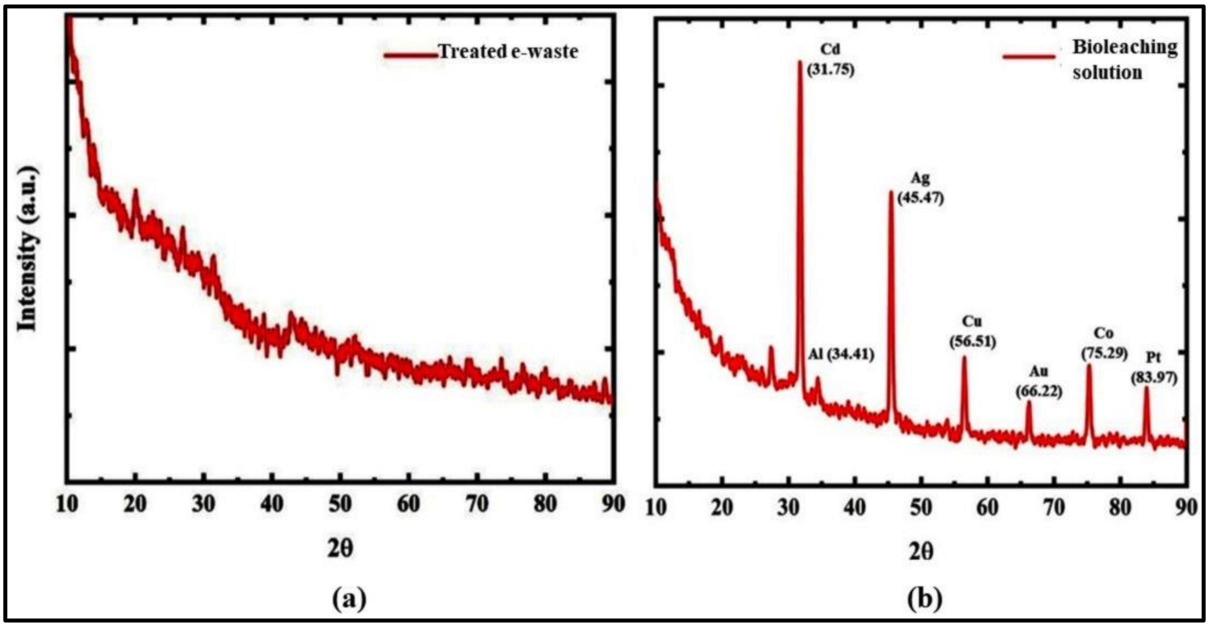


Figure 4.19 XRD spectrum to assess the mineralogical difference in bioleachate solution and CPCBs residue left after bioleaching (a) spectrum of CPCBs residual e-waste after bioleaching treatment by *B. sporothermodurans* ISO1 **(b)** XRD spectrum depicting the sharp peaks at 2θ value for metals leached into liquid solution from shredded dust CPCBs solid waste.

4.10 SWOT analysis

To comprehensively understand the dynamics of the bioleaching process, a thorough SWOT analysis meticulously evaluated the overall features of the bioleaching process. The bioleaching process has numerous advantages over conventional methods but also possesses limitations. This strategic assessment delves into identifying internal attributes, such as strengths and weaknesses, and external factors encompassing opportunities and threats to the bioleaching process.

STRENGTH	WEAKNESS
High specificity: Bioleaching processes through certain microbes are particular to certain metals, allowing for targeted extraction. **Environmental friendliness:** Bioleaching is often considered more environmentally friendly than traditional mining methods. **Lower energy consumption:** Bioleaching requires less energy than conventional methods, reducing operational costs. **Potential for recovery of low-grade ores:** Bioleaching effectively extracts metals from low-grade ores that may not be economically viable using other methods.	**Longer processing times:** Bioleaching processes can be slower than chemical extraction methods. **Sensitivity to environmental conditions:** The effectiveness of bioleaching can be influenced by factors like temperature, pH, and nutrient levels. **Limited metal specificity:** Some bioleaching processes may not be as specific to certain metals, leading to the co-extraction of unwanted elements. **Initial capital costs:** Setting up bioleaching facilities may require significant initial investments.

OPPORTUNITIES	THREATS
Growing demand for sustainable mining practices: The increasing focus on sustainable and environmentally friendly mining practices presents bioleaching opportunities. **Advances in biotechnology:** Ongoing advancements in biotechnology may lead to improvements in bioleaching processes. **Increasing metal prices:** Rising metal prices can make bioleaching more economically viable for extracting metals from low-grade ores. **Collaboration with mining companies:** Partnerships with traditional mining companies may present opportunities for integrating bioleaching technologies at an industrial scale.	**Market fluctuations:** Fluctuations in metal prices and market demand can impact the economic viability of bioleaching projects. **Competition from traditional mining methods:** Bioleaching may face competition from established mining methods, especially for certain ores. **Public perception and acceptance:** Lack of public awareness or acceptance of bioleaching may hinder its adoption.

4.11 Economical evaluation of the bioleaching process

The exact economic evaluation of the biocyanidation process at the research level is challenging due to infeasible initial cost estimations. However, an efficient way to gauge the economic aspects is to compare general costs with those of a typical chemical cyanidation process. Biocyanidation-related costs primarily involve cyanogenic bacteria for cyanide production, encompassing bio-reactor operation, nutrients, and glycine. Although this bioprocess step may be costlier than a chemical cyanidation process, biocyanidation incurs minimal additional expenses compared to chemical cyanidation. For example, the in-situ production of bio-CN eliminates the need to transport or store hazardous cyanide chemicals. Assuming an average gold mine operation requires up to 70,000 tonnes of NaCN annually (AGR, 2002), a successful biocyanidation process can save cyanide delivery and storage costs at the operation site [213, 234].

Moreover, this approach has the potential to significantly decrease environmental hazards and processing costs compared to traditional cyanidation. Bio-CN is produced at a relatively low concentration, and the discharge solution can be designed to have a minimum free cyanide concentration, avoiding substantial amounts of cyanide-containing waste. Cyanogenic bacteria may degrade residual cyanide using leftover nutrients in waste streams, reducing expenses for tailing management and cyanide remediation. Traditional leaching methods involving electricity and chemical reagents incur additional costs, limiting commercial viability. Despite a bioleaching bioreactor's high initial setup cost, operational costs can be minimized through variable optimization and cost-effective materials for microbial growth [231].

Further substantiating the economic viability of bioleaching is its extensive adoption across various industries, as highlighted in [section 2.7]. Furthermore, a notable case study from the Olympic Dam mine in Austria emphasizes the economic feasibility of this environmentally friendly approach. They successfully upscale the initial bioleaching pilot plant to an industrial level. [235]. The success of bioleaching highlights several advantages, including enhanced resource recovery, reduced environmental impact compared to traditional methods, and improved overall process efficiency. This case study serves as a compelling demonstration of the potential of bioleaching as a sustainable and economically competitive method for processing low-grade copper ore and e-waste.

CHAPTER 5

CONCLUSIONS AND FUTURE PROSPECTS

CONCLUSIONS

The relentless growth of e-waste, coupled with the abundance of various metals, positions e-waste as a valuable secondary mining source. The metal recovery process offers an opportunity to preserve natural mine sources, making it a sustainable metallurgical approach. This work aimed to provide a sustainable approach using a potential bacterial isolate to address the increasing volume of valuable waste. While this study focuses on the fundamentals of the bioleaching process, the significant outcomes serve as a compelling foundation for future research in the bioleaching of e-waste with the reported bacterial isolate. The key findings are summarized as follows:

The characterization of shredded dust of waste CPCBs for its elemental composition produced during the mechanical processing revealed this waste as an enriched source of various metals with significant amounts of Cu, Ag, and some other metals (Fe, Ni, Al, Pt, and Au). Therefore, it is essential to collect dust during physio-mechanical processing and utilize it in the metal leaching process rather than discarding it as low-value waste. Morphological and mineralogical analyses unveiled diverse toxic metallic and non-metallic constituents within e-waste, potentially influencing bacterial growth and the efficiency of bioleaching processes. These factors should be considered when exploring the bacterial community for bioleaching potential.

Screening and characterization of bacterial isolates acclimated to a metal-rich environment led to the identification of *Bacillus sporothermodurans* ISO1, which exhibited higher toxicity tolerance (EC50=425g/L) compared to another indigenous bacterial isolate, *Pseudomonas balearica* SAE1. The optimization of different parameters that facilitate the *Bacillus sporothermodurans* ISO1 growth and metabolically activities suggested that 40^{o}C temperature, pH 9, glycine concentration 7.5g/L, and pulp density 10g/L were favorable conditions for efficient bioleaching of Cu and Ag metals by *B. sporothermodurans* ISO1 through two-step bioleaching process.

The higher toxicity tolerance and efficient leaching potential of this potent bacterial isolate compelled further improvement of the metal's leaching efficiency to strengthen the industrial-scale potential of the bioleaching process. The leaching through a chemo-biohydrometallurgical approach with chemical lixiviant (i.e., $FeCl_3$) and biological lixiviant (bio-cyanide) enhanced the leaching capacity of *B. sporothermodurans* ISO1 and *P.*

*balearica*SAE1 for other precious metals in economical and eco-friendly manner. In addition, the present findings revealed methionine as a cyanogenesis stimulant that may enhance the availability of biogenic cyanide, conquer the specificity and selectivity limitation of the biocyanidation leaching process, and limit the use of chemical lixiviant in chemo-biohydrometallurgical approach.

In addition, to assess the sustainability of this potent bacterial strain for industrial-scale implementation, the bioleaching was demonstrated in pilot-scale bioleaching operation at subsequent amounts of pulp density with optimized process parameters and the significant dissolution of Cu, Au, Ag, Ni, Co. illustrates the sustainability and implementation of this ecological and environment-friendly approach for industrial-scale application of biometallugry process for e-waste urban mining approaches.

In conclusion, indigenous bacterial strains that are habituated to metal existence sites exhibit metabolic machinery towards the toxic constituents of e-waste, and thus, they are promising contenders for resource recovery from e-waste in industrial-scale bioleaching operations. Our findings indicate that bioleaching of metals from low-grade shredded dust e-waste sources is significant. Meanwhile, for efficient leaching on an industrial scale, a hybrid technology such as chemo-biohydrometallurgy through suitable chemical lixiviant such as $FeCl_3$ may accomplish a proficient and sustainable approach.

FUTURE SCOPE OF THE STUDY

The current study unveiled a new bacterial isolate (i.e., *B. sporothermodurans* ISO1) that has exhibited an efficient bioleaching potential. While the current study primarily focuses on the initial investigation of the bioleaching process, there are opportunities for further research involving this promising bacterial strain. Hence, the outcomes of the current study can be expanded to the following aspects of biohydrometallurgy to increase the practicality of this novel metal bioleaching strain for industrial-scale operation.

- Genomic and proteomic analysis of *B. sporothermodurans* ISO1 may lead to another diverse research area for revealing the accurate mechanism of the bioleaching process.
- The quantitative analysis of lixiviant production may lead to a new approach to protein engineering to enhance the enzymatic activity for the metal leaching process.
- The optimized parametric conditions can be employed at the fermenter scale to assess the feasibility of metals leaching with this potential bacterial strain.
- The methods to recover metals in pure form can be investigated to fulfil the sustainable development goal of "urban mining."

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APPENDICES

APPENDICES

APPENDIX A

A. 1 MEDIA COMPOSITIONS

All the media were prepared in distilled water and sterilized by autoclaving at 15 psi at 121°C for 15 minutes.

1. Nutrient Broth Media

Ingredients	Amount (g/L)
Yeast extract	1.5
Peptone	5.0
Sodium chloride	5.0
Meat extract	1.5
Final pH 7 ± 0.2 Suspend 13 grams in 1000ml distilled water	

2. Nutrient Agar media

Ingredients	Amount (g/L)
Yeast extract	1.5
Peptone	5.0
Sodium chloride	5.0
Meat extract	1.5
Agar	15
Final pH 7 ± 0.2 Suspend 28grams in 1000ml distilled water	

3. Kings Medium B Base

Ingredients	Amount (g/L)
Protease peptone	20
Dipotassium hydrogen phosphate	1.5
Magnesium sulphate	1.5
Agar	15
Final pH 7.2±0.2 Suspend 38 grams in 100ml distilled water	

4. Blood Agar Media

Ingredients	Amount (g/L)
Yeast extract	1.5
Peptone	5
Sodium chloride (NaCl)	5
Meat extract B	1.5
Agar	15
Final pH7.4±0.2 Suspend 28 grams in 1000ml distilled water	

Note: Add 20mlL^{-1} filter sterilized blood to the sterilized medium.

5. Simmons Citrate Agar Media

Ingredients	Amount (g/L)
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1
Dipotassium phosphate	1
Sodium citrate	2
Sodium chloride	5
Bromothymol blue	0.08
Agar	15
Final pH 6.8±0.2 Suspend 24.28 grams in 1000 ml distilled water.	

6. Starch Agar Media

Ingredients	Amount (g/L)
Yeast extract	1.5
Peptone	5
Sodium chloride (NaCl)	5
Meat extract B	1.5
Agar	15
Gram iodine	1-2 drops
Final pH 7.4±0.2 Suspend 28 grams in 1000ml distilled water.	

7. Glycerol stocks 50% (v/v) Solution

Ingredients	Amount (mL)
Glycerol	50
Distilled water	50

APPENDIX B

B.1 Procedure for biochemical characterization

1. Gram staining

- Prepare a culture smear on a glass slide.
- Gently flood the smear with primary stain (i.e., crystal violet) and keep it for 1 min.
- Rinse with tap water
- Gently flood the smear with Gram's iodine and let stand for 1 minute.
- Discard the Gram iodine solution, wash it off with a decolorizer (i.e., 95% ethanol), and immediately rinse with tap water.
- Flood the safranine on the slide to counter-stain the smear for 45 seconds.
- Rinse with tap water and air dry.

2. Endospore staining

- Prepare a thin smear and heat fix on the glass slide.
- Cover the bacterial smear with a piece of absorbent paper and place the slide over a container of boiling water.
- Saturate the absorbent paper with malachite green stain solution and steam for 5 mins.
- Remove the absorbent paper using forceps, allow the slide to cool down
- Rinse with distilled water
- Counterstain with safranin for 30 seconds, wash the slide with water, and blot dry the slide.
- Examine the slide under the oil immersion lens for the presence of endospores.

3. Catalase test

- A 3% hydrogen peroxide drop was added to a clean glass slide.
- The freshly grown bacterial culture was transferred with a sterile inoculation loop.
- Mix the inoculum properly.
- After 10 seconds, observe for catalase activity.

4. Methyl red test

- A bacterial culture was inoculated in a nutrient broth medium and incubated at 37^{o}C for 24 hours.
- The methyl red indicator solution was added to a fully grown bacterial culture and incubated for 10 minutes.
- After incubation, the tube was observed for color change.

5. Haemolytic test

- An autoclaved blood agar medium was supplemented with 2% human blood.
- A bacterial culture was streaked on blood agar medium and incubated at 37^{o}C for 24 hours.
- After 24 hours of incubation, a plate was observed for lysis of blood cells.

6. Cyanide production assay

The qualitative cyanide production of bacterial strains was tested using the Castric method. In this method, a change of color in the filter papers from yellow to brown demonstrates the ability to produce hydrogen cyanide (HCN).

- A bacterial culture was streaked on King's B agar medium.
- 1 cm absorbent paper strip was dipped in picric acid solution and fixed on the lid of the plates.
- The plate was kept at 37^{o}C for 7 days. After incubation, the plate was observed for a change in the color of the absorbent paper.

7. Starch hydrolysis test

- The bacterial culture was streaked on starch agar medium and incubated at 37^{o}C for 24 hrs.
- Gram iodine solution was added to the freshly grown culture medium.
- The plate was observed after 5 mins for color change.

8. Simmons Citrate agar test

- The sterilized Simmons citrate agar was streaked with a culture medium.
- The culture was allowed to grow at 37^{o}C for 24 hrs.
- After 24 hours of incubation, the plate was observed for color change.

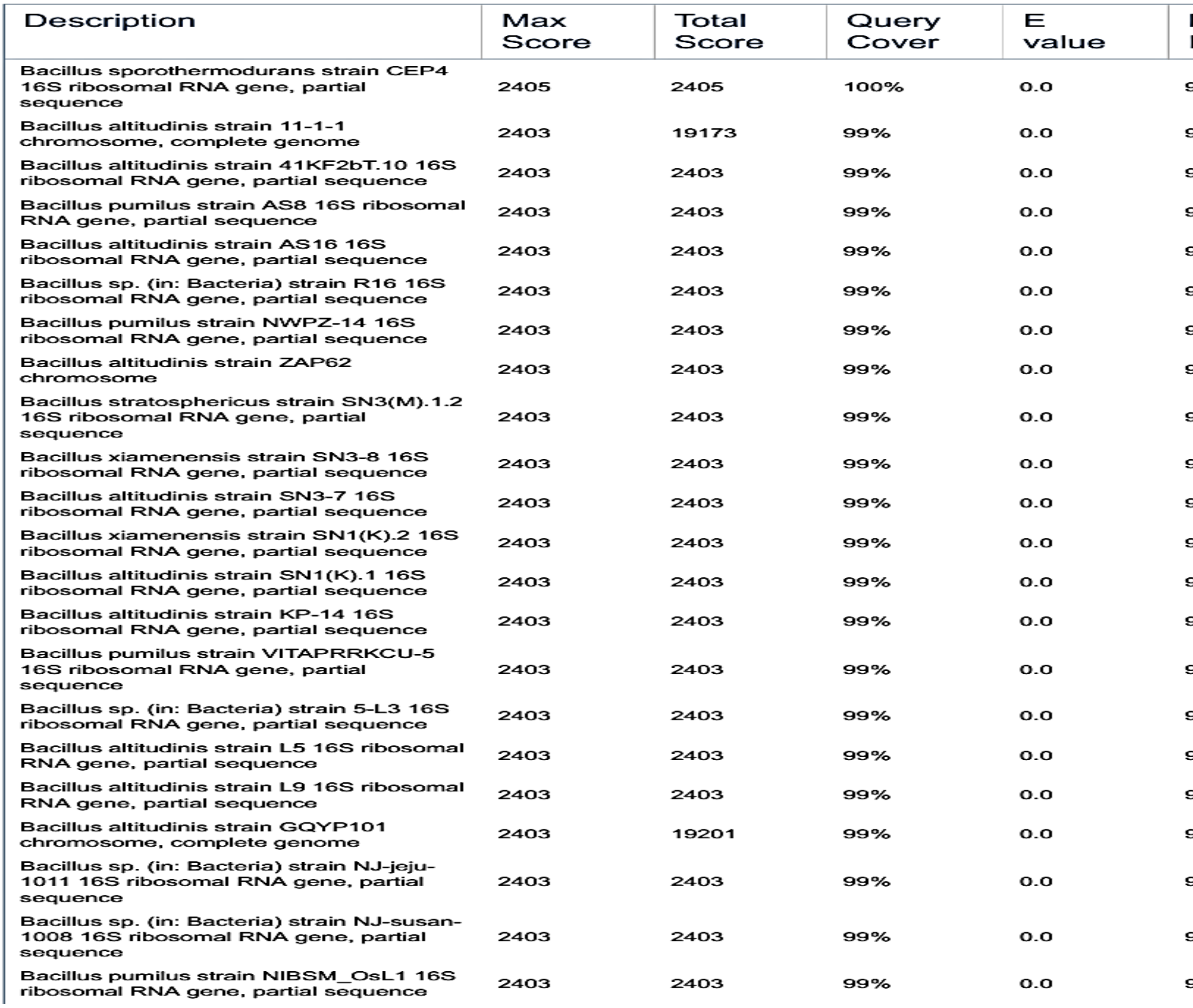
APPENDIX C

C.1 16S rRNA gene sequence

>AATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGCTGTCA
CTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGAC
GATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGAC
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAAC
GCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTG
CAAGAGTAACTGCTTGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGC
CAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGG
CTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATT
GGAAACTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAA
TGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACG
CTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTA
AACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAA
GCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT
GACATCCTCTGACAACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGT
GCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC
CCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACC
GGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTG
CTACAATGGACAGAACAAAGGGCTGCGAGACCGCAAGGTTTAGCCAATCCCACAAATCT
GTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATC
GCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACC
ACGAGAGTTTGCAACACCCGAAGTCGGTGAGGTAACCTTTATGGAGCCAGCCGCCC

RESULT: ***Bacillus sporothermodurans***

C.2 BLAST analysis

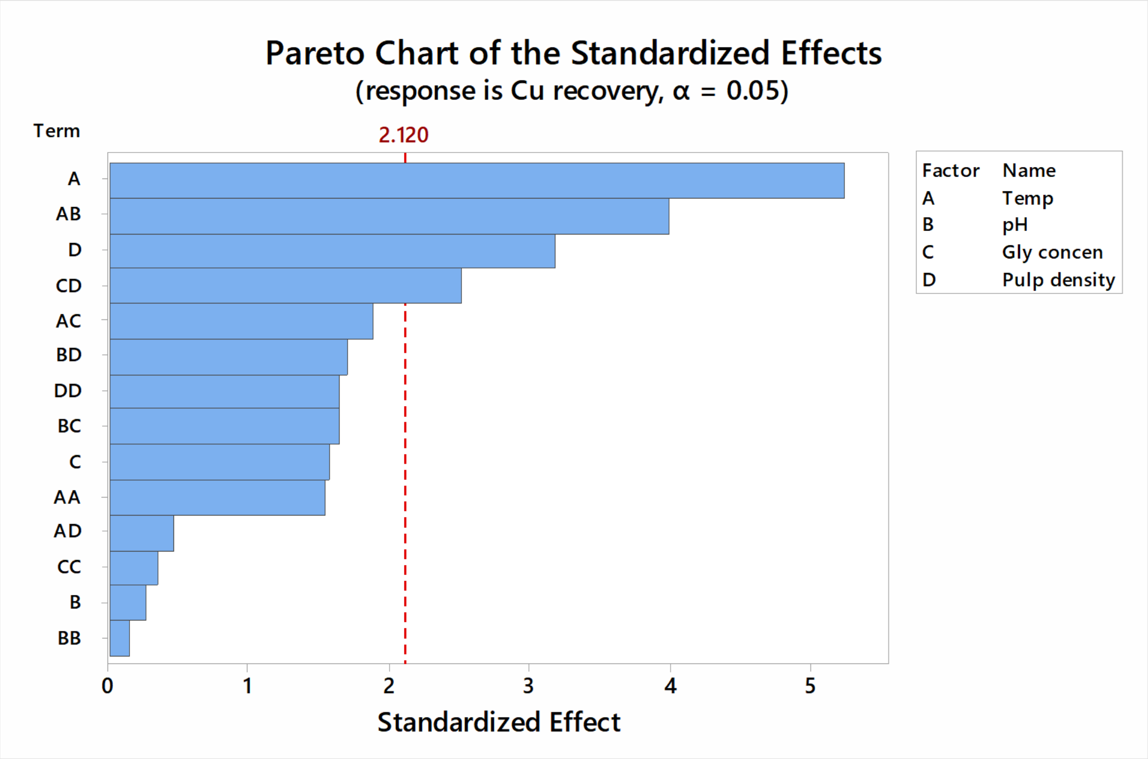


Description	Max Score	Total Score	Query Cover	E value
Bacillus sporothermodurans strain CEP4 16S ribosomal RNA gene, partial sequence	2405	2405	100%	0.0
Bacillus altitudinis strain 11-1-1 chromosome, complete genome	2403	19173	99%	0.0
Bacillus altitudinis strain 41KF2bT.10 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus pumilus strain AS8 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus altitudinis strain AS16 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus sp. (in: Bacteria) strain R16 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus pumilus strain NWPZ-14 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus altitudinis strain ZAP62 chromosome	2403	2403	99%	0.0
Bacillus stratosphericus strain SN3(M).1.2 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus xiamenensis strain SN3-8 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus altitudinis strain SN3-7 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus xiamenensis strain SN1(K).2 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus altitudinis strain SN1(K).1 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus altitudinis strain KP-14 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus pumilus strain VITAPRRKCU-5 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus sp. (in: Bacteria) strain 5-L3 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus altitudinis strain L5 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus altitudinis strain L9 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus altitudinis strain GQYP101 chromosome, complete genome	2403	19201	99%	0.0
Bacillus sp. (in: Bacteria) strain NJ-jeju-1011 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus sp. (in: Bacteria) strain NJ-susan-1008 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0
Bacillus pumilus strain NIBSM_OsL1 16S ribosomal RNA gene, partial sequence	2403	2403	99%	0.0

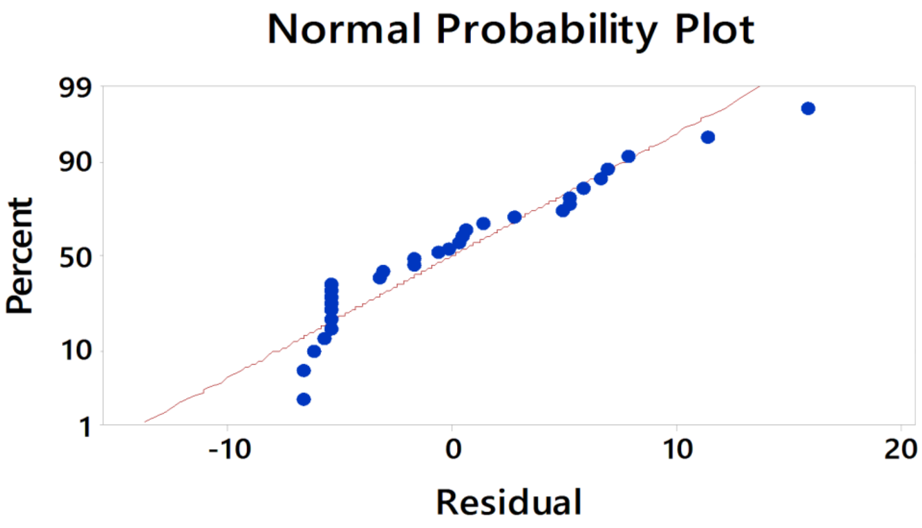
C.3 Statistical analysis of parameters' significance

1. One-way ANOVA analysis for significant recovery of Cu

The number of CPCBs (i.e., P.D) incubation temperature and pH of the medium significantly affected Cu recovery at 95% C.I. in linear form, whereas temp*pH and glycine concentration*pulp density showed significance in their interactive way.

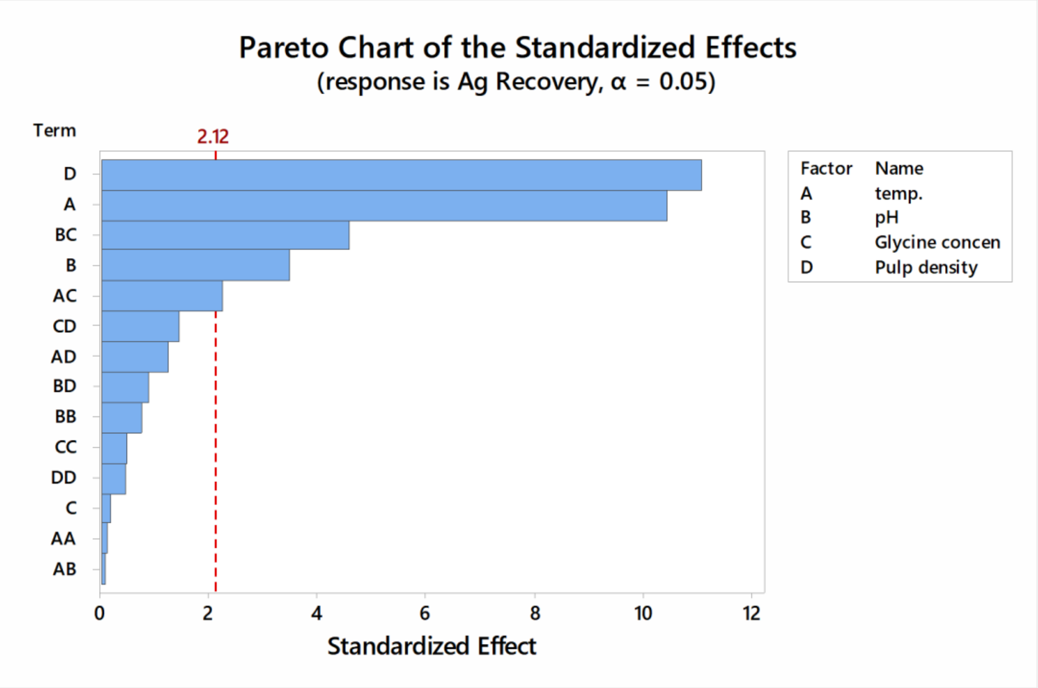


2. Analysis of experimentally obtained Cu recovery and software predicted response to assess the model validation of Mini@Tab 2018 software.

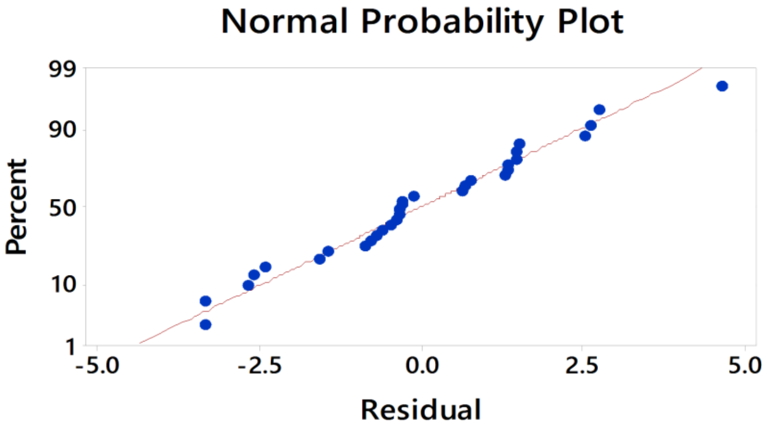


3. One-way ANOVA analysis for significant recovery of Ag

Temperature, pH, and pulp density showed significant effects on Ag recovery in a linear way, whereas, in a two-way, pH*glycine concentration was found to be a significant factor for efficient recovery of Ag metal.



4. Analysis of experimentally obtained Ag recovery and software predicted response to assess the model validation of Mini@Tab 2018 software.



APPENDIX D

JCPDS Data file

S. No	Metals	Peak	JCPDS No.	System /Lattice
1	Cu	56.51	86-1869	Rhombo, Centered
2	Cd	31.75	85-1328	Hexagonal, Primitive
3	Co	75.29	15-0806	Cubic, Face Centered
4	Al	34.41	85-1327	Cubic, Fcae Centered
5	Ag	45.47	03-0931	Cubic, Face Centered
6	Au	66.22	04-0784	Cubic, Face Centered
7	Al	34.41	85-1327	Cubic, Face Centered
8	Pt	83.97	01-1311	Cubic, Face Centered
9	Hg	27.40	74-0039	Hexagonal Primitive

LIST
OF
PUBLICATIONS

LIST OF PUBLICATIONS

1. P. Thakur and S. Kumar, "Evaluation of different amino acids as precursor for metals bioleaching from waste printed circuit boards by indigenous *Bacillus* sp. ISO1," International Microbiology, pp. 1-11, Oct, 2023 (IF 3.1, SCIE).

2 P. Thakur, and **S. Kumar,** "Exploring bioleaching potential of indigenous Bacillus sporothermodurans ISO1 for metals recovery from PCBs through sequential leaching process," Waste Management and Research, Mar 2023 (IF-4.432, SCIE).

3. P. Thakur, and **S. Kumar,** "Pretreatment of low-grade shredded dust e-waste to enhance silver recovery through biocyanidation by Pseudomonas balearica SAE1," 3 Biotech, vol. 11, no. 11, pp. 454, Oct 2021 (IF 2.893, SCIE).

4. P. Thakur, and **S. Kumar**, "Evaluation of e-waste status, management strategies, and legislations," International Journal of Environmental Science and Technology, vol. 19, no. 7, pp. 6957-6966, Jul 2021 (IF 3.519, SCIE).

5. P. Thakur, and **S. Kumar,** "Metallurgical processes unveil the unexplored "sleeping mines" e-waste: a review," Environmental Science and Pollution Research, vol. 27, no. 26, pp. 32359-32370, Jun 2020 (IF 5.190, SCI).

WORKSHOPS ATTENDED

1. Workshop on "Mass Spectrometry" organized by Division of Biological Sciences, IISc, Bangalore, 2-8 Jan, 2023.

2. One day workshop on "IPR AND Patent Filing" organized by Division of Intellectual Patent Rights and Consultation Cell Himachal Pradesh University, Shimla, 15th Nov, 2019.

3.Workshop on "Approached for screening and characterization of pre-clinical drug candidates" organized at Jaypee University of Information Technology, 8- 14 Dec, 2022.

4. Workshop on "Demonstration and Hands on training on HPLC Training" organized by Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, 25-29 Sep, 2018.

5. Workshop on “Demonstration and Hands on training on Fluorescence Microscopy” organized by Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, 10-30 Oct, 2018.

6. One day workshop on “Patent Filing Procedure and Geographical Indication of Himachal Pradesh at Jaypee University of Information Technology, Waknaghat, Solan, 30th Nov, 2018.

GENBANK SUBMISSION

1. P. Thakur and S. Kumar, “Bacillus sp. (in: firmicutes) strain ISO1 16S ribosomal RNA gene, partial sequence. Accession OQ373005, GenBank, FEB-2023.

3 Biotech (2021) 11:454
https://doi.org/10.1007/s13205-021-02977-4

ORIGINAL ARTICLE

Pretreatment of low-grade shredded dust e-waste to enhance silver recovery through biocyanidation by *Pseudomonas balearica* SAE1

Pooja Thakur[1] · **Sudhir Kumar**[1]

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Abstract

Shredded dust originated during mechanical dismantling of waste electrical and electronic equipments (WEEEs) is enriched source of some valuable metals which might be lost as unworthy waste. Composition analysis of shredded dust printed circuit boards (PCBs) revealed the presence of 12.75 mg g^{-1} copper (Cu) and 10.34 mg g^{-1} silver (Ag) along with some other metals (Fe, Ni, and Au). Low concentration of precious metal, such as gold (Au i.e., 0.04 mg g^{-1}) classified this shredded dust as low-grade scrap. Despite low concentration of Au this e-waste can be considered as potential "secondary ore" to recover other valuable metals like Ag. To improve the efficiency of Ag bioleaching using *Pseudomonas balearica* SAE1, pretreatment of e-waste was done using cost-effective ferric chloride ($FeCl_3$) chemical lixiviant. The concentration of $FeCl_3$ lixiviant was optimized to recover Cu metal prior to bioleaching process. Bioleaching of Ag was done under optimized conditions by *Pseudomonas balearica* SAE1 using 100 mL Luria Broth (LB) medium, 5 g L^{-1} glycine, pH 9, temperature 30 °C and 150 rpm. 95% Cu was recovered with 1% $FeCl_3$ prior to bioleaching. Ag solubilization was increased for treated e-waste (36%) as compared to untreated e-waste PCBs (25%). Prerecovery of Cu enhanced Ag bioleaching, as available cyanide was utilized by Ag metal. Therefore, this study provides an economical hybrid method to enhance retrieval of precious metal (Ag) by *Pseudomonas balearica* SAE1 with economic and ecofriendly redox lixiviant even from low-grade e-scrap.

Keywords WEEE · e-waste · PCB · Secondary ore · Biohydrometallurgy · *Pseudomonas balearica* SAE1

Introduction

Progressive advancement in the modern world has made our life more comfortable, and prompt us to stay with the flow and remain updated with this technological era. However, this progressive modernity in the technology world have also been leading to incorporate a new solid waste stream which is known as "e-waste". Nowadays, e-waste has excelled the highest among the entire municipal solid waste stream, expected to reach 50 million ton by 2021 with 20–25 Mt per year production rate (Xia et al. 2018; Benzal et al. 2020). This waste is known to consist complex mixture of base metals (Cu, Al, Zn, Ni, Fe), precious metals (Au, Ag, Pt, Pd), toxic metals (Cd, Cr, Be) along with some flame retardants and hazardous organic compound, such as polybrominated diphenyl ether (Khatri et al. 2018). The presence of hazardous and toxic components poses adverse effects of e-waste on human health and environment as well.

Printed circuit boards (PCBs) are considered as core constituent with total 3–6% weight contribution among all the electric and electronic equipment, which have been used 30% share of global market with production rate of 1.1×10^9 m^2 (Thakur and Kumar 2020). Therefore, PCBs e-waste contribute majorly among all the WEEEs. This typical e-waste is a diverse and heterogeneous mixture of metallic (28–30%) and non-metallic (70–72%) components, such as plastic, polymers, and ceramics (Chauhan et al. 2018). Cu is predominated metal (10–20%) found in PCBs along with some valuable (Au, Ag, Pt) and hazardous metals (Pb, Ni) (Priya and Hait 2017; Chatterjee and Abraham 2017). The amount of Ag and Au in PCBs waste is estimated to be approximately 1000 g and 250 g per ton, respectively, in comparison to natural deposits (< 10 g per ton) which make this waste as core of "urban mining" and recently being used as "secondary ore" (Kumar et al. 2018; Jagannath et al. 2017; Wu et al. 2018). On the basis of inherent

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valuable metals (Au, Ag) concentration, PCBs e-waste are generally classified into high, medium, and of low-grade level. Precious metals concentration is less in low grade as compared to high and medium grade scrap (Xia et al. 2018). Shredded dust originated during the mechanical treatment (Dismantling of WEEE) is an example of low-grade scrap.

In metallurgical methods biohydrometallurgy offers a promising "green technology", which is the most active research area, and has drawn more attention in the last two decades (Sodha et al. 2017; Hubau et al. 2020). Biohydrometallurgy-assisted biological extraction of metals utilizes the potential of naturally inhabitant microorganisms and their metabolites; therefore, referred as "Bioleaching" (Arshadi et al. 2019; Boxall et al. 2018). This highly economic and environment friendly technology has overwhelmed the traditional approaches, such as pyrometallurgy and hydrometallurgy (Valix 2017; Wang et al. 2016). *Acidithiobacillus thiooxidans, Acidithiobacillus ferrooxidans,* and *Chromobacterium violaceum, Pseudomonas* sp., and *Bacillus megaterium* are some of the chemolithotrophic and cyanogenic bacteria, respectively, which have been explored for retrieval of metals from e-waste as well as low-grade mining ore (Brandl et al. 2001). *Chromobacterium violaceum* and *Pseudomonas* sp. are major cyanogenic strains used for solubilization of precious metals from waste PCBs. These bacteria exhibit potential to release cyanide (CN^-) as a secondary metabolite by utilizing glycine as direct precursor through oxidative decarboxylation reaction at the early stationary phase. At neutral pH, cyanide exist as hydrogen cyanide (HCN) with p*K*a value 9.3 which is detoxified by bacteria itself at late stationary phase by converting into β-cyanoalanine (Natarajan and Ting 2014; Kumar et al. 2018; Pardhan and Kumar 2012). Researchers are continuously trying to scale-up bioleaching process and make its industrial scale application distinctive. Sodha et al. (2020) optimized parameter condition for bioleaching of copper by *Leptospirillum ferriphilum* in batch reactor and ultimately recovered 99% Cu through cementation process. Garg et al. (2019a,b) demonstrated the effect of higher pulp density on bench scale bioreactor to scale up the bioleaching process at large scale activity whereas Garg et al. (2019b) observed bioleaching process in stirred tank reactor and stated that fed batch bioleaching result better than batch process bioleaching. They obtained 98% copper recovery in fed batch bioreactor with 3.5% w/v pulp density. However, time requirement, specificity, and selectivity are still some of limitations of biohydrometallurgy process and bring it to its infant stage, which have long been trying to improve. Researchers used many chemical treatments in combination with biological methods (i.e., chemo-biohydrometallurgical method) to overwhelme specificity and to improve the efficiency of bioleaching process, because no method is much efficient to leach out metals from WEEE alone (Das and Ting 2017; Kumar et al. 2018). Hence, the use of hybrid method is an effective way to enhance metal retrieval potential biohydrometallurgical technique. Therefore, the present work focused on enhancement of silver leaching using *Pseudomonas balearica* SAE1 through pretreatment of PCBs sample by $FeCl_3$ chemical lixiviant. $FeCl_3$ pretreatment was selected on the basis of its multiple advantages including low cost and pollution-free process. In the present study, following parameters were examined: (a) effect of prerecovery of Cu (highest amount) with $FeCl_3$ lixiviant; (b) optimization of $FeCl_3$ concentration for maximum solubilization of Cu metal; (c) optimization of time, required for formation of stable complex.

Materials and methods

Procurement and compositional analysis of shredded dust PCBs sample

Shredded dust generated during the mechanical processing of e-waste was collected from e-waste recycling unit at Panipant Haryana (i.e. Exigo Recycling Pvt. Ltd). Particles size of the pulverized PCBs was ≤ 150 µm. To determine the metallic composition, 1% w/v PCBs powder in 100 mL aqua regia ($HCl:HNO_3 = 3:1$) solution was heated at 100 °C for 1 h. (Sahni et al. 2016). Volume lost during heating was added up by sterile distilled water. Solution was filtered through Whatman filter paper followed by 0.45-µm glass fiber filters (PALL-GF-A/E-I). The concentration of dissolved metals in particle free leachate was analyzed through atomic absorption spectrophotometer (AAS, AAnalyst400, Perkin Elmer).

Pretreatment with ferric chloride to recover copper

All the chemical and biological experiments were performed in duplicates with experimental volume of 100 mL. For the pretreatment and recovery of Cu, 10 g L^{-1} pulp density of sterilized e-waste was added to different concentration of ferric chloride ($FeCl_3$) solution (i.e. 5 g L^{-1}, 10 g L^{-1}, 20 g L^{-1}) followed by incubation in orbital shaker at 50 °C and 150 rpm for different time periods (1 h, 2 h, 3 h). After incubation, filtration was done through Whatman filter paper followed by 0.45-µm glass fiber filter. The particle free supernatant was subjected for Cu content analysis through AAS, whereas pellet was washed, dried, and subjected for silver metal extraction through bioleaching process by *Pseudomonas balearica* SAE1.

Bioleaching experiment

Pseudomonas balearica SAE1 was obtained from the depository of Jaypee University of Information Technology Waknaghat, Solan (H.P); earlier isolated from e-waste habituating site. Bioleaching experiment was completed in 250 mL Erlenmeyer flasks (nonbaffled) with 100 mL Luria Broth (LB) medium (consisting tryptone: 10 g L^{-1}, yeast extract: 5 g L^{-1}, NaCl: 10 g L^{-1}) supplemented with 5 g L^{-1} glycine, pH 9, temperature 30 °C, rpm 150 and 1% w/v pulp density. A two-step bioleaching was implemented to reduce the toxicity effect of e-waste on lixiviant production and to improve precious metals recovery (Kumar et al. 2018). Therefore 4% (v/v) bacterial culture with viable cell count of 3×10^{11} cfu/mL was grown at 30 °C, 150 rpm in the absence of e-waste for 48 h to produce maximum cyanide lixiviant after then pretreated sample was added to culture flask and kept under same incubation conditions for biocyanidation reaction for 8 days. Bioleaching with untreated sample was run in parallel as control. After bioleaching, filtered leachate was analyzed for the presence of metal ions through AAS.

Analytical methods

Determination of metals recovery

Recovery of metals through chemical and biological processes was calculated by applying following formula:

$$m = c\,(\mathrm{mg\ L^{-1}}) \times v\,(\mathrm{mL})/1000, \tag{1}$$

m = metal concentration, c = concentration of metal analyzed by atomic absorption spectrophotometer, v = volume of lixiviant taken for leaching, mg/L to mg/g conversion, M/w = metal content, w = weight of e-waste used for metal recovery

$$\%\ \text{age metal recovery} = \text{concentration of metal in e - waste} \,/\text{concentration of metal present in leachate after leaching reaction} \times 100.$$

Thermodynamic study of chemical reaction

Chemical reaction occurred during the chemical treatment with ferric chloride was analyzed for thermodynamic study. Thermodynamic reaction was studied by applying the following formula

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{2}$$

ΔG^0 = change in standard Gibbs free energy, ΔH = change in standard enthalpy, T = temperature, ΔS^0 = change in standard entropy.

Surface structure characterization of treated and untreated e-waste

The surface structure of untreated and biological treated PCBs sample was depicted through Scanning Electron Microscope (SEM). Biological treated (*Pseudomonas balearica* SAE1) sample was dried and mounted for morphological characterization. SEM micrograph analysis was performed at Panjab University, Chandigarh.

Results and discussion

Metallic constituents of shredded dust PCBs e-waste

Metal composition of PCBs e-waste was analyzed by aqua regia digestion. The concentration ($\mathrm{mgg^{-1}}$) of different metals present in waste PCBs is represented in Table 1. Cu, Fe, Al, Ni are the base metals majorly found in waste PCBs along with Au and Ag like precious metals. Cu was found to be in the highest concentration among all the base metals whereas Au concentration was least, which classified this shredded dust e-waste as low-grade scrap. Metals concentration except Ag was depicted significantly lower than those reported by Kumar et al. (2018) for virgin PCBs e-waste with similar particle size (≤ 150 μm). The variable metals concentration was observed, because of PCBs sample used for analysis. In present study shredded dust originated during physio-mechanical process was utilized for metal retrieval, whereas Kumar et al. used virgin PCBs sample. Metallic concentration of base and precious metals varies with the change in particles size, more ductile nature of Cu, distribute it more in larger size fraction as compared to precious metal (Au) (Huang et al. 2014). Subsequently high concentration of Cu entraps and hinders the presence of Au and other precious metals. However, the

Table 1 Major metals composition of shredded waste PCBs (≤ 150 μm) using aqua regia digestion method

Metals	Concentration (mg g^{-1})
Cu	12.75 ± 3.8
Ag	10.34 ± 2.46
Fe	2.46 ± 0.85
Al	0.39 ± 1.02
Ni	0.24 ± 1.22
Au	0.04 ± 1.39

size reduction might have released the hindered precious metals which increased their aqua regia digestion (Kumar et al. 2018); therefore, high Ag concentration was obtained in the small size shredded dust sample (≤ 150 µm). The results of the present study support earlier reported metal composition by Sun et al. (2016); they found Cu metal predominant in PCBs with particle size 1–8 mm, whereas precious metals were higher within the particle size ≤ 0.2 mm. Chauhan et al. (2018) have also been reported the compositional analysis of shredded dust originated during physio-mechanical processing. They also indicated the highest concentration of organic matter (73.1%) followed by some base metals such as Al (4.65%), Fe (4.55%), and Cu (2.67%). Therefore, there is need of good dust collection to prevent loss of this enriched shredded waste.

Pretreatment (Cu recovery) with ferric chloride and thermodynamic study of chemical reaction

Copper content in the sample was found to be at the highest concentration, which form complexes with biogenic cyanide and cause hindrance in the retrieval of other valuable metals (Kumar et al. 2018). Many chemical lixiviant has been studied to recover Cu metal prior to bioleaching of precious metals from PCBs. Shah et al. (2014) used ferrous sulphate for recovery of base metals (Cu, Zn, Ni) from PCBs prior to bioleaching of precious metals by iron-oxidizing consortium. They obtained 84.16% Cu recovery through 3 g ferrous sulfate. Das and Ting (2017) reported 85.5% Cu recovery through strong sulfuric acid with hydrogen peroxide. Khatri et al. (2018) recovered 99% Cu within 5 days of incubation by using ferric and ferrous sulfate (70:30). In present study, mild acid $FeCl_3$ is used to recover Cu prior to bioleaching in order to enhance Ag recovery through biocyanidation process by *P. balearica* SAE1. $FeCl_3$ reacts with PCBs and leach out Cu metal into liquid solution. The $FeCl_3$ lixiviant lead to 95% Cu recovery without significantly altering the concentration of Ag. We obtained the maximum recovery of Cu in the shortest time period in comparison to above-mentioned reported studies. In $FeCl_3$, Ferric ions (Fe^{3+}) act as strong oxidizing agent, whereas chloride ions (Cl^-) are strong complexing agent, which accelerate the solubilization of Cu metal. $FeCl_3$ mediated Cu recovery is represented in Eqs. (3), (4), and (5). 3 h incubation at 50 °C was found to be optimum period to leach out maximum Cu (Fig. 2a). $FeCl_3$ is first dissociated into Fe^{3+} and Cl^- ions; Fe^{3+} oxidize Cu metal and reduced itself into Fe^{2+} (ferrous ion) whereas Cl^- ion finally make complex with oxidized Cu metal to form $CuCl_2$. $CuCl_2$ also act as oxidizer (shown in Eq. 3) to oxidize Cu when $FeCl_3$ is completely utilized. So $FeCl_3$ provides two oxidizing agents to reduce the concentration of lixiviant (Xu et al. 2016). No significant difference was found between 1% (10 g/L) and 2% (20 g/L) $FeCl_3$ solution, therefore 1% (1 g/L) ferric chloride solution was considered as optimum concentration. Thermodynamic nature of chemical reactions occurred during the chemical treatment with $FeCl_3$ lixiviant is calculated in term of Standard Gibb's free energy (ΔG^0). Initial reactions between $FeCl_3$ and Cu are unfavorable, nonspontaneous and highly endergonic ($\Delta G > 0$) hence require energy for complex formation, whereas overall reaction is favorable, spontaneous, and exergonic ($\Delta G < 0$) in nature.

$$FeCl_3 + CuCl \longrightarrow FeCl_2 + CuCl_2 \quad \Delta G^0 = +749.76 \tag{3}$$

$$CuCl_2 + Cu \longrightarrow 2CuCl \quad \Delta G^0 = +10,011.82 \tag{4}$$

$$\text{Overall reaction: } FeCl_3 + Cu \longrightarrow FeCl_2 + CuCl \quad \Delta G^0 = -9370.1. \tag{5}$$

Chemical kinetics

Overall, chemical reaction (5) is following second-order reaction and kinetics is as:

$$1/[A] = 1/[A]_o + kt. \tag{6}$$

Here, $[A]$ and $[A]_o$ are changes in initial concentration with time and initial concentration.

If we plot $1/[A]$ vs t (time), it will provide straight line where half time ($t_{1/2}$) is:

$$t_{1/2}\ (M^{-1}\ S^{-1}) = 1/k\ [A]_o. \tag{7}$$

In the present experiments, $[A]_o$ 12.75 mg/g.

Statistical analysis

An one-way ANOVA statistical analysis was applied to compare copper recovery rate at $p \leq 0.05$ (Fig. 1). It has been noticed that Cu recovery with 0.5% $FeCl_3$ was significantly lower than 1% and 2% $FeCl_3$ concentration. In comparison to initial concentration of Cu (12.75 mg g^{-1}) 87% recovery was obtained with 0.5% $FeCl_3$, whereas 94% and 95% Cu was recovered with 1% and 2% $FeCl_3$ concentration, respectively. The recovery rate variation was significant between 0.5%, 1%, and 2 % $FeCl_3$, but among 1% and 2% recovery difference was non-significant at $p \leq 0.05$. Hence 1% $FeCl_3$ was considered as optimum concentration for maximum recovery of Cu. Time required to complete the chemical reaction between lixiviant ($FeCl_3$) and e-waste was another important parameter

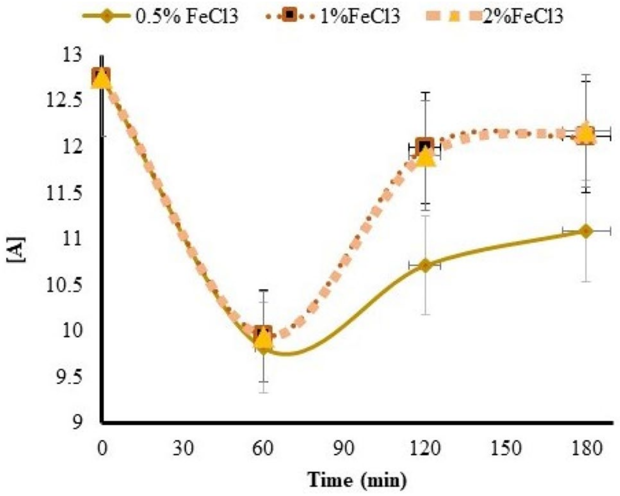

Fig. 1 Reaction kinetics analysis of ferric chloride at 0.5%, 1%, and 2% concentrations with different time interval at $p \leq 0.05$

determining the maximum recovery of Cu metal. Significant variation was observed during Cu recovery from 0 min to 180 min. 0.5% $FeCl_3$ leached 77%, 84%, 87% Cu at 60 min, 120 min, 180 min, respectively. Whereas 78%, 94%, 95% Cu was leached out with 1% $FeCl_3$ at 60 min, 120 min, 180 min, respectively. Significant variation was observed between 60 min and 180 min but among 120 min and 180 min recovery difference was non-significant at $p \leq 0.05$. Therefore, 1% w/v $FeCl_3$, 1% w/v e-waste pulp density and 180 min incubation at 50 °C were considered as optimum conditions for maximum recovery of Cu from shredded dust of PCBs.

Biocyanidation recovery of silver by *Pseudomonas balearica* SAE1

High concentration of copper and other base metals (Fe, Ni) may cause hindrance and toxicity to bacterial growth. Therefore, pretreatment and two-step bioleaching were opted to increase silver leaching efficiency. *P. balearica* SAE1 is an indigenous bacterial strain that exhibit high toxicity tolerance (EC_{50} = 325.7 g PCBs/L) (Kumar et al. 2018) and release hydrogen cyanide (HCN) as lixiviant. At alkaline pH cyanide solubilizes Au as dicyanoaurate and Ag as dicyanoargentate through biocyanidation reaction as shown in Eqs. (8) and (9), respectively. Kumar et al. (2018) claimed recovery of Au (68.8%) and Ag (33.8%) by *Pseudomonas balearica* SAE1 from virgin PCBs e-waste. Therefore, in the present study *P. balearica* SAE1 was utilized for solubilization of Ag metal. In shredded dust PCBs sample; higher concentration of Ag (10.34 mg g^{-1}) than Au (0.04 mg g^{-1}) contributed predominant solubilization of Ag through biocyanidation in comparison to Au solubilization. Maximum solubilization of Ag metal was observed within 8 days of incubation period at pH 9. Silver recovery was observed to be enhanced for pretreated e-waste (36%) as compared to untreated e-waste (25%) as shown in Fig. 2b. In comparison to other reported studies, the present study observed higher Ag solubilization using cynogenic microorganism. Brandl et al. (2008) stated 5% Ag solubilization by *Pseudomonas plecoglossicida* whereas Kumar et al. (2018) described 33.8% Ag and 68.5% Au recovery as dicyanoargentate and dicyanoaurate, respectively. In the present study, Ag leaching efficiency of *P. balearica* SAE1 increased due to the pretreatment of e-waste with $FeCl_3$, as Cu was recovered prior to bioleaching, hence during biocyanidation reaction the maximum cyanide was available for

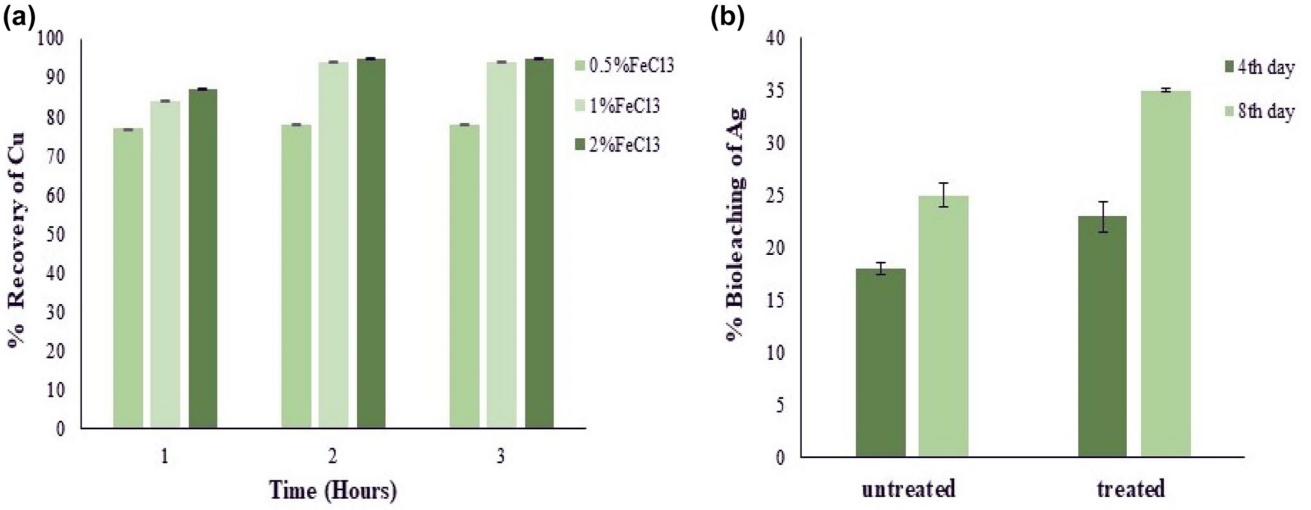

Fig. 2 Metals recovery through chemical and biological treatment: **a** recovery of Cu by ferric chloride, **b** % bioleaching of Ag by *P. balearica* SAE1

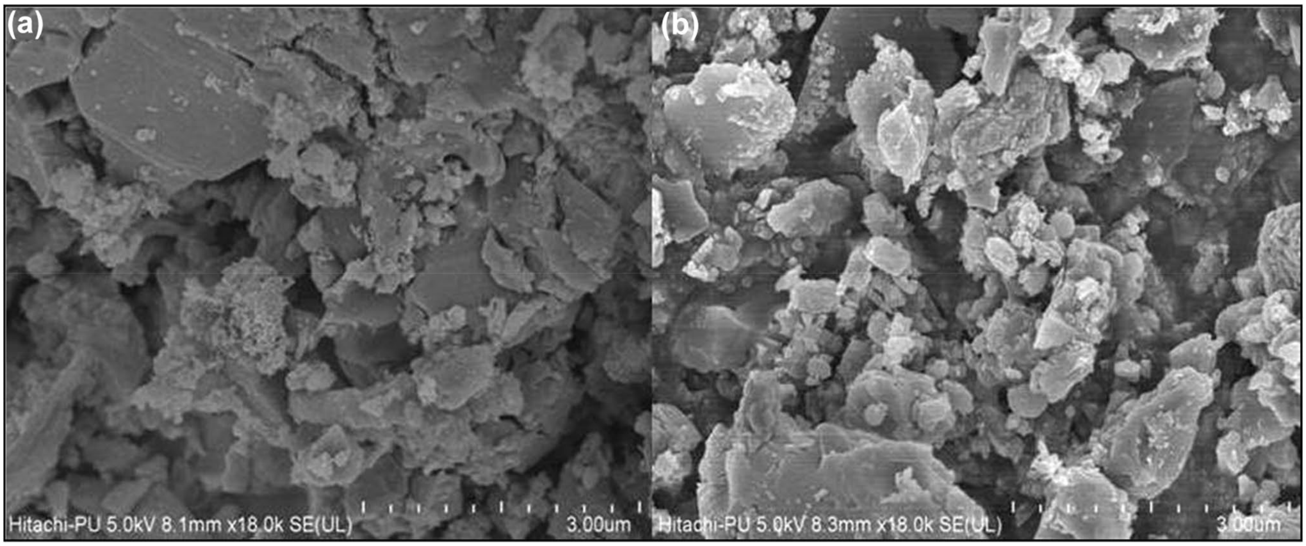

Fig. 3 Scanning electron micrographs (18,000X magnification) before and after bioleaching process: **a** untreated sample, **b** transformation occurred after bioleaching process with *Pseudomonas balearica* SAE1

Ag solubilization. Efficiency of *P. balearica* SAE1 has been increased up to 36% by pretreatment with $FeCl_3$, in comparison to untreated e-waste as well earlier reported study by Kumar et al. (2018); under optimized conditions.

pH is a vital factor for bioleaching of metals by cynogenic microorganism as p*K*a value of released biogenic HCN lixiviant is 9.3, therefore alkaline pH (i.e., 9) can increase the metal solubilization rate through upsurge CN^- ion production. The alteration in pH was likewise seen during bioleaching of Ag metal. Prior to e-waste addition, the pH of nutrient solution reduced from 9 to 8.5 (data not shown). This alteration might be attributed to bacterial secondary metabolites production, such as glyoxylic acid, cyno-formic, etc. (Liang et al. 2014; Kumar et al. 2018). However, after adding e-waste, pH initially increased from 8.7 to 8.9, and then raised up to 9.4 until 8 days of incubation period. This increased pH determined the alkaline nature of waste PCBs and biocyanidation reaction of HCN with Ag metal to form dicyanoargentate complexes. Arshadi and Mousavi (2015), Sahni et al. (2016) and Kumar et al. (2018) have reported comparable finding regarding pH change during metals recovery with *Bacillus megaterium*, *Chromobacterium violaceum* and *Pseudomonas balearica* SAE1, respectively. So, from the present study, recovery of precious metals and base metals by *P. balearica* SAE1 and $FeCl_3$, respectively at alkaline pH will be promising at large scale application.

$$4\mathrm{Au} + 8\mathrm{CN}^- + \mathrm{O}_2 + 2\mathrm{H}_2\mathrm{O} \longrightarrow 4\ \mathrm{Au(CN)}_2^- + 4\ \mathrm{OH}^- \tag{8}$$

$$4\mathrm{Ag} + 8\mathrm{CN}^- + \mathrm{O}_2 + 2\mathrm{H}_2\mathrm{O} \longrightarrow 4\ \mathrm{Ag(CN)}_2^- + 4\mathrm{OH}^-. \tag{9}$$

Surface structure characterization of e-waste

Morphological transformation of e-waste after bioleaching with *P. balearica* SAE1 was compared with untreated e-waste through SEM. Figure 3a represents the structure characterization of untreated e-waste sample, which revealed the occurrence of irregular clusters of metal particles having heterogeneity in sizes and texture. Most of the fraction was rough, deteriorated with some crystals and flakes on the surfaces of plastic and fiber components . Heterogeneity occurred due to shearing force applied during physiomechanical process (Das and Ting 2017; Natarajan and Ting 2014). After treatment in Fig. 3b, the predominant metal content (Cu, Ag) was leached out, so most of the metallic clusters were separated and more distant than in untreated e-waste. Metallic particles were also observed in treated e-waste, as not all metals can be leached out during bioleaching of e-waste under given set of conditions.

Future prospect

Shredded dust PCBs e-waste is a prodigious “secondary source” of many valuable metals which is neglected as useless dust during physiomechanical processing. Low metal concentration in shredded dust PCBs reduces the toxicity limitation of microbial leaching even at high pulp density therefore this e-waste can be utilized as unusual sample source for large scale bioleaching. Hence industrial scale metal recovery can be attained with this low-grade electronic scrapy using this cost-effective chemo-biohydrometallurgical technique, as by optimizing parameters for large scale production.

Conclusion

Compositional analysis of shredded dust revealed the presence of high concentration of copper and silver. Therefore, dust should be collected during physiomechanical processing and screwed up for metals recovery. Higher concentration of base metal (Cu) contributed toxicity to bacterial growth and reduced bioleaching of precious metal (Ag) therefore pretreatment and two-step bioleaching is suggested to enhance the recovery of metals. $FeCl_3$ is a mild acid consist chloride ion (Cl^-) as strong complexing agent and promote the leaching of copper and decrease the required concentration of lixiviant ($FeCl_3$). Hence pretreatment with $FeCl_{3,}$ established to be environmentally sound and cost effect. *P. balearica* SAE1 improved recovery of silver metal for treated e-waste through two-step bioleaching. $FeCl_3$ and biocyanidation-based Cu and Ag leaching showed high recovery rate with many advantages including less investment, low energy consumption, and ecofriendly behavior. Therefore, a hybrid method validated its suitability for transformation of this solid waste into wealth at industrial scale.

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Authors contributions: Conceptualization: SK; Methodology: SK and PT; Formal analysis and investigation: SK and PT; Writing—original draft preparation: PT; Writing—review and editing: SK and PT; Supervision: SK.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethics approval We declare that there are no ethical issues for human or animal rights in the work presented here.

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Exploring bioleaching potential of indigenous *Bacillus sporothermodurans* ISO1 for metals recovery from PCBs through sequential leaching process

Pooja Thakur and Sudhir Kumar

Abstract

The low efficiency and selectivity limitations of biohydrometallurgy technique compel the researchers to explore novel microbial strains acclimated to metal existence site with higher toxicity tolerance and bioleaching capability in order to improve the role of bioleaching process for e-waste management. The current study aimed to explore bioleaching potential of indigenous *Bacillus sporothermodurans* ISO1; isolated from metal habituated site. The statistical approach was utilized to optimize a variety of culture variables including temperature, pH, glycine concentration and pulp density that impact bio-cyanide production and leaching efficiency. The highest dissolution of Cu and Ag, 78% and 37% respectively, was obtained at 40 °C, pH 8, glycine concentration $5\,g\,L^{-1}$, and pulp density $10\,g\,L^{-1}$ through One Factor at a Time (OFAT), which was further increased up to 95% Cu and 44% Ag recovery through the interactive effect of key factors in the Response Surface Methodology (RSM) approach. Furthermore, Chemo-biohydrometallurgy approach was utilized to overwhelm the specificity limitation; as higher concentration of Cu in computer printed circuit boards (CPCBs) causes interference to recover other metals. The sequential leaching through ferric chloride ($FeCl_3$), recovered Cu prior to bio-cyanidation by *B. sporothermodurans* ISO1 and resulted in the improved leaching of Ag (57%), Au (67%), Pt (60%), etc. The current work reports on *B. sporothermodurans* ISO1, a new *Bacillus* strain that exhibits highest toxicity tolerance ($EC50 = 425\,g\,L^{-1}$) than earlier reported stains and has higher leaching potential that can be implemented to large-scale biometallurgical process for e-waste treatment to achieve the agenda of sustainable development goal (SDG) under the strategies of urban mining.

Keywords

CPCBs, *Bacillus sporothermodurans* ISO1, sequential leaching, OFAT, RSM, cynogenic

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Introduction

The obsolete and non- functional electric and electronic equipment known as e-waste has carved out a niche and special attention among all the solid waste streams. E-waste is classified according to its type, source of development, content and the mechanism of its decomposition. Though, the most widely accepted definition of e-waste is ‘any item that uses a power source and has reached the end of its useful life’ (Mudali et al. 2021). The short life span and upgrading of technology have been continuously increasing the quantum of e-waste by 2 Mt/annum and has currently reached 57.4 Mt (7.3 kg per person) worldwide and further anticipated to exceed 74.7 Mt by 2030 (Forti et al. 2020). Since the beginning, Asia has continued to be the leading producer of e-waste among the continents, and in 2019 it still holds that position with 24.9 Mt; according to the most recent figures, China is the top producer in the world with 10.1 Mt. With 3.2 Mt of e-waste produced, India came second in Asia and third overall. Additionally, America is second among the continents with 13.1 Mt, trailing by only Europe (12 Mt), and then Africa (2.9 Mt). Oceania produces the least amount of electronic trash overall, at 0.7 Mt (Modoi and Mihai 2022; Pan et al. 2022). This complex mixture of metallic components consumed the secondary raw material of approximately 57 billion USD, in which copper (Cu) and gold (Au) contributes majorly worth 10,960, and 9481 million USD, respectively (Arya and Kumar 2020; Mudali et al. 2021). These significant quantities of valuable metals keep e-waste on priority in solid waste streams and become a hotspot among researchers and recycler units as well (Forti et al. 2020; Kumar et al. 2021). Lithium-ion batteries, cathode ray tubes (CRT), Liquid crystals display, printed circuit boards (PCBs), etc., are some of the major sources for metallic constituents of waste

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electric and electronic equipment (WEEE) (Arya and Kumar 2020). But, among all the PCB is the fundamental component of any electronic device which solely consumes huge number of base metals such as copper (Cu), iron (Fe), nickel (Ni), zinc (Zn), precious metals like gold (Au), silver (Ag), platinum (Pt), hazardous metals like cadmium (Cd), lead (Pb) and mercury, (Hg) and rare earth metals (e.g. scandium (Sc) and yattrium (Y)) (Sodha et al. 2020; Thakur and Kumar 2021a, 2021b). This obsolete component of electronic products is estimated to constitute approximately 100 times more Au, >100 times more platinum group elements and 50-folds supplementary Cu than their primary sources. Therefore, PCBs make e-waste an 'urban mine' or 'secondary source' of various precious metals (Awasthi et al. 2018; Thakur and Kumar 2020).

Landfilling and incineration – two usually adopted informal ways to tackle this astonishingly growing solid waste stream – are most harmful methods, causing release of toxic leachates in the ground and emission of gases to the environment. CRT that are disposed of as electronic garbage produce Pb and Cd, which eventually seep into groundwater and endanger aquatic life (Arya and Kumar 2020). Additionally, exposure to poisonous gases and chemicals has the potential to have a negative influence on a person's health working in informal sector. According to a report by WHO, 18 million teenagers and adults are actively involved in informal e-waste processing and 12 million women endangered themselves and their unborn children by exposure to e-waste toxicity (Murthy and Ramakrishna 2022). With all these occupational hazardous threats to the environment and human health, e-waste management is indisputably connected to the 2030 Agenda for Sustainable Development Goals (SDGs) (i.e. SDG 8 on decent work and economic growth, SDG 3 on good health and well-being, etc., SDG 12 focusing on origin of raw material and domestic use of raw material) (Chatziparaskeva et al. 2022; Modoi and Mihai 2022; Papamichael and Zorpas 2022). Therefore, it was advised that all nations continue to manage e-waste sustainably, with a goal of reducing its amount. In addition, within the framework of a circular economy approach, this 'sleeping mine' should be seen as a significant supply of secondary raw materials to augment natural resource conservation, economic value, and infrastructure for formal management systems (Arya and Kumar 2020; Murthy and Ramakrishna 2022). In the purview of all critical scenario of e-waste generation and its management many initiatives have been taken such as Basel convention system, European Union WEEE directive, nations' individual laws and legislations to curb the menace of e-waste and explore key solutions for its management (Perkin et al. 2014; Patil and Ramakrishna 2020; Thakur and Kumar 2021b). Extended Producer Responsibility is one such vital systems which sustains producer's responsibility for total life cycle improvements of product system up to its final disposal. (Murthy and Ramakrishna 2022; Pan et al. 2022).

The conventional procedures such as pyrometallurgy and hydrometallurgy have been used since decades on an industrial scale to extract various metals because of their high efficiency rate, short processing time, and simultaneous recovery of many metals. But, release of toxic fumes and use of hazardous acids associated with these techniques are a concern for human health and environmental pollution. Despite the industrialization of these technologies, high energy and economic demand, detrimental effects to human and environment are limitations that prompt researchers to pursue new economical and eco-friendly methods (Arshadi et al. 2019; Auerbach et al. 2019; Brandl et al. 2001; Chauhan et al. 2018). Biohydrometallurgy is an alternative method which has been explored in the past few years to mobilize metals from their mine sources as well as e-waste. Biohydrometallurgy is a microbe-mediated method, known as 'bioleaching' that encompasses benefits like less energy consumption, economic, eco-friendly, easy in operation, etc., and therefore considered as green technology for treating mine wastes and e-waste (de Andrade et al. 2019; Marra et al. 2018; Kumar et al. 2018a, 2018b). The sulphur-reducing and cynogenic microorganisms such as *Acidithiobacillus ferrooxidans* and *Chromobacterium violaceum* are the foremost microorganisms exploited for metals extraction from numerous mineral sulphide ores and oxidic wastes (Arshadi et al. 2019). Lately, more bacterial as well as fungus species (e.g., *Pseudomonas, Bacillus.*, and *Aspergillus)* have been reported for recovering various precious metals. The cynogenic bacteria manifested themselves with utmost production of cyanide ion (CN^-) and hydrogen cyanide (HCN) as secondary metabolites via consuming glycine as primary precursor, attributes them to make complexes with many metals such as $[Cu(CN)_2]^-$, $Au(CN)_2]^-$ and $[Ag(CN)_2]^-$ to dissolve them in alkaline solution (Faramarzi et al. 2004; Tauetsile et al. 2019). The low efficiency, higher feed concentration, specificity, time requirement, etc., are some of the limitations associated with bioleaching that laid the commercial scale potential at its infant stage (Kumar et al. 2021; Thakur and Kumar 2020). The researchers have continuously been developing many methods to overwhelm the limitations and to manifest this technology for industrial scale applications. The effect of synergistic behaviour on bioleaching activity have also examined with different cynogenic and sulphur reducing bacterial strains (Han et al. 2022; Shittu et al. 2021). Pradhan and Kumar (2012) utilized mixed culture of *Chromobacterium violacium* and *Pseudomonas fluorescence* to enhance the Cu recovery. Moreover, Sahni et al. (2016) used chemo-biohydrometallurgy method to enhance the efficiency of cynogenic strains for recovery of various metals. Whereas, Natarajan and Ting (2014) advanced the process with metabolic engineering to construct strain with higher cyanide production ability. Despite these aforementioned methods used for bioleaching through biogenic cyanide lixiviant, the level of cyanide production is still not sufficient to make the process economically viable (Arshadi and Mousavi, 2015b).

The toxicity of hazardous metals present in e-waste is also another important constraint for efficient bioleaching rate as this inhibits the cyanide production ability of cyanogen (Pradhan and Kumar 2012). Therefore, the continuous search for the potent microorganisms that exhibit bioleaching potential and are indigenous to various metal habituated sites have been going through as these bacteria possesses a specific metabolic system that make them capable to tolerate high concentration of various metals and expected to solubilize more metals and overcome the specificity limitation of bioleaching methods (Chauhan et al. 2018; Kumar et al. 2021). Hence, the key objective of this study was to explore potent indigenous bacterial strains from the metal habituated site that have high toxicity tolerance and overwhelm the limitation of specificity and selectivity of earlier reported microbes. So, soil sample from dump yard of vehicles, source of various metals was screened to isolate the microbial species followed by optimization of the effective parameters which play an important role in bacterial growth, cyanide production and bioleaching of metals. In addition, the further enhancement in leaching efficiency and solubilization of both base and precious metals was performed through chemo-biohydrometallurgy approach.

Materials and methods

E-waste acquirement and metal determination

The sample used for analysis was PCBs shredded dust produced during the physical and mechanical treatment, attained from Exigo Recycling Pvt. Ltd., an e-waste processing firm situated at Panipat, Haryana, India. The metals' compositional analysis of pulverized sample, particles sized $\leqslant 150\,\mu m$ was done through standard aqua regia digestion method (Kumar et al. 2018a; Sahni et al. 2016), followed by initial filtration through Whatman filter paper and then 0.45 μm glass fibre filters (PALL-GF-A/E-I). The sterile distilled water was added to cover up the lost volume during heating process. The various metals solubilized in leachate were analysed through atomic absorption spectrophotometer (AAS, AAnalyst400, Perkin Elmer, Waltham, Massachusetts, United States).

Isolation of metal habituated bacterial strains

The native microorganism inhabitant to metal presence site are more companionable to various metals present in e-waste (Maza et al. 2019). Therefore, the bacterial strain was isolated from metal enriched area underlying dump yard of cars, located at Tara Devi (Shimla, Himachal Pradesh) through sequential enrichment method. A 1 mL sample was inoculated into 100 nutrient broth (NB) media followed by 3 days incubation at 37 °C at 150 rpm. After 3 days incubation period, 1 mL culture was transferred to 100 mL NB supplemented with $10\,g\,L^{-1}$ e-waste and allowed to grow in incubator shaker (Thermo Scientific MAXQ800, Waltham, Massachusetts, United States) at 37 °C and 150 rpm for 3 days. This continuous sequential enrichment selection was performed up to $100\,g\,L^{-1}$ e-waste concentration for a total of 15 days in time interval of 3 days incubation. After 15 days of sequential transfer, 100 μL culture was spread on nutrient agar (NA) media followed by 24 hours incubation at 37 °C. To obtain pure culture, further streak plate method was performed on NA media.

Characterization of isolated strain

The isolated strain was primarily examined for its morphological and biochemical characteristics using techniques like Gram's staining, KOH string test, endospore staining, haemolytic assay, hydrogen peroxide catalase assay, methyl red assay, etc. Then molecular identification was done through 16S rRNA gene sequencing. The genomic DNA extraction using the EXpure Microbial DNA isolation kit was followed by amplification of 16S rRNA genes through universal primers 27 F (5′ AGAGTTT GATCTGGCTCAG 3′) and 1492 R (5′ TACGGTACCTTGTTA CGACTT 3′). Polymerase chain Reaction amplified 16S rRNA gene was sequenced through single pass sequencing process followed by electrophoresis in an ABI3730xl sequencer at Yaazhxenomic Pvt. Ltd. at Tamil Nadu. The acquired nucleotide sequence was compared with the GenBank database using the NCBI blast sequence similarity search tool. The program MUSCLE 3.7 was employed for multiple sequence alignment analysis followed by phylogenetic analysis through PhyML 3.0 program tool.

Toxicity tolerance assessment

Determination of toxicity tolerance is the major parameter to obtain the potential bioleaching strains. Toxicity tolerance reveals the potential of bacterial strain to grow with different metals present in e-waste. Toxicity tolerance towards the PCBs e-waste was determined through colony forming unit (CFU) counting method at 200, 250, 300, 350, 400, 450, $500\,g\,L^{-1}$ pulp density. The experiment was performed in duplicates in 250 mL Erlenmeyer flasks. 1% v/v inoculum (4×10^6 CFU mL^{-1}) was added into 100 mL NB medium (pH 7) supplemented with respective concentration of sterile e-waste. All the experimental flasks were kept in incubator shaker (Thermo Scientific MAXQ 800) at 37 °C, 130 rpm for 24 hours followed by enumeration of CFU counting by spread plate method. Incubation of bacterial culture in the absence of e-waste was taken as control.

Toxicity tolerance was considered in percentage inhibition response and represented in the form of dose response curve and calculated by following formula (shown in equation (1))

$$\%IR = \frac{\text{control} - \text{test}}{\text{control}} \times 100 \tag{1}$$

IR implies inhibition response, control signifies bacterial growth in the absence of e-waste, and test signifies growth of bacteria in the presence of e-waste.

Parameter optimization through OFAT and RSM

Considering previous studies, incubation temperature, pH, e-waste amount (i.e. pulp density), glycine concentration and time are the major factors which affect the bioleaching process of bacterial strains (Kumar et al. 2018b); therefore, these factors were optimized in the present study for maximum recovery of metals via OFAT method. The optimization was initiated at 37 °C with 4% (v/v) inoculum, 130 rpm for 48 hours incubation period. The bioleaching optimization was performed at temperature range of 25 °C–40 °C, pH 6–9, pulp density 1–20% (w/v), glycine concentration 1–10 g L^{-1}. All the preceding optimization experiments were performed in duplicates along with a set of control experiment. In controlled experimental set, nutrient media without inoculating cultural medium was operated at similar conditions. For optimizing glycine concentration, controlled set was inoculated with cultural medium without glycine precursor.

The significant variables selected from OFAT method was applied to response surface methodology (RSM) to observe the synergetic effect that can enhance the bioleaching potential of bacteria at different levels. The central composite design (CCD) was implemented in Minitab 2018 software to optimize the range of temperature, pH, glycine concentration and pulp density at coded level of $-\alpha$, 0, $+\alpha$. The software generated two-level factorial design for 31 experimental sets with 16 trials at cubic points, 7 trials centre points and 8 trials at axial points. Out of 31 experiment sets, one set was repeated six times, therefore total 25 sets were experimentally performed in duplicates with inoculum size 4% v/v in 100 mL NB.

Sequential leaching for recovery of base and precious metals

The higher Cu content in PCBs is a barrier for recovery of Ag and other metals, as most of the biogenic cyanide produced by microorganism is utilized by Cu and cause hindrance for other metals. Therefore, sequential leaching through chemo-biohydrometallurgy method using chemical lixiviant (a mild acid i.e. $FeCl_3$) followed by biological lixiviant (cyanide produced by bacteria) can enhance the recovery of metals other than Cu (Thakur and Kumar 2021a, 2021b). Chemical treatment of e-waste with $FeCl_3$ was performed as per our pervious study (Thakur and Kumar 2021). After the treatment with 1% $FeCl_3$, 1% w/v e-waste was washed with distilled water followed by sterilization through autoclave, and proceeded for two-step bioleaching of other metals through bio-cyanide (a biological lixiviant) by 4% v/v (4×10^8 CFU mL^{-1}) inoculum of isolated bacterial strain grown in 100 mL of NB medium under optimized conditions. Metals analysis was done through AAS after 8 days of incubation period. All the experiments were performed in duplicates alongside control experiment. Bioleaching control set was run with equal volume of nutrient medium and pulp density without inoculating bacterial culture and incubated under optimized growth conditions (i.e. temp. 40 °C, 130 rpm).

Results and discussion

Metal composition of CPCBs

Metals concentration present in computer printed circuit boards (CPCBs) is illustrated in Table 1. The e-waste was found to be enriched with metals. Copper (Cu), aluminium (Al), iron (Fe), nickel (Ni) silver (Ag), gold (Au) and platinum (Pt) were some of the major base metals and precious metals, respectively, found in CPCBs, where Cu was predominant among all the metals. The earlier investigation by Chauhan et al. (2018) also indicated the higher concentration of some base metals (e.g. Al 4.65%, and Cu 2.67%) than precious metals in shredded dust of e-waste originated during physio-mechanical processing. A variation in metallic composition may be attributed to source of e-waste and particles size generated during mechanical processing of the products. Higher concentration of Cu is due to increased usage of the metal during manufacturing of the electronic products, and higher ductility of Cu, distributing it more in e-waste than precious metals such as Ag, Au in larger size fraction (Huang et al. 2014). Furthermore, higher amount of Cu hinders and obstructs the exposure of Au and other precious metals and reduces the reactivity with aqua regia solution. However, in the present study, Ag that was obtained in higher amount than in other reported studies, may be attributed to size reduction of the particles (shredded dust with particle size ⩽ 150 µm), which aids in the exposure of precious metals and enhance their reactivity with aqua-regia solution (Kumar et al. 2018a, 2018b). Similar results were reported by Sun et al. (2016); they determined the metal composition with different particle size of e-waste and obtained less Au than Cu in particle size 1–8 mm, whereas precious metal concentration increased within the particle size ⩽0.2 mm.

Table 1. Shredded dust of CPCB consisting of various base and precious metals (Thakur and Kumar 2021).

Sr. No	Metals	Concentration (mg g^{-1})
1	Cu	12.751
2	Ag	10.34
3	Fe	2.46
4	Al	0.39
5	Ni	0.24
6	Au	0.04
7	Co	0.02
8	Pt	0.08

Isolation, characterization and identification of indigenous bacterial strain

The sequential enrichment method leads to screening of a potential microbial strain that is able to tolerate various metals present in PCBs e-waste. The single microbial strain isolate 1 (ISO1) was obtained at 100 g L^{-1} pulp density, which was utilized for all succeeding experiments. The primary characterization revealed the morphological and biochemical features of ISOI and found to be Gram positive (+ve) with ability to form endospores, haemolysis and cyanide production. Furthermore, 16S rRNA sequencing followed by Basic Local Alignment Search Tool (BLASTN) sequence similarity search to NCBI GenBank database revealed the highest sequence similarity (99.85%) with *Bacillus*

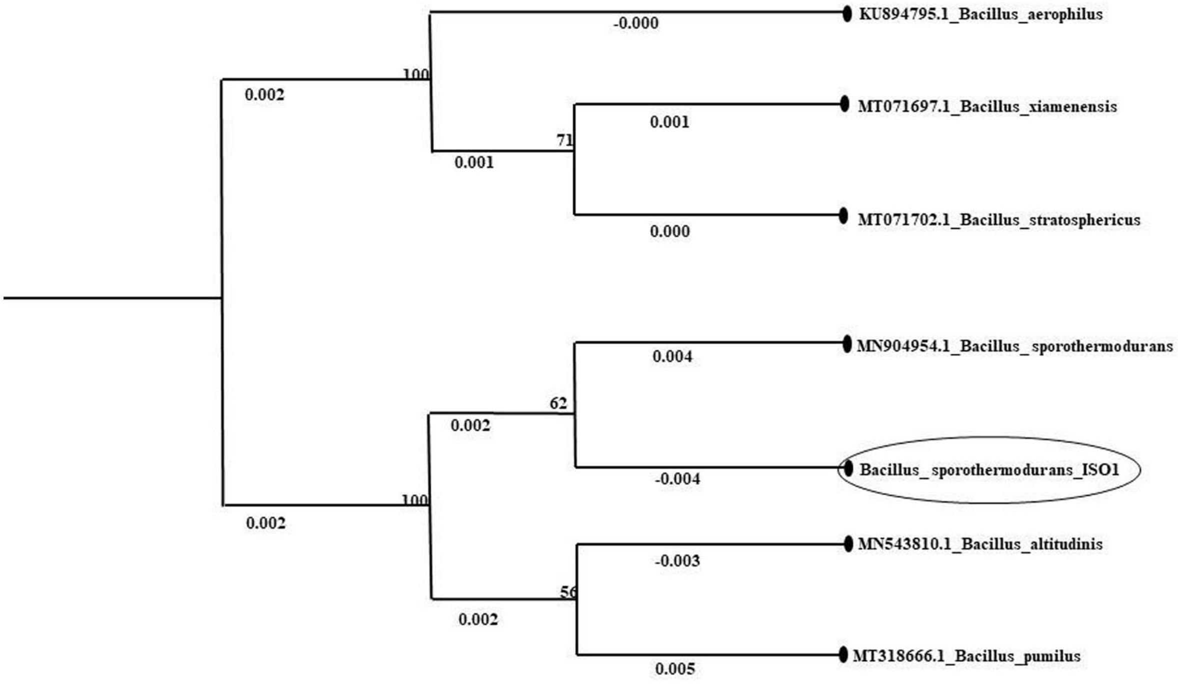

Figure 1. Phylogenetic tree to depict the evolutionary relationship of *B. sporothermodurans* ISO1 with other *Bacillus* species.

sporothermodurans. The phylogenetic analysis through multiple sequence alignment showed maximum similarity index with different species of genus *Bacillus* as shown in Figure 1. So, *B. sporothermodurans* ISO1 is a new strain which has been explored for the first time for its bioleaching potential. As per authors' knowledge *Bacillus megaterium* is the only *Bacillus* sp. that have been reported in bioleaching aspects.

Toxicity tolerance assessment

E-waste is a source of various toxic components including toxic metals and brominated and chlorinated compounds; which cease the bacterial growth (Arab et al. 2020). Therefore, toxicity tolerance is an important potential of microorganism to guarantee feasible and efficient bioleaching. The toxicity assessment through dose-response method is a measurable analysis that determines the toxicity tolerance at maximum treatment concentration and 50% effective concentration (EC50) suitable for metals bioleaching (Kumar et al. 2018a, 2018b). Hence, dose response curve was generated for CFU mL^{-1} obtained from the toxicity assessment at various e-waste concentrations. Toxicity tolerance was assessed in terms of percent inhibition response. EC50 value of e-waste was assessed through dose response curve shown in Figure 2 and it was found to be greater than 400 g L^{-1} e-waste pulp density which was found to be comparatively higher than *Pseudomonas balearica* SAE1 and *C. violacium* studied by Kumar et al. (2018a). However, this may be attributed by metallic constituents' variations in e-waste, used for the study. Kumar et al. used virgin PCBs e-waste with higher metal concentration whereas in the present study shredded dust PCBs e-waste was used which has less metal concentration than that used by Kumar et al. (2018a). Furthermore, habituate of *B. sporothermodurans* ISO1, may attribute to developed metabolic machinery towards tolerance of different metals that may resist the effect of toxic e-waste at higher concentration.

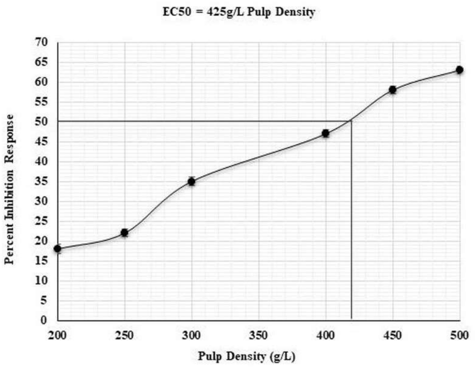

Figure 2. Toxicity effect of e-waste on *B. sporothermodurans* ISO1 in term of dose response curve.

Optimizations of effective factors

Effect of temperature. Bacterial growth and activity may vary in wide temperature range. Many studies have determined mesophilic temperature range (i.e. 20 °C–40 °C) as best temperature

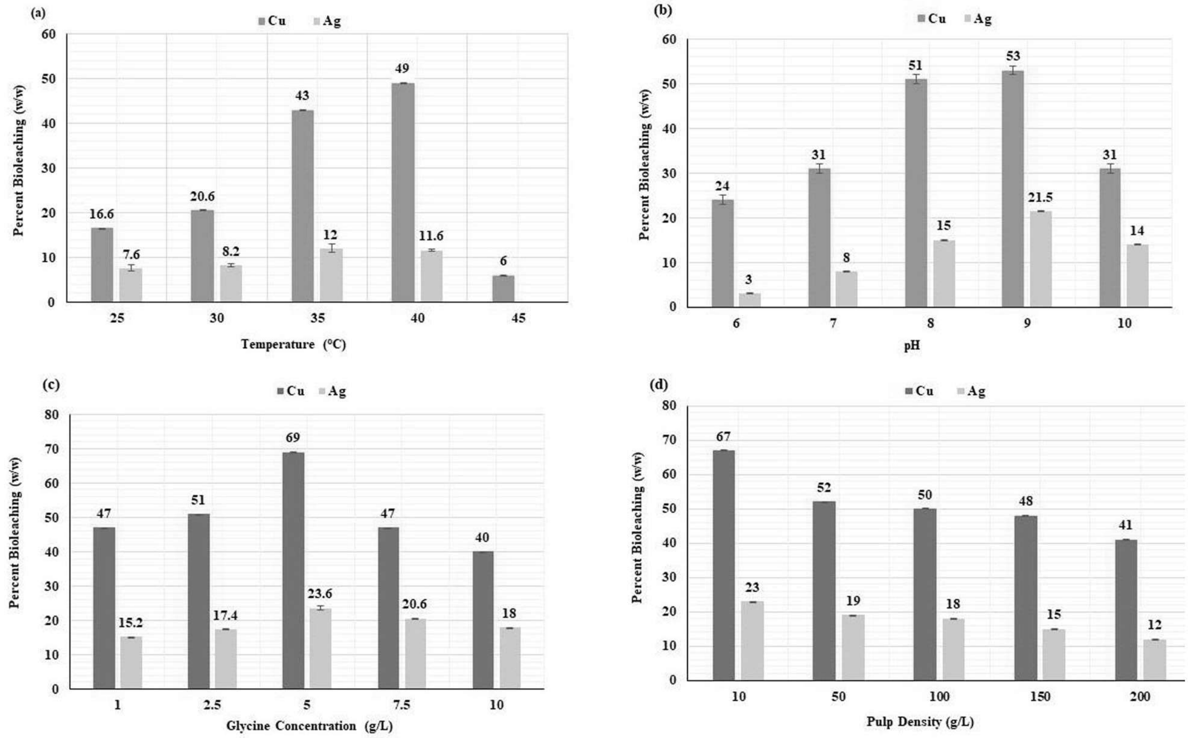

Figure 3. Different parameters that affected the bioleaching activiy of *B. sporothermodurans* ISO1. (a) Optimazation of temperature range (25 °C–40 °C). All values are the means of two replicates and ±SD values. (b) Change in the bioleaching potential of *B. sporothermodurans* ISO1 with change in the pH of the medium. (c) Optmization of glycine as a primary precursor of cyanide production. (d) Effect of pulp denisity on the bioleaching of Cu and Ag by *B. sporothermodurans* ISO1.

conditions for effective cyanide production and bioleaching activity by cyanogenic strains such as *C. violaceum* and *Pseudomonas* (Sodha et al. 2020). Therefore, indigenous cyanogenic strain was examined for its effective bioleaching potential in the mesophilic range of 20 °C–40 °C. It is depicted from the Figure 3(a) that the *B. sporothermodurans* ISO1 bioleaching activity increased with increase in temperature. The earlier investigations reported optimum temperature range of 25 °C–30 °C for *C. violaceum* (Kumar et al. 2018a; Yuan et al. 2018), 35 °C–37 °C for *Pseudomonas* sp. and *B. megaterium* (Pradhan and Kumar 2012). In our study, the maximum metals solubilization was obtained from 35 °C to 40 °C (i.e. 49% Cu and 12% Ag at 40 °C) as shown in Figure 3(a). But, further increase in the temperature, that is, 45 °C, bioleaching activity was declined sharply as growth of *B. sporothermodurans* ISO1 ceased above 40 °C. However, our finding is conflicting Arab et al. 2020, as they found bacterial strain (NS23) that showed maximum cyanide production (1.6 mg L^{-1}) at 20 °C. Furthermore, they suggested that low temperature reduces the volatilization of HCN and reduce the oxygen consumption during bacterial growth. But, *B. sporothermodurans* ISO1 attained maximum growth at temperature 40 °C; hence, maximum cyanide production and leaching was obtained at respective temperature. *B. sporothermodurans* ISO1 was found to be the first prominent cyanogen showing efficient bioleaching ability at 40 °C unlike earlier reported cynogenic microorganisms and offers a low energy and economic bioleaching process for large-scale fermenter system.

Effect of pH

pH is a very vital factor that influences the production of biogenic cyanide $(CN)^-$. The earlier reported studies claimed higher yields of biogenic cyanide under alkaline conditions rather than acidic conditions as pK_a value of HCN is 9.3; therefore alkaline environment increases the availability of CN^- and enhances the metal solubilization proportion (Brandl et al. 2008; Kumar et al. 2021; Pradhan and Kumar 2012). Mostly microorganisms' growth is favoured at physiological pH range (i.e. 7–8). Therefore, the influence of initial pH of medium on cyanide production by *B. sporothermodurans* ISO1 was investigated at pH 7–10. The Figure 3(b) inferred that bioleaching efficiency is higher at alkaline conditions and maximum metals leaching (Cu 53%; Ag 21%) obtained at pH 8–9. This is because a low pH (acidic pH) favours the release of more HCN instead of CN^- ions, a volatile and less water-soluble gas, whereas, alkaline environment shifts equilibrium towards aqueous cyanide ions (CN^-) formation to form metals–cyanide complex (Natarajan and Ting 2014; Kumar et al. 2021). In the present study, pH fluctuation was observed during two-step bioleaching process, where maximum bacterial growth was obtained at initial pH 9 and pH got reduced to 8.3 on 2–3 day of incubation period. Subsequently, pH increased up to

9.3 on addition of e-waste due to its alkaline nature and favoured the condition of CN^- (shown in equation (2)) ion formation and metal solubilization as pH was maintained near the pK_a value of cyanide. Hence maximum bioleaching was obtained at initial pH 8. Further, increase in the pH (i.e. 10), decreased the bacterial cell population (maximum OD: 0.427); therefore, reduced bioleaching occurred at initial pH 10. The study by Shin et al. (2013) also discloses that higher pH (i.e. 10) decreases cyanide production by inhibiting bacterial growth. Another study by Arshadi and Mousavi (2015a) also investigated the pH variation by *B. megaterium* during the bioleaching of Cu and Ag. They found pH decline from 10 to 8.8 on first day of bacterial growth and then finally increased up to 9.4 with $0.5\,g\,L^{-1}$ glycine concentration.

$$HCN \longleftrightarrow H^+ + CN^- \qquad (2)$$

Effect of glycine concentration. The cyanide production ability of cyanogenic microorganisms is stimulated not only on the carbon source but also some amino acid precursors. Glycine is considered as the primary precursor that can directly convert into HCN; hence, an optimum addition of glycine to the culture media can enhance cyanide production (Arab et al. 2020). Based on earlier reported investigations, glycine concentration $>10\,g\,L^{-1}$ may become toxic and attenuates bacterial growth, hence reduction in the cyanide production (Arab et al. 2020). Therefore, effect of glycine concentration was determined at a concentration of 1, 2.5, 5, 7.5, $10\,g\,L^{-1}$. Figure 3(c) reveals that *B. sporothermodurans* ISO1 peaked the cyanide production from 2.5–$5\,g\,L^{-1}$ (maximum with $5\,g\,L^{-1}$, i.e. 69 % Cu and 23% Ag), whereas amounts of glycine above $5\,g\,L^{-1}$ adversely affected the bioleaching potential of *B. sporothermodurans* ISO1. This is due to production of cyanide-consuming compound such as β-cyanoalanine and degradation of cyanide owing to the toxicity of excessive CN^- at excessive glycine concentration (Arab et al 2020). The obtained trend for glycine concentration was also supported by other reported bioleaching studies, as Kumar et al. (2018a, 2018b), Arab et al. (2020) also obtain maximum bioleaching with $5\,g\,L^{-1}$ glycine precursor.

Effect of pulp concentration. The Figure 3(d) inferred the trend of bioleaching affected by increasing the amount of e-waste into bioleaching medium. It can be depicted from the figure that *B. sporothermodurans* ISO1 was able to leach efficient copper metal up to $50\,g\,L^{-1}$ e-waste concentration. But, further increase of amount from 50 to $200\,g\,L^{-1}$ resulted in significant decrease in the metal solubilization. The Cu and Ag leaching at $200\,g\,L^{-1}$ was 44 and 12%, respectively. The increased amount of e-waste attributes the higher toxic effect of various metals presents in e-waste that inhibits the bacterial metabolism and cyanide lixiviant production (Kumar et al. 2018a, 2018b). To the best of authors' knowledge, there have not been reported any study yet which showed efficient bioleaching higher than $10\,g\,L^{-1}$ pulp density. *B. sporothermodurans* ISO1 showed higher Cu leaching efficiency than indigenous bacterial strains isolated by Arab et al. 2020 from the landfill of e-waste. They obtained 81.17, 41.72 and 31.52% of Cu solubilization using N37, N23 and N41 bacterial isolates, respectively.

Aforementioned parameters play important roles in the growth of microorganism and therefore all of them are highly significant in the bioleaching efficiency of *B. sporothermodurans* ISO1. But, pH and pulp density were found to be predominat factors that affected the bioleaching efficiency of *B. soprothermodurans* ISO1 adversely. The various studies claimed that the production of biological cyanide $(CN)^-$ solely depends on the pH of the medium, as pK_a value of HCN is 9.3, therefeore alkaline pH (>9) favours the formation more CN^- and metal ion complexes. Whereas, at low pH most of CN^- is lost under volatilization of HCN gas (Arab et al. 2020; Kumar et al. 2018a; Thakur and Kumar 2021a). Pulp denisty or amount of e-waste is another significant aspect that impacts the bioleaching potential of microorganism. As the concentration of e-waste increases, the amalgam of hazradous metals, inorganic pollutants in e-waste bacome toxic to microorganism and ceases the growth of micoorganism during bioleaching process and therefore reduce the production of lixiviant and metals solubilization significantly (Han et al. 2022; Mudali et al. 2021). These observations are based on the effect of one parameter at a time. Although, the potential of microorganism for bioleaching processes may vary with interactive effect of different variables, as favourable factor may dominate the negative impact of another unfavourable factor. Thus, the interactive effect of multi-variate factor was analyzed through RSM approach.

Response of different parameters through RSM analysis

To evaluate the interactive effect of multi-variate factors, RSM is a powerful statistical tool which develop the functional relationship between response and variable. RSM provides rapid and accurate optimum conditions of variable which leads to highest and lowest range of responses (Karim and Ting 2018). The 31 experimental sets of four parameters, that is, temperature, pH, glycine concentration and pulp density designed in RSM through CCD with their experimental and software predicted Cu and Ag recovery are listed in Table 2. The maximum leaching was obtained with 13th experimental run where temperature was 40 °C, pH 8, glycine concentration $7.5\,g\,L^{-1}$ and pulp density $10\,g\,L^{-1}$. The bioleaching ability of *B. sporothermodurans* ISO1 was enhanced up to 95% Cu and 44% Ag solubilization through synergetic effect of these factors. The statistical significance of designed model was confirmed through analysis of variance (ANOVA), where F-value was 7.12 and 19.74 for Cu and Ag, respectively (data shown in Supplemental File). The p-value $>$ F-value at $\alpha = 0.05$ implies the statistical significance of model (Kumar et al. 2018a, 2018b). The ANOVA analysis showed factors with significant p-values, which influenced bioleaching of Cu and Ag at linear, quadratic and interactive level. ANOVA analysis depicted that temperature and pulp

Table 2. The experimental and software predicted values of Cu and Ag recovery for experimental runs designed by CCD-RSM in Minitab18 software.

Sr. no.	Temp. (°C)	pH	Glycine concentration (g L^{-1})	Pulp density (g L^{-1})	Experimental Cu recovery (% w/w)	Predicted Cu recovery (% w/w)	Experimental Ag recovery (% w/w)	Predicted Ag recovery (% w/w)
1	37	9	5	55	39	44.432	18	21.368
2	40	10	7.5	10	53	59.7852	31	31.6092
3	37	9	5	55	39	44.432	18	21.368
4	37	9	5	55	39	44.432	18	21.368
5	37	9	5	55	39	44.432	18	21.368
6	37	9	5	55	39	44.432	18	21.368
7	34	10	7.5	10	47	46.4102	14	14.3176
8	40	9	5	55	70	62.1627	27	27.904
9	40	10	7.5	100	61	62.7991	21	19.5398
10	37	9	5	100	58	46.6071	16	15.3484
11	37	9	5	55	39	44.432	18	21.368
12	34	10	7.5	100	53	53.1741	7	5.49811
13	40	8	7.5	10	95	88.4102	44	42.7064
14	40	8	7.5	100	71	77.6741	28	28.387
15	34	8	7.5	100	34	35.7991	13	14.5953
16	40	10	2.5	100	57	57.6741	21	20.4426
17	34	9	5	55	47	42.1627	16	15.2373
18	40	8	2.5	10	84	90.2852	39	35.6092
19	34	8	2.5	100	33	32.6741	9	9.49811
20	40	10	2.5	10	80	74.9102	39	36.262
21	37	9	2.5	55	46	49.1627	23	20.4596
22	37	9	7.5	55	59	43.1627	17	20.6818
23	37	9	5	55	39	44.432	18	21.368
24	37	9	5	10	60	58.7182	28	28.7929
25	40	8	2.5	100	62	59.2991	19	17.5398
26	34	8	7.5	10	37	42.7852	27	25.6648
27	34	10	2.5	10	77	76.7852	24	24.7203
28	37	10	5	55	51	44.1627	19	20.4596
29	37	8	5	55	49	43.1627	26	24.6818
30	34	8	2.5	10	65	59.9102	24	24.3176
31	34	10	2.5	100	60	63.2991	12	12.1509

density are the two major vital factors which have central impact on bioleaching at linear level, whereas temperature, pH, and glycine concentration-pulp density have effect at their interactive level. The determination of model fits to obtained data was evaluated by finding the correlation between experimentally obtained and software predicted bioleaching values in terms of R^2 values. R^2 values were 0.86 and 0.93 for Cu and Ag, respectively and indicates 86 and 93% variations in Cu and Ag leaching response. The R^2 value close to 1 indicates that data fits better to model fit regression line. Hence R^2 value for obtained data values assured good correlation for software predicted and actually obtained bioleaching values.

The Figure 4 depicts the 3D surface model of significant interactive effect of decisive factors. 3D model is significant to interpret the value of response variable that is affected by two independent predictor variables. Figure 4(a) and (c) showed that temperature and pH were two independent variables that affected the bioleaching rate of Cu and Ag metal respectively, and Figure 4(b) and (d) depicts the interactive effect of pH and glycine concentration on Cu and Ag metal recovery.

Regression kinetics

The reaction kinetics for Cu and Ag recovery was generated through non-linear regression analysis. The non-linear regression analysis expresses the fit data model in mathematical function. The regression analysis model excluded non-significant parameters and generated second-order kinetics for Cu and Ag metal recovery with most influenced variables analyzed through ANOVA analysis (shown in Supplemental Table). The final coded equation, represents the correlation between linear, quadratic and interactive effect of bioleaching factors and obtained response. Equations (3) and (4) depict the percentage recovery of Cu and Ag metal, respectively, with different variables such as temperature, pH, glycine concentration and pulp density in their linear, square and interactive way.

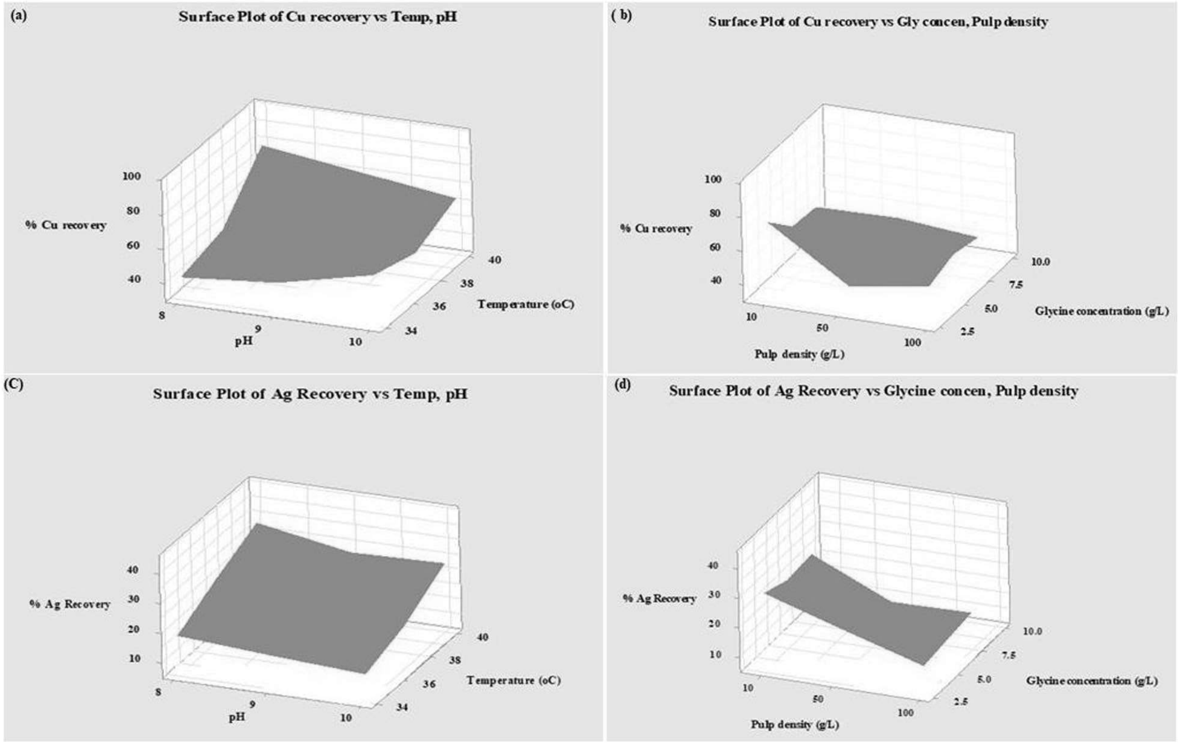

Figure 4. 3D surface plot for recovery of Cu and Ag influenced by interactive effect of vital factors at *P*-value > *F*-value at $\alpha = 0.05$; (a) Cu recovery with interactive effect of temperature and pH, (b) effect of pH and glycine concentration on bioleaching of Cu, (c) surface plot for Ag recovery with interactive effect of temperature and pH, and (d) Ag recovery via interactive effect of glycine and pulp density.

$$\begin{aligned}\text{Cu recovery} = {} & 238 - 38.2\text{A} + 116.2\text{B} - 13.3\text{C} - 1.237\text{D} \\ & + 0.859\text{A}^2 - 0.77\text{B}^2 + 0.277\text{C}^2 + 0.00406\text{D}^2 \\ & - 2.687\text{AB} + 0.508\text{AC} - 0.0069\text{AD} - 1.325\text{BC} \\ & + 0.0764\text{BD} + 0.0450\text{CD}\end{aligned} \tag{3}$$

$$\begin{aligned}\text{Ag recovery} = {} & 82 - 0.4\text{A} - 19.3\text{B} + 4.35\text{C} - 0.119\text{D} \\ & + 0.023\text{A}^2 + 1.20\text{B}^2 - 0.128\text{C}^2 + 0.000347\text{D}^2 \\ & + 0.021\text{AB} + 0.1917\text{AC} - 0.00602\text{AD} \\ & - 1.175\text{BC} + 0.0125\text{BD} + 0.00833\text{CD}\end{aligned} \tag{4}$$

Where A, B, C, D signify incubation temperature (°C), pH, glycine concentration (g L^{-1}) and pulp density (g L^{-1}), respectively in linear, square and interactive sets.

Sequential leaching for Cu and other metals

The maximum leaching potential of *B. sporothermodurans* ISO1 is shown in Figure 5, which reveals higher Cu recovery rate compared to other metals present in PCBs e-waste sample. The similar trend was observed by Thakur and Kumar (2021a) with *P. balearica* SAE1. The limited cyanide production and highest Cu concentration in CPCBs attributes the decreased leaching of precious and other base metals such as Ag and Fe. Therefore, sequential leaching with $FeCl_3$ followed by biogenic cyanide was observed to be an economical and eco-friendly method to improve the leaching rate of other metals even in the presence of Cu as bulk metal to overwhelm the specificity limitation of bioleaching method. 1% $FeCl_3$ leached out maximum Cu (i.e. 83%) in 3 hours of incubation period. Recovery of Cu in $FeCl_3$ solution was attributed by Fe^{3+} ions and Cl^- ions which act as strong oxidising and strong complexing agents, respectively (equation (5)) (Thakur and Kumar 2021a). Biocyanidation reaction by *B. sporothermodurans* ISO1 leached out maximum metals present in PCBs e-waste, are shown in Figure 6, where silver recovery was enhanced from 44 to 57% along with sufficient recovery of other metals such as Au (67%), Pt (60%), Fe (49%). The recovery of aforementioned metals inferred higher cyanide production potential of *B. sporothermodurans* ISO1. Whereas prior recovery of Cu provides bio-cyanide availability for other metals and overwhelm the specificity limitation of bioleaching method. The cyanide has more potential to make complex with precious metals in comparison to base metals; hence recovery of Au, Ag and Pt was higher in comparison to Fe through bioleaching method (Kumar et al. 2018b). Thus, present chemo-biohydrometallurgical, a hybrid method was found to be a significant way to recover various metals form e-waste. In terms of environmental aspect, the use of $FeCl_3$, a mild acid, may prove to be favourable and can surpass the other strong acids used in hydrometallurgy technique such as Hydrochloric acid (HCl) and Nitric acid

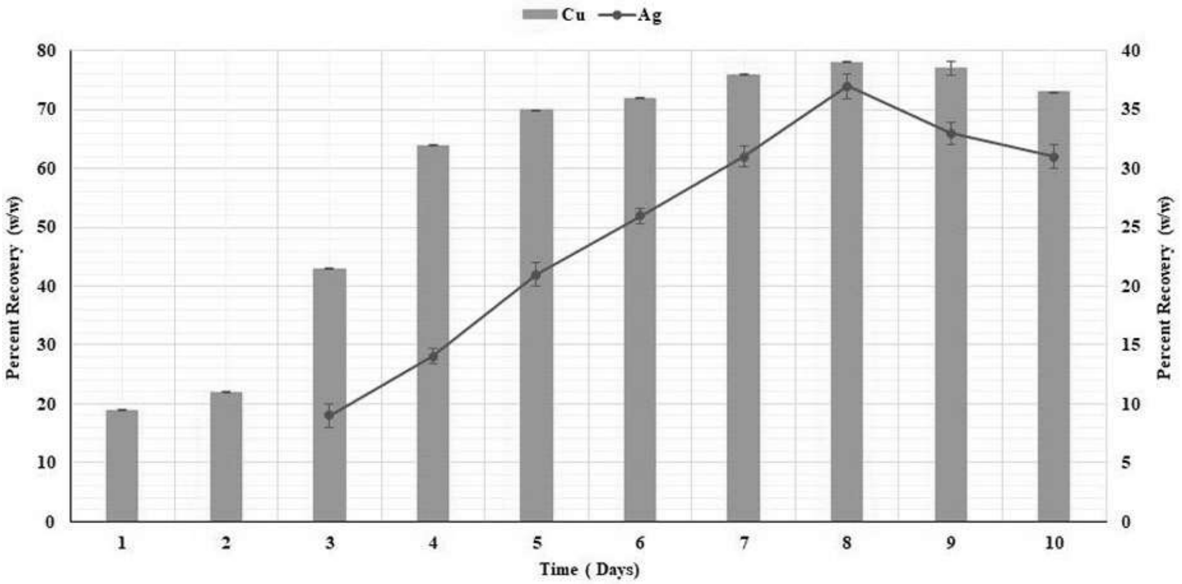

Figure 5. Solubilization of Cu and Ag from 10 g L^{-1} pulp density through bio-cyanide produced by *B. sporothermodurans* ISO under optimized conditions.

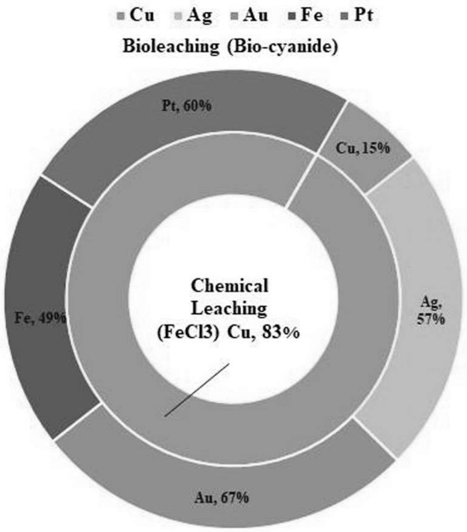

Figure 6. Percentage recovery of metals from PCBs e-waste through sequential leaching with chemical lixiviant and biological lixiviant.

(HNO_3) (Thakur and Kumar 2021b). In addition, bioleaching efficiency of *B. sporothermodurans* ISO1 was improved for metals other than Cu that can mitigate the selectivity constraint of bioleaching process. So, this method may be considered as a potential way to recover metals from e-waste at industrial scale bioleaching operation; as such hybrid methods have gained the attention of many biometallurgy industries over conventional methods (i.e. Pyrometallurgy and hydrometallurgy) to recover metals for e-waste at large-scale operations to sustain the urban mining strategies. A Mint innovation is one of the biometallugry corporations in Auckland, New Zealand that pioneered the adoption of chemo-biohydrometallurgy process for recovery of base metals and precious metals from e-waste. In addition, Mintek, BacTech, N2S are some other firms that are commercializing the biohydrometallurgy technique for recycling e-waste at large-scale operations. (Han et al. 2022).

$$FeCl_3 + Cu \longrightarrow FeCl_2 + CuCl \tag{5}$$

Conclusion

The cynogenic microorganisms have been taking prodigious attention in the field of biohydrometallurgy. Many cynogenic microorganisms such as *Pseudomonas sp., C. violaceum*, *Leptosprillium* sp., etc., have been utilized in two-step bioleaching for solubilization of various metals along with application of different chemical lixiviant ($FeCl_3$) in chemo-biohydrometallurgy methods to overwhelm the specificity limitations of these microorganisms towards leaching of single metal. The present study focused on exploration of bacteria that are habituated to metals existence environment succeeded by the optimization of different factors which affects the growth of *B. sporothermodurans* ISO1 and its bioleaching activity. The *B. sporothermodurans* ISO1 exhibited satisfactory e-waste toxicity tolerance (EC50 = 425 mg L^{-1} pulp density) along with efficient leaching of metals from PCBs e-waste at 40 °C, pH 9, glycine concentration 5 g L^{-1} and pulp density 10 g L^{-1}. The present study unveils new *Bacillus* strain (i.e. *B. sporothermodurans* ISO1) along with its optimized growth and bioleaching conditions. The sequential leaching with chemical lixiviant ($FeCl_3$) and biological lixiviant (bio-cyanide) enhanced leaching capacity for precious metals by *B. sporothermodurans* ISO1 in economical and eco-friendly system. The high toxicity tolerance and significant leaching efficiency than earlier reported microorganism can attribute to the sustainability of this potent bacterial strain for industrial-scale bioleaching operations, so process optimization for scale-ups is a vital step; as kinetics and mass flow of the microorganism can vary with scale-up activity that can impact the bioleaching efficiency at large scale. Furthermore, the exploration of exact mechanism exhibited by *B. Sporothermodurans* ISO1 towards bioleaching would be a very vital future aspect that can impose the role of genomic and proteomic engineering to further enhance its leaching efficiency and slow process limitation to promote its role in industrial scale biometallugry process to sustain the circular economy system through urban mining strategies.

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Author contributions

Conceptualization: [Sudhir Kumar] and [Pooja Thakur]; Methodology: [Sudhir Kumar] and [Pooja Thakur]; Formal analysis and investigation: [Sudhir Kumar] and [Pooja Thakur]; Writing – original draft preparation: [Pooja Thakur]; Writing – review and editing: [Sudhir Kumar] and [Pooja Thakur]; Supervision: [Sudhir Kumar].

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Reference

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RESEARCH

Augmentation in bioleaching potential of indigenous *Bacillus* sp. ISO1 for metals recovery from waste computer-printed circuit boards

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Abstract

The bio-cyanidation process of various cyanogenic microorganisms is found to be a sustainable and effective method for metals recovery from primary and secondary sources. This process has surpassed the limitations of the chemical cyanide treatment process; thus, prioritized as a promising approach for e-waste “urban mining” strategies. The main focus of the study was to enhance the bioleaching capacity of indigenous *Bacillus* sp. ISO1 and to implement optimized parameters in large-scale bioleaching operations. The assessment of various amino acids unveiled that like other cyanogenic microorganisms *Bacillus* sp. ISO1 also preferred glycine as a prime precursor for cyanide synthesis, as maximum metal solubilization was achieved with glycine amino acid. Other amino acids influenced the bacterial growth but not significantly affected the biocyanidation process. The evaluation and optimization of methionine as a lixiviant stimulator demonstrated that the addition of 1 mg/L methionine effectively enhance the production of glycine-utilizing cyanide lixiviant, that led to a significant solubilization of Cu (86%), Au (75%), and Ag (63%) metals. Furthermore, the kinetics of metal solubilization and operating conditions were explored at increased volume (i.e., 3 L working volume) of bioleaching medium to assess the industrial scale potential of this potent bacterial strain with optimized parameters such as temperature, pH, pulp density, and inoculum size. The significant recovery of Cu (˃ 60%) and other metals at this substantial volume suggested the implementation of a bioleaching process with this potent bacterial strain at industrial scale operations.

Keywords E-waste · Urban mining · Biocyanide · Glycine · *Bacillus* sp

Introduction

Technological advancement and economic expansion have led to a decrease in the lifespan of electronic and electrical equipment (EEEs) and astonishingly increased the pace of waste electronic and electrical equipment (WEEEs) and electronic trash or “e-waste” production (Arya and Kumar, 2020). Printed circuit boards (PCBs) are the central element of all electronic equipment and hence contributed majorly to e-waste generation than other constituents such as batteries, LED screens, etc. This waste is known to comprise 20 distinct elements with heterogenous metallic compositions of noble, base, precious, and rare earth metals (Chauhan et al., 2018; Thakur and Kumar, 2021). Copper (Cu) is the most prevalent metal in all forms of PCBs and is used frequently due to its high conductivity and relatively low cost. It is estimated that the technology sector annually demands 72% Cu metal which is attained from natural resources via extensive and energy-intensive process (Mudali et al., 2021; Pan et al., 2022; Valero et al., 2018). Therefore, it is believed that discarded PCBs contain 50 times more Cu than their natural ores. Additionally, gold (Au) is 50–100 times and platinum group elements (PGEs) are 7–200 times more abundant in PCBs; hence, this waste emerges as the core of “urban mining” (Islam et al., 2020; Mudali et al., 2021; Pan et al., 2022; Rigoldi et al., 2018; Sukhomlinov et al., 2019; Valero et al., 2018). The unsustainable demand for various metals has a large carbon footprint in addition to the depletion of natural resources, as the mining process has led to 127 Mt CO_2eq of greenhouse gases worldwide (Arora et al., 2017; Baniasadi et al., 2021; Forti et al., 2020). Therefore, recycling of WEEEs has become a key strategy to address the pressing issue of natural resource conservation and environmental protection. Over the past few years, “urban

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mining," has gained attention in alleviating the burden of e-waste by promoting a circular economy approach and recycling of WEEEs (Arora et al., 2017; Arya and Kumar, 2020; Baniasadi et al., 2021; Forti et al., 2020; Ilyas et al., 2021).

Over the last two decades, biotechnology applications in the form of biohydrometallurgy and bioleaching methods have been widely utilized and surpassed the hydrometallurgy and pyrometallurgy approaches on account of environmental benefits (Baniasadi et al., 2021; Chauhan et al., 2018; Faramarzi et al., 2020; Ilyas et al., 2013; Kumar et al., 2021; Pan et al., 2022). The high energy consumption, economic demand, environmental pollution, and human health risks are important drawbacks of conventional hydrometallurgy and pyrometallurgy techniques. These limitations have compelled to explore this biocyanidation approach in biohydrometallurgy field. Bioleaching of metals by biocyanidation approach is economical and environment friendly, thus considered as green approach. The biohydrometallurgy technique trails the principle of the hydrometallurgy technique in which potential microorganisms substitute the use of strong chemical lixiviants for the extraction of metals in an economical and eco-friendly way (Merli et al., 2022; Sahni et al., 2016; Thakur and Kumar, 2020). Cyanidation (use of cyanide lixiviant) is one of the most widely used hydrometallurgy methods practice at the industrial scale for precious metals retrieval from primary and secondary ore. However, the hazardous effects of chemical cyanide have prompted to explore biogenic cyanide; produced by various potential microorganisms in the bioleaching process (Chauhan et al., 2018; Guarnieri et al., 2020; Ha et al., 2010; Kuyucak and Akcil, 2013; Merli et al., 2022; Reck and Graedel, 2012; Sahni et al., 2016; Thakur and Kumar, 2020). Furthermore, a comparative analysis of bioleaching and hydrometallurgy processes for recovery of Cu and Zinc (Zn) from PCBs has shown that bioleaching has a less negative environmental impact with 0.4 kg CO_2 eq carbon footprint than hydrometallurgy 0.7 kg CO_2 carbon footprint (Faraji et al., 2022; Wang et al., 2021). In addition to its environmental benefits, comparatively lesser investment in the bioleaching process favors its adoption as a promising technology for the treatment of WEEEs, and more particularly for spent PCBs at the industrial scale (Thakur and Kumar, 2020; Wang et al., 2021; Wen et al., 2015).

The cyanogenic microorganisms such as *Chromobacterium violaceum*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens* are well-reported microorganisms that have established a captivating biocyanidation process for extraction of various metals in cost-effectively and ecologically manner (Arya and Kumar, 2020; Brandl et al., 2001; Chauhan et al., 2018; Mudali et al., 2021; Pradhan and Kumar, 2012). These cyanogenic bacteria are able to release biogenic cyanide (bio-CN) as a secondary metabolite that is known to form stable complexes with various metals. Furthermore, they can consume additional cyanide during the process hence reducing the risk of human exposure and environmental contamination (Brandl et al., 2001; Garg et al., 2019; Kumar et al., 2021; Shittu et al., 2021). Nutrient medium plays a crucial role in bioleaching as it has a major impact on the growth and metabolism of microorganisms which can significantly regulate cyanide production and ultimately their leaching efficiency. Earlier, cyanogenic bacteria had only been known to utilize glycine as a primary precursor for cyanide production; however, recent studies evaluating the effect of different amino acids on cyanogenic microorganisms and has suggested that depending on the bacterial strains, other amino acids can also replace the glycine for enhanced production of cyanide (Aminian-Dehkordi et al., 2020; Faraji et al., 2021, 2022). Therefore, it is vital to determine the influence of various precursor mediums on the substantial production of cyanide and the leaching capability of any novel cyanogenic bacteria.

In the current study, a novel *Bacillus* sp. ISO1 isolated and characterized for bioleaching potential in our previous study (Thakur and Kumar, 2023) has been explored for lixiviant production and bioleaching potential. Moreover, it has been reported that certain stimuli can influence the production of lixiviants in numerous cyanogenic bacteria. Hence, the present study intended to assess the impact of methionine amino acid as a cyanogenesis stimulant, and the optimization of its concentration for lixiviant production by indigenous *Bacillus* sp. ISO1 to enhance the metals solubilization. Additionally, the present study was projected to assess the dynamic process of biocyanidation at increased volumes (3 L) of bioleaching solution with an optimal pulp density of 10 g/L. The significant recovery of various base and precious metals at this substantial volume demonstrated the potential of this potent bacterial strain for scaling up the bioleaching process from bench to industrial scale operations. Further investigations at the genomic and proteomic levels can elucidate the precise bioleaching mechanism employed by this bacterial strain. This will upsurge the industrial-scale application of this promising bioleaching process by utilizing the potential of this indigenous bacterial strain.

Materials and methods

Microbial culture for biocyanidation and metal solubilization

Bacterial culture *Bacillus* sp. *ISO1* utilized for the current study was isolated earlier from the metals habituated site and optimized for maximum metal leaching potential in our previous study (Thakur and Kumar, 2023). The culture was revived in a nutrient broth medium consisting peptic digestion of animal 5 g/L, beef extract 1.5 g/L, yeast extract 1.5 g/L, and sodium chloride 5 g/L. The 16S rRNA nucleotide sequence of this bacterium has been submitted to NCBI GenBank Data Base (Accession No. OQ373005).

e-waste procurement, characterization, and metals analysis

The shredded dust of waste computer-printed circuit boards (CPCBs) with particle size ≤ 150 µm was obtained from Exigo Recycling Pvt. Ltd. unit in Panipat, Haryana. To assess the metallic content of CPCBs, the standard aqua regia digestion procedure was employed, in which 1% of e-waste was added to an HCl: HNO_3 (3:1) solution and heated at 100 °C for 1 h. Filtration with Whatman filter paper was proceeded by 0.45 µm glass fiber filters (PALL-GF-A/E-I) to make the solution particle-free (Sahni et al., 2016; Thakur and Kumar, 2021). The concentration of various metals dissolved in leachate was analyzed through an atomic absorption spectrometer (AAS) [AAnalyst400, Perkin Elmer].

The morphological and mineralogical analysis of the e-waste sample was done through scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM-EDS). The e-waste sample was dried and mounted for characterization at the Indian Institute of Science (IISc) Bengaluru. To strengthen image resolution, the sample was placed on adhesive carbon tubes and coated with a thin gold coating. The energy-dispersive X-ray spectroscopy also revealed another elemental concentration of CPCBs.

Furthermore, the elemental intensity and elemental core structure of CPCBs were validated by X-ray diffraction (XRD) analysis at Himachal Pradesh University (HPU), Shimla. The dried fine particles were introduced into the matrix's internal core along a peak range of 0 to 380 at a 2Ø value followed by the interpretation of specific peaks through the Joint Committee Powder Diffraction Standards (JCPDS) statistical tool.

Precursor selection for cyanide production by Bacillus sp. ISO1

The bacterial isolate was utilized to assess the influence of different amino acids on bacterial growth and Cu solubilization. The bacterial growth medium (i.e., nutrient broth) was supplemented with a 5 g/L concentration of different amino acids (e.g., histidine, tyrosine, asparagine, alanine, cysteine, phenylalanine, leucine, glycine, lysine, methionine) and adjusted at pH 9 with 1 N NaOH. The 4% v/v (approximately 4×10^8 CFU/mL) culture of *Bacillus* sp. *ISO1* was added into each sterilized medium and incubated at 40 °C for 30 h proceeded by the addition of 1% pulp density into each flask for bioleaching of Cu metal. Then, 2 mL leaching solution from each flask was taken every day to assess pH change and bacterial growth. Another flask with similar nutrient conditions except for the addition of any precursor source was run as a control.

Effect of methionine additive on cyanogenesis

Amino acids are one of the nutritional sources for bacterial growth and physiological functions (Ferla and Patrick, 2014). Therefore, the effect of methionine as a nutritional source and cyanogenesis stimulator was investigated. Nutrient broth (NB) supplemented with different concentrations such as 0.5 g/L, 1 g/L, and 5 g/L of methionine additive and 5 g/L glycine as a primary precursor for cyanide production. The 4% v/v culture medium was added to the medium and kept at optimized growth conditions in the absence of e-waste for maximum lixiviant production. Then, 1%w/v pulp density was added into the bioleaching solution and further incubated for 8 days for the metals solubilization process. The efficiency of methionine supplementation on bioleaching of PCBs by *Bacillus* sp. *ISO1* was evaluated by the amount of Cu, Au, and Ag recovery. The nutrient medium without supplementation of amino acid was inoculated with equal volume of bacterial culture and run as control set.

Metals solubilization efficiency at the increased volume of leaching solution

Most of the bioleaching studies have been conducted with small volumes at the experimental level into non-baffled flasks. But the kinetics of microorganisms varies with changes in the volume of the growth medium as mass flow, nutrient availability, and other factors may differ in comparison to small-scale medium. Hence, the dynamics of the biocyanidation process were evaluated at large volume (3 L working volume) bioleaching solution with optimized pulp density (i.e., 10 g/L). The 3 L nutrient medium in a 5 L non-baffled flask was supplemented with optimized methionine additive and glycine precursor. The pH of the medium was adjusted at pH 9 with NaOH. The sterilized medium was inoculated with *Bacillus* sp. ISO1 and incubated at 40 °C and 180 rpm. Further, 10 g/L pulp density was added into the bioleaching solution after obtaining maximum bacterial growth (OD_{600} = 1.234) and cyanide production. The 5 mL sample was taken every day for assessing, pH change, and metal solubilization.

Determination of metals recovery

Metals recovery by *Bacillus* sp.ISO1 through biocyanidation processes at large-scale bioleaching operation was calculated by applying the following formula

$$m = c\left(\mathrm{mg\,L^{-1}}\right) \times v\,(\mathrm{mL})/1000 \tag{1}$$

m = metal concentration, c = concentration of metal analyzed through AAS after aqua regia digestion, v = volume of leaching solution

mg/L to mg/g conversion

M/w = Metal content

w = amount of e-waste added to the leaching solution

$$\%ge\ metal\ recovery = \frac{\text{concentration of metal in the leachate solution obtained through bioleaching process}}{\text{concentration of metal present in CPCBs obtained through aqua regia digestion}} \times 100 \quad (2)$$

Result and discussion

Characterization of PCBs e-waste

Metal composition analysis revealed the existence of various base and precious metals. The metal content of PCB shredded dust e-waste is enlisted in Table 1. The table shows that the concentration of Cu and Ag (12.75 mg/g and 10.34 mg/g) were significantly high, but precious metals such as Au and Pt were in trace amounts. Excluding the Ag metal, the concentration of all identified metals was found to be lower in comparison to Kumar et al. 2021 (e.g., 23.39 mg/g Cu and 0.08 mg/g Au). The differences in metal content have been found due to variations in the type of e-waste sample utilized for the investigation. In the present study, we utilized shredded dust of PCBs e-waste that originated during the physio-mechanical process, while Kumar et al. (2021) used virgin PCBs sample. Furthermore, the highest concentration of Cu metals contributed because of its more ductile character, which distributes it more in bigger size fractions, therefore limiting the presence of other valuable metals (e.g., Ag, Au, Pt). Although, the small size of the shredded dust PCBs sample facilitates the interaction of silver (Ag) metal with aqua regia solution. Thus, Ag was obtained in significant amounts (Kumar et al., 2021). The findings of the current study have been supported by prior reports on the metal composition by Sun et al. (2017) (Sun et al., 2017). Additionally, in 2018, Chauhan et al. demonstrated compositional analysis of shredded dust e-waste, revealing substantial concentrations of several base metals such as aluminum (Al) (4.65%), iron (Fe) (4.55%), and Cu (2.67%), and corroborate the consideration of this waste for metals recovery (Chauhan et al., 2018). Henceforth, an effective dust collection system needs to be implemented to prevent the loss of this enriched waste during the physical dismantling process of any WEEEs.

Table 1 Major base and precious metals unveiled in shredded dust CPCBs through aqua regia digestion method

Sr. No	Metals	Concentration (mg/g)
1	Cu	12.75 ± 3.8
2	Ag	10.34 ± 2.4
3	Fe	2.46 ± 0.85
4	Al	0.39 ± 1.02
5	Ni	0.24 ± 0.22
6	Au	0.04 ± 1.39
7	Co	0.02 ± 0.82
8	Pt	0.08 ± 0.06

Morphological and mineralogical characteristics of CPCBs e-waste

SEM revealed that CPCBs fine particles have a variety of textures and an uneven structure. Figure 1a inferred the existence of smooth, rod-like, polygonal-shaped particles of plastic, silica, or ceramics made up of Al, Si, Ca, etc. These particles were embedded with rough, amorphous crystal pieces of different metallic constituents. The distinct peaks in the EDS spectra in Fig. 1b further confirmed the presence of significant non-metallic silicon, carbon, and other fragments along with the initial concentration of different base and precious metals. The presence of these non-metallic constituents contributed to the toxicity of e-waste and affects the leaching potential of bacteria.

X-ray diffraction is used to detect the structure regularity and molecular arrangements that contribute to different properties of the material present in PCBs fine particles. XRD spectrum (Fig. 1c) interpretation reveals that most of the material was amorphous in PCBs powder; primarily contributed by the non-metallic components such as ceramics, gels, and polymers since no long-order or well-defined diffraction planes, and no sharp peaks were obtained. In addition, the presence of five peaks at 2θ values of (24.3), (31.831), and (39.7), (43.317), (44.377), (45.593), (50.568), (60.623) unveil various metals constitution like Hg, Cd, Pt, Cu, Al, Ag, Au, and Co respectively, inside the e-waste fine particles scrutinized using joint committee powder diffraction (JCPDS) standard data file. Somehow, similar constituents were obtained by Garg et al. (2019) through mineralogical and morphological analysis of mobile phone PCBs (Garg et al., 2019). Therefore, substantiated assessment of all non-metallic and metallic fractions in PCBs elucidates the need for prospective microorganisms that exhibit high toxicity tolerance and improved metabolic mechanisms toward the various harmful constituents.

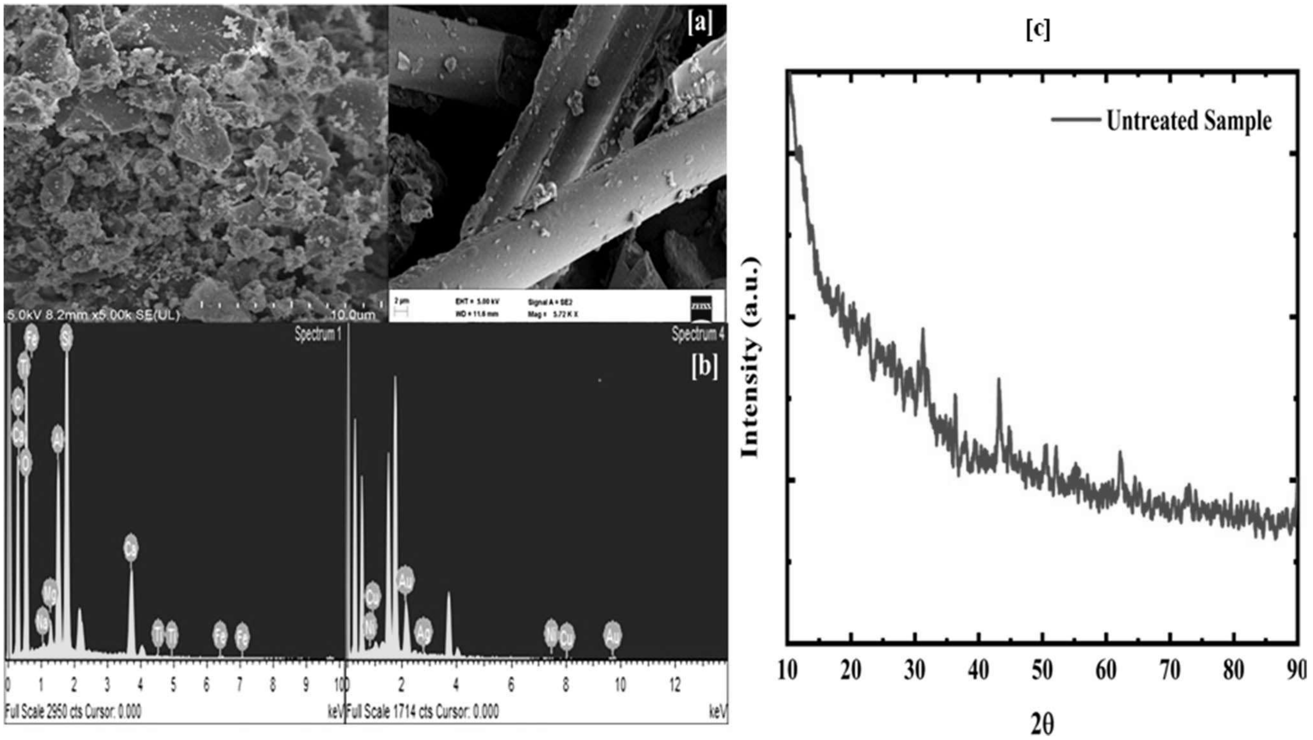


Fig. 1 Characterization of shredded dust CPCBs. **a** Morphological analysis of CPCBs at 5.00KX magnification. **b** Mineralogical analysis through EDS revealing the elemental composition of shredded dust CPCBs. **c** XRD spectrum depicting the amorphous structure of materials present in PCBs e-waste

Precursor for cyanide production

Glycine is used as a primary metabolic precursor by many cyanogenic bacteria for the production of cyanide during the biocyanidation process. The carbon in CN^- and CO_2 is derived from the CH_2^- and COOH groups of glycine, while the C-N bond remained intact (Wang et al., 2021). Although glycine utilization for cyanide production is endothermic and non-spontaneous, the complete metabolic mechanism involved is unexplained yet. The biocyanidation process may be influenced by several other amino acids since they are a vital nutrient source for bacterial growth. Therefore, the effect of 10 amino acids for cyanide production by *Bacillus* sp. *ISO1* was assessed and illustrated in Fig. 2a. In comparison to all the amino acids, glycine surpassed the Cu solubilization and inevitably contributed to maximum cyanide production; therefore, *Bacillus* sp. ISO1 preferred glycine as a prime precursor for biocyanidation. Additionally, threonine, histidine, and lysine also stimulated cyanidation significantly, even though the influence on bacterial growth (Fig. 2a) was minimal with these amino acids. Phenylalanine exhibited a minimum effect on bacterial growth and cyanide formation, hence no considerable Cu solubilization was achieved. Methionine favored bacterial growth but not significantly contributed to cyanide production. The precise metabolic mechanism of these amino acids cannot be described, but their effect must be achieved through an indirect route, such as regulation of the glycine pool, regulation of glycine transportability, etc. The influence of amino acids is not specific to all microorganisms. Dehkordi et al. (2020) conducted in-silico studies to investigate the influence of various 13 amino acids on genes involved in cyanide generation by *B. megaterium* DSM319, and observed that cysteine, glutamine, and histidine had the most pronounced influence on cyanide generation, as their removal was accompanied by 50% decrease in cyanide production. They also observed that isoleucine, proline, threonine, and valine could have only an influence on biomass generation (Aminian-Dehkordi et al., 2020). Furthermore, Faraji et al. (2021) also evaluated the effect of threonine and glutamine on *B. megaterium* cyanide production for Au recovery in comparison to glycine and found that threonine has greater potential for bacterial

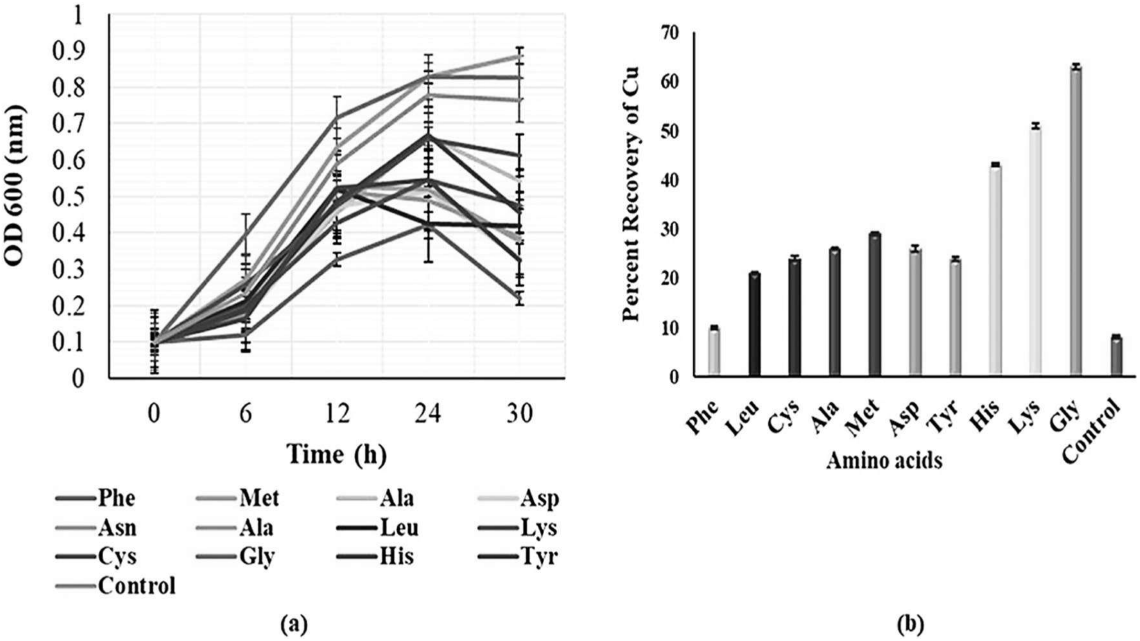


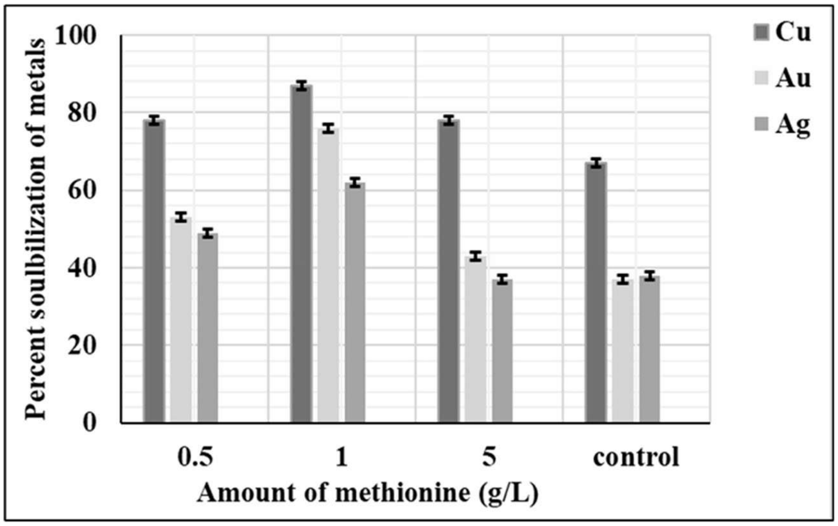
Fig. 2 Effect of various amino acid on growth and biocyanidation process (**a**) effect of different amino acids on growth of *Bacillus* sp. ISO1 (**b**) recovery rate of copper metal in the presence of different amino acids

growth stimulation than glutamine and glycine, but biocyanidation influenced equally by glutamine and threonine which is higher than glycine, so threonine and glutamine are considerable precursor for cyanide production (Faraji et al., 2021). Although the exact mechanism of the utilization of this amino acid has not been reported yet.

Stimulation of cyanogenesis by methionine additive

Methionine is a proteinogenic amino acid that plays an important role in the initiation of translation and *S*-adenosyl methionine cofactor; a main cellular carrier of methyl groups (Ferla and Patrick, 2014). The concentration of methionine was optimized to influence the biomass production of *Bacillus* sp. ISO1 and cyanogenesis process. It is depicted in Fig. 3 that 1 g/L methionine along with 5 g/L of glycine precursor was optimum for stimulating biomass production and cyanogenesis, which implied the maximum solubilization of metals. Further, 1 g/L methionine led to provide the cyanide for solubilization of 86% Cu and 75% Au metal. Whereas, more than 1 g/L methionine had no significant impact since it may even become toxic and cease the growth of *Bacillus* sp. ISO1. During the initial investigation of the biocyanidation process of cyanogenic microorganisms, Peter and Caster

Fig. 3 Percentage solubilization of Cu, Au, and Ag metals using methionine additive with glycine precursor



(1977) proposed the use of methionine, betaine, dimethyl glycine, or choline, etc. for elevation in bio-CN yield. They evaluated the effect of methionine additive, on *Pseudomonas aeruginosa*, to enhance glycine-stimulated cyanogenesis at glycine concentration of between 1 and 20 mM and found that above these levels of glycine, methionine does not affect the cyanogenesis (Castric, 1977). Whereas, methionine concentrations between 5 and 30 mM do not stimulate cyanide production in the absence of glycine. Afterward, Harris et al. 1987 also claimed that methionine stimulated cyanogenesis in *C. violaceum*. The precise mechanism of methionine utilization in cyanogenesis is uncertain, but these studies implied that the methylation of homocysteine to generate methionine is a key utilization of the C-1 tetrahydrofolate pool that influences cyanogenesis in *C. violaceum* (Harris et al., 1987). Moreover, not many studies have been reported related to this concept, and the chemo-biohydrometallurgy approach has been emphasized to improve the role of the bioleaching process over the past two decades. But now, a recent study by Aminian -Dehkordi (2020) investigated the role of some other amino acids which can influence cyanide production. They suggested the role of cysteine in cyanide overproduction by *B. megaterium* for gold leaching from waste PCBs (Aminian-Dehkordi et al., 2020).

Bioleaching efficiency of Bacillus sp. ISO1 at large volume

The initial assessment of metal leaching is often performed in shake flasks with optimized parameters, where pulp density is limited to a few grams to correspond to concentrations approximately 10 g/L (optimum concentration for maximum leaching). However, the heterogeneous nature of e-waste hinders the representation of such a small size sample in the initial bioleaching batch and fluctuates continuously in composition and metals solubilization, therefore becomes challenging to assess the outcomes of respective operations (Garg et al., 2019). The assessment of leachate from a substantial bioleaching volume enables the outcomes of small-scale bioleaching to be consistent in a repeatable and representative manner. Hence, the kinetics of bacterial growth and biocyanidation were studied in a large volume (3 L) with an optimal pulp density (10 g/L). The rate of Cu solubilization and pH change of the medium by *Bacillus* sp. ISO1 at an increased volume of leachate solution has been inferred in Fig. 4. The figure shows that *Bacillus* sp. ISO1 exhibited gradual enhancement in Cu leaching and attained maximal recovery (˃ 60%) on the 13th day of the incubation period, which indicates the efficient production of cyanide during the initial phase of two-step bioleaching that formed metal-cyanide complex after e-waste addition into the leachate medium. The progressive rise in metal recovery was facilitated by increasing the pH of the medium, as more alkaline pH (˃ 9) reduces the volatility of HCN and provides availability of more CN^- into the solution, which forms different complexes with the Cu metal (Faraji et al., 2022; Faramarzi et al., 2020; Kumar et al., 2018). Furthermore, the recovery of other base metals was also facilitated after Cu solubilization, as the presence of Cu causes hindrance to forming the complex formation of cyanide with other metals. Figure 5 illustrated all the metals solubilized by *Bacillus* sp. ISO1 in increased volume of leachate medium by utilizing glycine as key precursor and methionine as a stimulant.

The variation in pH was observed continuously after the initiation of two-step bioleaching (shown in Fig. 4). It is depicted from the figure that the initial pH was decreased up to 8.6 which is attributed to bacterial growth and HCN production during the early stages of bioleaching. The

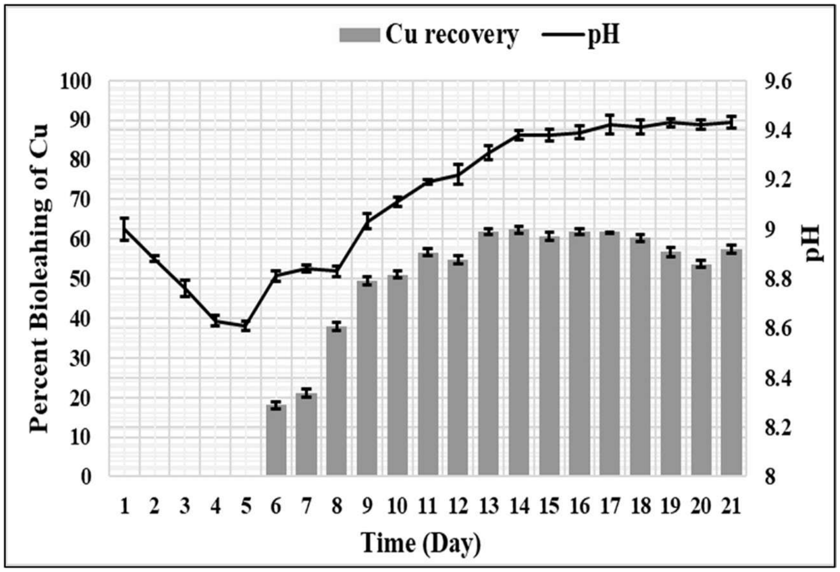


Fig. 4 Cu solubilization and change in pH of the medium during bioleaching process by *Bacillus* sp. *ISO1* at 3 L volume of leaching medium

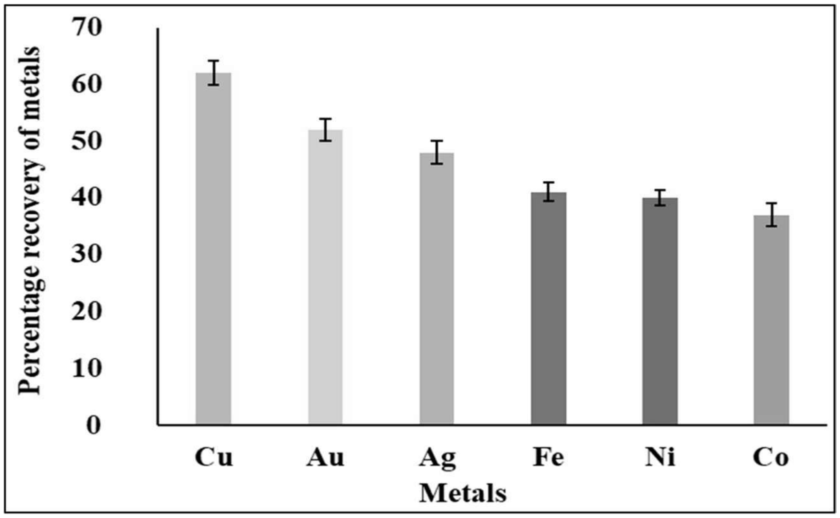


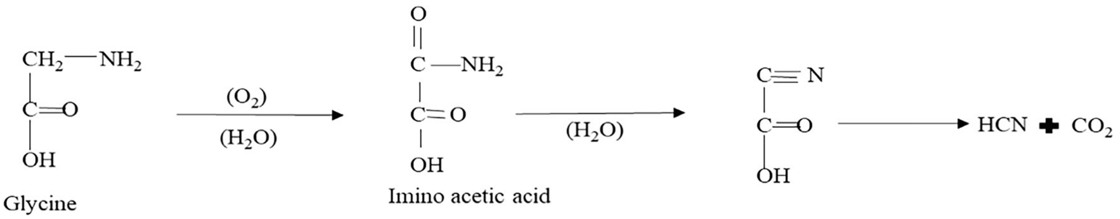
Fig. 5 Percentage recovery of various metals under optimized conditions at 3 L working volume

most plausible explanation for this initial decrease is the mild acidification process during the conversion of organic compounds into carboxylic acids such as glyoxylic, cyanformic, and oxamic acids and HCN production. Furthermore, the release of metabolites in the early phase influences biomass production and HCN production. Subsequently, a progressive increase in pH was observed after the addition of e-waste, which was contributed by metal-cyanide complex formation. The final pH reached almost the pKa value of HCN (9.3) aided to form more CN^- and enhanced the solubilization of metals later in the bioleaching phase. Many similar findings have been reported on pH variations. Arshadi and Mousavi (2015) and Kumar et al. (2018) found a similar trend of pH change during metals extraction from waste PCBs using cyanogenic bacterium *B. megaterium* and indigenous *Pseudomonas balearica* SAE1, respectively (Arshadi and Mousavi, 2015; Kumar et al., 2018). Moreover, Arab et al. (2020) demonstrated the bioleaching process by various indigenous cyanogenic strains and found that the pH variation with the initial pH of 7–10 converged to approximately 8.2 during the initial growth phase and then elevated to approximately 9.2. All these outcomes suggested that pH is a significant parameter in the bioleaching process that influences the availability of CN^- and affects the metals solubilization (Arab et al., 2020). Hence, the optimization of initial pH and maintenance of an alkaline environment during the bioleaching process is an important factor for efficient metals recovery.

To strengthen the industrial-scale implementation of this effective bioleaching method, the present study assessed the metal leaching efficiency at 3L working volume. The significant recovery of metals at this substantial volume (shown in Fig. 5) remarks the practicability of bioleaching potential of this bacterial strain at large scale bioleaching operations. The numerous studies have been reported the kinetics of the process and optimized the conditions at a large scale. Garg et al. (2019) investigated the bioleaching potential of iron-oxidizing bacteria in a 2 L baffled glass reactor, with a working volume of 1 L. They attained 98–99% of copper recovery together with reasonable extraction of Zn, Al, and Ni from 15% pulp density under lab-scale optimizations conditions. The study conducted by Erust et al. (2020) aimed to impend the optimal values of crucial parameters at the shake-flask level and assess the practicability of these optimal conditions in bench-scale bioreactors. The optimized parameters were utilized in laboratory-scale experiments that led to attain maximum recovery Cu (98.1%) for Ni (79.5%), and Al (55.9%) within a period of 8 days. Furthermore, some researcher extended the viability of shake flask bioleaching conditions to the large-scale operation and enlightened industrial scale application of this eco-friendly and economical process. Erust et al. (2021) demonstrated the semi pilot bioleaching with 10 L working volume and found that10% v/v mixed inoculum of *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, and *Acidithiobacillus thiooxidans* recover 95% Cu within 8 days of bioleaching process. In addition, Junjun et al. (2015) reported the efficiency of *Pseudomonas chlororaphis* at 5 L bioleaching working volume. They suggested that 22.5 °C temperature, pH 7, and addition of 4.4 g/L glycine and 2 g/L methionine is optimum for cyanide lixiviant production that led to recover 88.1% au and 76.6% Cu from PCB at 5 L bioleaching working operation. As per the authors' knowledge, this is the first study that explored the *Bacillus* sp. ISO1 for bioleaching at this substantial volume (3 L). Although *Bacillus megaterium* has been reported for bioleaching at laboratory scale in shake flask. Thus, the significant findings of the reported studies and current study impelled the industrial-scale applications of this economical and eco-friendly technique for recycling vulnerable e-waste.

Biochemical mechanism of the bioleaching process

Cyanide is defined as a chemical compound formed of triple-bonded carbon–nitrogen radical with +2 oxidation of carbon and − 3 of nitrogen (Kuyucak and Akcil, 2013). Several cyanogenic microorganisms such as *Chromobacterium violacium*, *P. aeruginosa*, *B. megatherium*, etc. exhibit the potential to produce this chemical compound as a secondary metabolite in the mid to late exponential growth phase (Faraji et al., 2022). The enzyme responsible for cyanide generation is a membrane-associated HCN synthase also known as glycine dehydrogenase; it is a flavoenzyme that comprises iron cofactors and is encoded in most cyanogenic microorganisms via hcnABC operon (Arya and Kumar, 2020) (shown in Fig. 6). HCN synthase enzyme transforms glycine, a precursor, to iminoacetic acid by oxidative decarboxylation and subsequently hydrogen cyanide (HCN). During this process, HCN synthase produces four electrons that require an electron acceptor, which can be oxygen across the electron transport chain (Vakilchap et al., 2020). The genetic control of the hcnABC operon and the cyanogenesis process is both complex, so the biocyanidation process occurs only under specific environmental conditions. In bacteria, biocyanidation process is strictly regulated, such that cyanide concentration is usually 1 mM; tolerated by many living cells (Motaghed et al., 2014).



$$\mathrm{NH_2{-}CH_2{-}C({=}O){-}OH} \xrightarrow[(H_2O)]{(O_2)} \mathrm{NH_2{-}C({=}O){-}C({=}O){-}OH} \xrightarrow[(H_2O)]{} \mathrm{N{\equiv}C{-}C({=}O){-}OH} \longrightarrow \mathrm{HCN} + \mathrm{CO_2} \qquad (3)$$

Under physiological pH 7, cyanide is mainly in the form of hydrogen cyanide (HCN), a volatile gas and therefore rapidly diffuse into the environment. The cyanide equilibrium favors cyanide ions formation in alkaline conditions (Eq. 4), which forms various metal complexes. Based on the cyanide amount and pH conditions, anionic complexes of Cu are formed in various forms such as Cu (CN)$^{-}{}_{2}$, Cu (CN)$^{-2}$, Cu (CN)$^{-3}$, and Cu (CN)$^{-4}$, etc. Moreover, the formation of

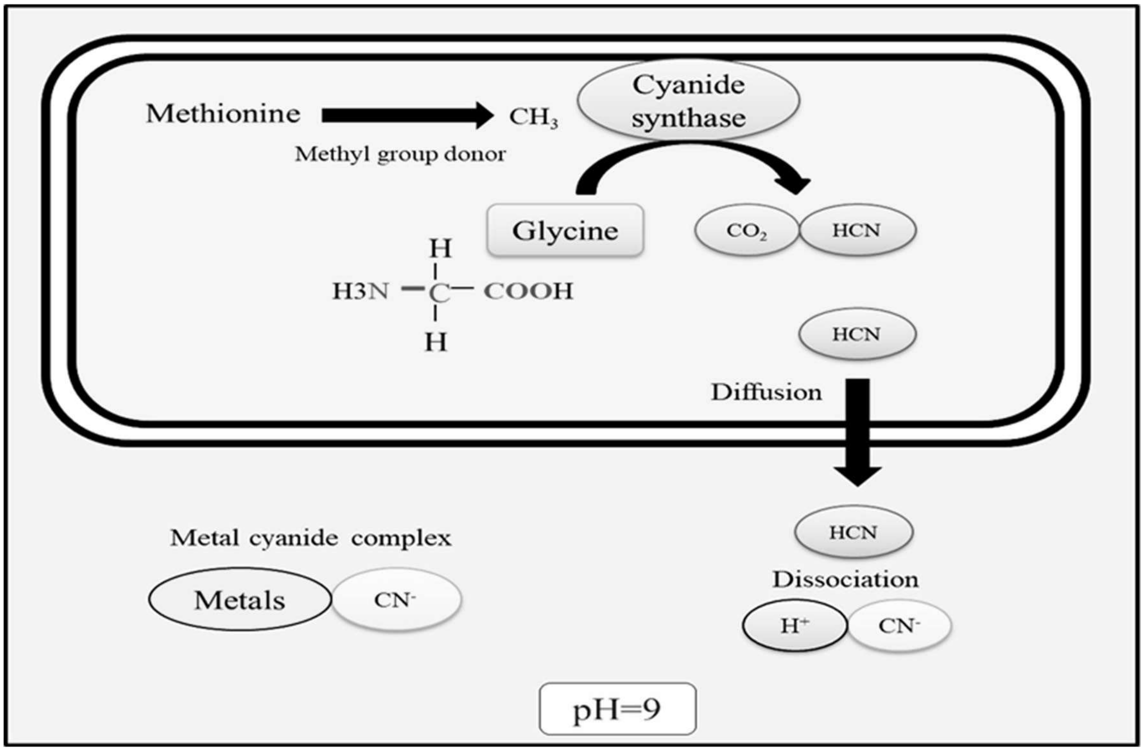


Fig. 6 Illustration of cyanide production mechanism by HCN synthase enzyme in the cyanogenic microorganism

dicyanoaurate anions [Au(CN)$^{2-}$] and others, such as [Pt (CN)$_4$]$^{2-}$, [Pd (CN)$_4$]$^{2-}$ and [Ni (CN)$^{2-}$] are stable metal-complexes over a wide range of redox and pH conditions (Wang et al., 2021). The complex formation is influenced by the standard electrode potential of these metals (i.e., E^0, Au = 1.83 V, E^0, Ni = 0.67 V, E^0, Fe = 0.44 V, E^0, Cu = 0.34 V). Cyanide form much faster complex with Cu than Au as its standard potential is lower than Au metal. In addition, higher content of Cu in the solid matrix of e-waste also restricts the biocyanidation of Au^{3+} (Vakilchap et al. 2020). Since the pKa of cyanide is 9.3, conducting a metal dissolution reaction under alkaline conditions increases its availability for metal solubilization complexation.

$$HCN \rightarrow H^+ + CN^- \tag{4}$$

$$4Cu + 8CN^- + O_2 + \overset{H_2O}{\rightarrow} 4\left[Cu\ (CN)_2\right]^- + 4OH^- \tag{5}$$

$$4Au + 8CN^- + O_2 + H_2O \rightarrow 4\ \left[Au\ (CN)_2\right]^- + 4OH^- \tag{6}$$

$$4Ni + 8CN^- + O_2 + H_2O \rightarrow 4\ \left[Ni\ (CN)_2\right]^- + 4OH^- \tag{7}$$

Conclusion

Cyanogenic microorganisms have gained attention in the field of biohydrometallurgy. Researchers have focused to enhance the biocyanidation potential of various cyanogen such as *Chromobacterium violacium*, *Pseudomonas* sp., and *Bacillus megaterium*. This study investigated novel indigenous *Bacillus* strain and explored suitable precursors and inducers to improve its biocyanidation and metal leaching capacity. Glycine was found to be the suitable precursor, a substrate for the production of cyanide lixiviant by the enzyme hydrogen cyanide synthase. The addition of 1 g/L of methionine as a stimulant effectively increased cyanide lixiviant production, resulting in efficient solubilization of copper (86%) and gold (75%) and silver metals (63%) under optimized conditions. *Bacillus* sp. ISO1 exhibited high toxicity tolerance and demonstrated significant leaching efficiency compared to other bacterial strains, indicating its suitability for large-scale bioleaching operations. Further investigation into the kinetics of mass flow and metal solubilization at substantial volume of leaching revealed that *Bacillus* sp. ISO1 exhibit efficient recovery of copper (60%) and other base metals that illustrates the sustainability of bioleaching potential of this potential bacteria strain and optimized conditions for industrial-scale e-waste urban mining strategy.

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Author contributions Conceptualization: [Sudhir Kumar] and [Pooja Thakur]; methodology: [Sudhir Kumar] and [Pooja Thakur]; formal analysis and investigation: [Sudhir Kumar] and [Pooja Thakur]; writing— original draft preparation: [Pooja Thakur]; writing— review and editing: [Sudhir Kumar] and [Pooja Thakur]; supervision: [Sudhir Kumar].

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Data availability All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no conflict of interest.

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REVIEW ARTICLE

Metallurgical processes unveil the unexplored "sleeping mines" e- waste: a review

Pooja Thakur[1] · Sudhir Kumar[1]

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Abstract

The aim of this review paper is to critically analyze the existing studies on waste electric and electronic equipment (WEEE), which is one of the most increasing solid waste streams. This complex solid waste stream has pushed many scientific communities to develop novel technologies with minimum ecological disturbance. Noteworthy amount of valuable metals makes e-waste to a core of "urban mining"; therefore, it warrants special attention. Present study is focused on all the basic conceptual knowledge of WEEE ranging from compositional analysis, global statistics of e-waste generation, and metallurgical processes applied for metals extraction from e-waste. This review critically analyses the existing studies to emphasize on the heterogeneity nature of e-waste, which has not been focused much in any of the existing review articles. Comprehensive analysis of conventional approaches such as pyrometallurgy and hydrometallurgy reveals that high costs and secondary pollution possibilities limit the industrial feasibilities of these processes. Therefore biohydrometallurgy, a green technology, has been attracting researchers to focus on this novel technique to implement it for metal extraction from WEEE.

Keywords E-waste · Urban-mining · Sleeping mines · Pyrometallurgy · Hydrometallurgy · Biohydrometallurgy

Introduction

In today's scenario, revolutionary development has connected everyone and created a digital economy. Everyday electronic companies are introducing products with new features and services at cheaper rates, which are increasing the demand for these electronic equipment. In parallel, this increased demand for electronic equipment is generating a high amount of waste electronic products considered as e-waste or WEEE, which is a prominent solid waste stream (Chauhan et al. 2018). Increased number of users, economic development, and innovation in technology are few factors responsible for this growing amount of e-waste. Numerous studies have been reviewed like consumption and usage of e-waste, market and distribution analysis, material flow analysis, and export analysis to estimate the e-waste. There could be a considerable variation in reports due to assumptions and strategies applied in different studies, making the quantification of WEEE generation difficult. These challenges have prompted researchers to regulate the increasing e-waste, as these accumulated wastes liberate the alternative pollutants and heavy metals (Perkins et al. 2014; Pant et al. 2012). There are many conventional as well as advanced strategies applied for e-waste treatment. Although, the traditional procedures are effective for recovering metals, yet need is felt for the development of a novel approach to create a standard system to treat e-waste and retrieve metals. Comprehended information is available regarding the existing approaches like hydrometallurgical and pyrometallurgical technologies for WEEE recovery. This review aims to highlight the international and national scenario of e-waste production, heterogeneity with metals concentration, which is not focused yet in any of the studies, discussion about the different techniques being used and improvements in biohydrometallurgical process.

Composition of e-waste

The equipment which works on the electromagnetic field or electric current is classified as electrical and electronic

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equipment (EEE). These equipment generate and measure the electromagnetic field or current (Perkins et al. 2014). Regular upgradation in all the electric and electronic equipment leads to generate tremendous amount of outdated products as e-waste. WEEE consists of various toxic substances, with three main categories, i.e., ceramics, metals, and organic materials (Xia et al. 2018). PCB (printed circuit board) is considered as principal constituent of major electronic products, made up of 305 types of plastics, 30% refractory oxides together with enol, and 40% metals (in which Cu holds 10–30%) (Jagannath et al. 2017). Therefore, WEEE is claimed to have a complex, diverse, and heterogeneous composition comprising iron steel (50%), non-ferrous metal (13%), plastics (21%), and other constituents (16%) such as ceramics, glass, and rubber (Anshu and Hait 2017; Uddin 2012). Copper, zinc, and nickel are classified as non-ferrous metals which are abundantly found in WEEE. Table 1 represents the major metallic constituents along with their sources in electric and electronic equipment. Polycyclic aromatic hydrocarbons (PAH), polybrominated dibenzo-p-dioxin (PBDD), and polybrominated diphenyl ethers (PBDEs) are classified as plastic, whereas brominated flame retardants (BFR), dibenzofurans, dioxins, and glass fibers have been considered into organic materials; additionally, alkaline earth oxides, alumina, barium titanate, and silica have been categorized in ceramic group (Julander et al. 2014; Imran et al. 2017). The liberation of some hazardous metals like cadmium, beryllium, mercury, and chromium into the ecosystem is responsible for toxicity of WEEE (Anshu and Hait 2018). Seeing the complexity, heterogeneity, and harmful effects of e-waste, management, and resources conservation has become the utmost concern to avoid resulting pollution.

Global statistics of WEEE generation

Quantification of e-waste is the critical step to consider the challenges coupled with WEEE. Significant amount of data related to e-waste will help us in regulating illegal dumping and e-waste generation. According to a survey conducted by United Nation University (UNU), in 2016, worldwide e-waste quantity was 44.7 Mt which is anticipated to excel more than 52.2 Mt by 2021 with 4% annual extension rate (Wang et al. 2016). Only 20 % (8.9 Mt) of total waste is subjected for its collection and recycling purpose whereas 35.8 Mt (80%) is undocumented and dumped illegally into the landfills. High gross domestic product (GDP) of developed countries is responsible for higher e-waste production rate in term of kilogram (kg) per inhabitant as compared with developing countries. Top ten countries with highest e-waste contribution are listed in Table 2. In spite of low rate of e-waste generation per inhabitant (4.2 kg/inh), Asia was found to be top producer with total 18.2 Mt (Jagannath et al. 2017; Wang et al. 2016). From Asian continent, China contributed the highest amount (7.2 Mt) followed by Japan and India with 2.1 and 2 Mt, respectively. Despite of greatest collection rate (35 %), Europe listed second rank among the continents with 12.3 Mt in parallel to 16.6 kg/inh. According to the global e-waste Monitor 2017, in Europe, Germany contributed highest quantity (1.9 Mt), followed by Great Britain and Russia 1.6 Mt and 1.4 Mt, respectively. Americas produce total 11.3 Mt of e-waste with 6.3 Mt contributions from United State of America (USA) itself. Brazil ranked second, with 1.5 Mt, followed by Mexico, with 1 Mt (Anshu and Hait 2017). Africa holds last rank where Egypt contributes 0.5 Mt and 0.3 Mt contributions from South Africa and Algeria. Oceania continent ranked last for total e-waste generation (0.7 Mt) with 6 % collection rate, but considered greatest producer in terms to per inhabitant (17.3 kg/inh) (Awasthi et al. 2016a). The rising rate of this municipal solid waste stream have been alarmed many countries for adopting e-waste legislation. Currently, national e-waste management policies and legislation covered 66% of the world population, which has been increased from 44% since 2014. Being highest inhabitant countries of Asia, 72% population of China and India has enacted strict e-waste legislation. China has showed

Table 1 Constituents of WEEE and their source

Constituents	Sources
Base metals	Printed circuit boards
Precious metals	Mobile chip resistors, printed circuit boards
Nickel (Ni)	Nickel-cadmium chargeable batteries
Lithium (Li)	Lithium-ions batteries
Barium phosphorus and heavy metals	CRTs
Chromium (Cr)	Plastic computer housing, CRTs and batteries
Brominated flame retardants (BFR)	Electronic equipment and circuit boards
Plastic and PVC	Cables and computer body
Mercury (Hg)	Switches and circuit boards
Cadmium (CD)	Chip resistors and semiconductors
Arsenic (As)	CRTs, LCD screen, printed circuit boards

Table 2 List of countries contributed highest amount of e-waste in 2016

Sr. No	Countries	E-waste generation million metric ton/year	E-waste generation Kg/inhabitant	Population (millions)
1	China	7.2	5.2	1378
2	USA	6.3	19.4	323,9
3	Japan	2.1	16.9	126
4	India	2	1.5	1309
5	Germany	1.9	22.8	82.5
6	UK	1.6	24.9	65.5
7	Brazil	1.5	7.4	206
8	Russia	1.4	9.7	143
9	France	1.3	21.3	64.5
10	Indonesia	1.2	4.9	258.8

strong responsibility also in formal recycling, and metals recovery from e-waste Awasthi and Li 2017. In 2011, National Television and Computer Recycling Scheme was implemented by Australian government, which resulted into the recycling of 7.5 % of total generated e-waste. The issues of poor e-waste management facilities of the low economic countries require urgent attention. Figure 1 represents the global e-waste generation along with recycling data for different continents.

Urban mining—an economic perspective

In 2016, the net worth of e-waste was estimated to be $ 61.05 billion, which is higher than the GDP of most developing countries. Moreover, e-waste is the reservoir of many valuable metals having concentrations 40 to 50 times higher than natural deposits, which makes this waste to "sleeping mines" (Kumar et al. 2018). More surprisingly, gold and silver are two precious metals consumed tremendously (i.e., 320 ton and 7500 ton, respectively) each year for production of new electronic products as reported by Environmental Protection Agency (EPA). Hence, about $ 21 billion worth precious metals are present in these devices. Altogether 60 different elements are found in the electronics devices, which can be recovered. E-waste generally contains high amounts of aluminum and iron along with plastic, which can be reused (Das and Ting 2017). UNU reported that e-waste globally contains secondary raw materials of worth approximately $ 60 billion as shown in Table 3, considering the significance of waste electronic products; there is a need for the establishment of a

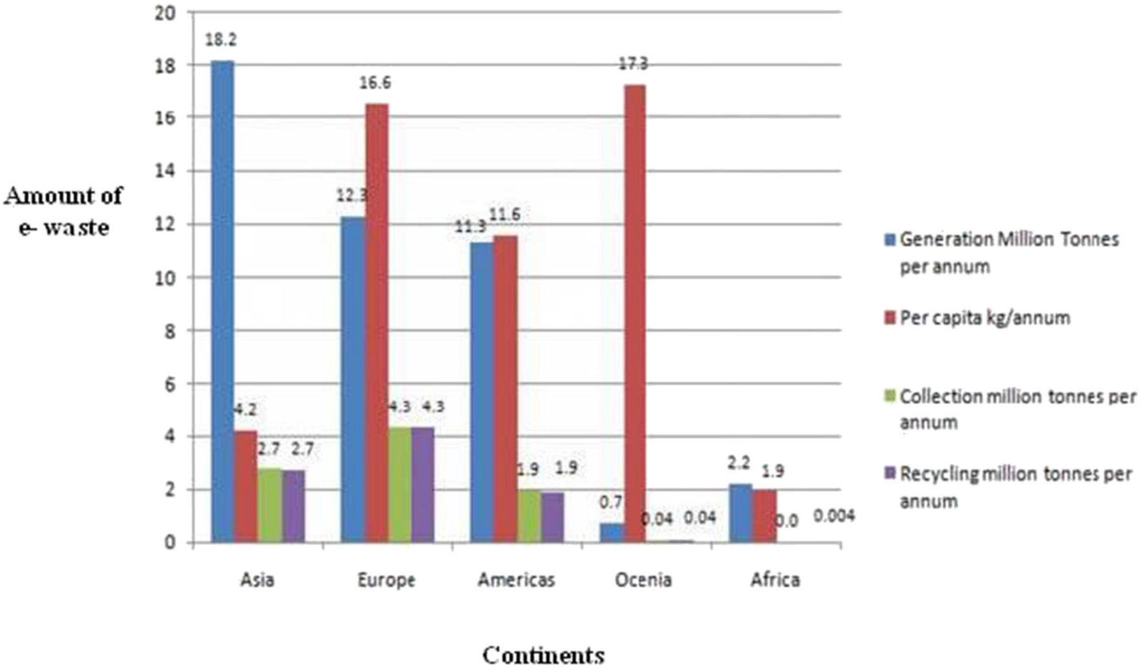

Fig. 1 Continents contributing total amount of e-waste along with collection and recycling rate

proper management system which allows the regulated recovery of valuable metals from dumped electronic equipment. EPA reports have stated that, by recycling 1 million cell phones alone, we can recover 10 tons copper, 0.01 tons of palladium, 0.275 tons of silver, and 0.025 tons of gold. Therefore, to utilize this chance, there is a need for framing new policies which aid in developing an infrastructure having advanced equipment capable of retrieving highly invaluable metals from e-waste.

Heterogeneity of e-waste

Heterogeneity of e-waste is the key challenge which prevents us to determine the precise amount of metals in same WEEE samples. PCBs (printed circuit boards) are considered to be the most vital component in most electronic products. As per the statistics provided by Ministry of Information Industry of China, PCBs production in 2010 was around 1.1 × 10^9 m^2 corresponding to 30% share in the global market (Chauhan et al. 2018). These facts impose threat of rapid increase in e-waste. Various studies have been conducted on PCBs to quantify different metal concentration in it. One-gram sample of waste PCB was assessed by Xiang et al. (2010) and found that PCB sample contains aluminum (26 mgg^{-1}), copper (231 mgg^{-1}), lead (29 mgg^{-1}), tin (19 mgg^{-1}), and zinc (17.5 mgg^{-1}). Pardhan and Kumar (2012) determined the metal composition of PCBs scrape. They found 152-mgg^{-1} copper, 46.3-mgg^{-1} aluminum, 32.5-mgg^{-1} iron, 29.3-mgg^{-1} lead, 17.5-mgg^{-1} nickel, 11.2-mgg^{-1} zinc, 0.58-mgg^{-1}silver, and 0.29-mgg^{-1} gold. Gu et al. (2017) analyzed PCBs of spent mobile phones with 203-mgg^{-1} copper, 48.39-mgg^{-1} nickel, 62.25-mgg^{-1} tin, and 1.11-mgg^{-1} gold. In 2018, Wu et al. (2018) found the presence of 0.85-mgg^{-1} aluminum, 65-mgg^{-1}copper, < 0.01-mgg^{-1} iron, 0.02-mgg^{-1} lead, 0.23-mgg^{-1}nickel, and < 0.01-mgg^{-1}zinc in 1-g PCB sample. Kumar et al. (2018) revealed the presence of 23.4-mgg^{-1} copper, 1.1-mgg^{-1} cobalt, 0.9-mgg^{-1}chromium, 22.2-mgg^{-1} of iron, 0.08-mgg^{-1} of gold, 2.0-mgg^{-1} of nickel, 0.4-mgg^{-1} of silver, and 0.7-mgg^{-1} of zinc in 1 g of PCB sample. Khatri et al. (2018) analyzed the metallic composition of cell phones PCBs and found 275.5-mgg^{-1} copper, 63.7-mgg^{-1} iron, 17.9-mgg^{-1} zinc, 19.6-mgg^{-1} nickel along with other base metals and toxic metals whereas silver and gold like invaluable metals were found to be very less, i.e., 0.02 mgg^{-1} and 0.08 mgg^{-1}. Amount of precious metals in PCBs categorizes this e-waste into high, medium, and low grade scrape. Precious metals are significantly more in high class and medium class PCBs material whereas in low grade material precious metals content is very less. Xia et al. (2018) reported the presence of aluminum (0.88 mgg^{-1}), copper (2.49 mgg^{-1}), lead (0.78 mgg^{-1}), tin (0.1 mgg^{-1}), and zinc (0.35 mgg^{-1}) in PCBs without any precious metals in low-grade PCBs. Copper is found to be predominant metal along with other base metals in all PCBs sample, whereas precious metal concentration varies along with the size and processing of PCBs samples. These studies show and highlight the different metal concentration within the same PCBs e-waste. This heterogeneity trend is not limited to PCB samples, but it is same for other e-waste streams. This heterogeneous nature of electronic scrap limits recovery and recycling of metals (Joshi et al. 2017).

Table 3 Raw materials value of 44.7Mt WEEE generated in year 2016

Material	Kilotons (Kt)	Million €
Silver (Ag)	1.6	884
Aluminum (Al)	2472	3585
Gold (Al)	0.5	18,840
Copper (Au)	2164	9524
Iron (Fe)	16,283	3582
Palladium (Pd)	0.2	3369
Plastics	12,230	15,043

Recycling processes and their effects

There are two major sectors that are involved in WEEE management and recycling, i.e., "Formal" and "Informal". E-waste treatment is done with appropriated machinery and designed facilities in case of former process, which ensure the safe working environment. However, high cost of equipment and facilities restrict the usage of formal processing in many developing countries. Although, formal e-waste recycling units have proper facilities, yet the exposure of toxicants can affect the workers and surrounding environment (Cucchiella et al. 2015; Chancerel et al. 2009). Generally, informal recycling units are home-based, in which metals are recovered in unskilled and unscientific manner without proper equipment. In spite of high rule and regulations for e-waste, India and China have numerous informal e-waste recycling units. As in Delhi, about 25,000 people work in informal recycling unit to process approximately 25,000 tons of discarded electronic products per year (Pardhan and Kumar 2014). These informal recycling activities are not only contaminating environment but imposing the health risk on people. Various studies have comprehended information about the occurrence of heavy metals in and nearby recycling yards above the regulated limits fixed by EPA and WHO. Pardhan and Kumar (2014) claimed that soil of Mandoli area (New Delhi) is contaminated with 17.08-$mgkg^{-1}$ arsenic (As), 1.29-$mgkg^{-1}$cadmium (Cd), 115.50-$mgkg^{-1}$ copper (Cu), 2645.31-$mgkg^{-1}$lead (Pb) 12.67-$mgkg^{-1}$ selenium (Se), and 776.84-$mgkg^{-1}$ zinc (Zn). Various studies have also claimed that informal recycling through traditional techniques can lead to different health issues like cancer and neurological disorders. Toxicants such as polychlorinated biphenyl (PCBs) are major components of e-waste, known to cause neuropsychological disorders such as cognition problems (Awasthi et al. 2016b; Alab et al. 2012; Annamalai 2015). Lead, a well-known toxic metal, also has imposed irreversible effect on nervous system causing anemia, behavioral change, brain damage, kidney failure, learning disruption, and miscarriages. Likely, cadmium (Cd) accumulation also leads to renal failure and osteoporosis. Even chromium (Cr) and mercury (Hg), used in batteries, printed wiring boards, relay, and switches, are associated with asthmatic bronchitis, convulsions, lung cancer, and skin damage as enlisted in Table 4 (Chatterjee and Abraham 2017; Awasthi et al. 2016a, b). Julander et al. (2014) investigated the soil of formal recycling units at Philippines and showed the abundance of Cu and other hazardous metals like Cd and Pb. These evidences possess great occupational threat on the workers occupied in formal recycling units and even to the environment. Thus, there is need of proper safety management to avoid harmful effects of WEEE on human as well as on the atmosphere.

Approaches employed to transform waste into wealth

E-waste is the core of urban mining with much higher copper (Cu) and gold (Au) content compared with natural mines. So it will be worthy to transform this waste into wealth by recovering valuable metals and conserving natural resources. Some studies reported that approximately17,000 tons of CO_2 is emitted during production of one ton of gold through natural mines, whereas ~ 10,000 and~ 14,000 tons of CO_2 is estimated for collection of palladium (Pd) and platinum (Pt), respectively (Chauhan et al. 2018). Hence, WEEE treatment for metals retrieval would be beneficial from both ecological as well as economic perspective. As no single approach is found to be satisfactory for the complete recovery process, therefore, various stages such as mechanical treatment, segregation, and metallurgical treatments are applied to develop a collective process. There are various methods as shown in Fig. 2, used to process WEEE for metals extraction.

Mechanical method

Prior to metals recovery, physical processes are applied for dismantling and segregation of WEEE. Selective or simultaneous are the two ways to perform disassembling. The selective dismantling is selected for recycled oriented operations. In the simultaneous approach, all components are removed together by heating in furnace at high temperatures. In spite of high efficiency rate, risk of components destruction, additional sorting process and high operation cost limits the use of simultaneous disassembling process (Chauhan et al. 2018). Low speed high torque shear shredder is one of the instruments, applied for primary crushing to reduce the size of WEEE. Ball milling and disc milling are two other methods that have been accounted for the pulverization of WEEE (Ghosh et al. 2015; Yoo et al. 2009). Loss of precious metals, release of dust containing hazardous metals, brominated flame retardant (BFRs), and dioxins are the problems associated with shredding process. After the shredding, particle fractions are forced through gravity-based separation system, which separate out metallic and nonmetallic components based on their magnetic and electrostatic property(Joda and Raschi 2012) . Due to difference in the magnetic properties of ferromagnetic metals, magnetic separators are predominantly used to separate ferromagnetic metals from nonferrous metals (Chauhan et al. 2018). Ghosh et al. (2015) reported the mineralogical analysis of shredded dust produced during mechanical processing of PCBs and indicated the consistence of 73.1% organic matter, 4.55% iron, 4.65% aluminum, 1.06% lead, and 2.67% copper. So there is need of good collection systems in order to prevent dust exposure and loss of metals.

Table 4 Health effects of some hazardous metals present in WEEE

Hazardous metals	Sources	Effect on human health
Lead (Pb)	Computer monitors, printed circuit boards	Cause damage to central nervous system, kidney, blood system, and reproductive system
Cadmium (Cd)	Chip resistors, semiconductors	Accumulated in the liver, kidney, pancreas, and thyroid
Mercury (Hg)	Thermostats, switches, mobile phones, batteries	Damage central nervous system as well as fetus
Chromium (Cr)	Corrosion protector of galvanized steel plates	Damage DNA, skin sensitization and kidney damage
Barium (Ba)	Protector to radiations	Brain swelling, muscles weakness, damage to heart, liver, and spleen
Berrilium (Be)	Mother board and finger clips	Cause lung cancer and skin disease

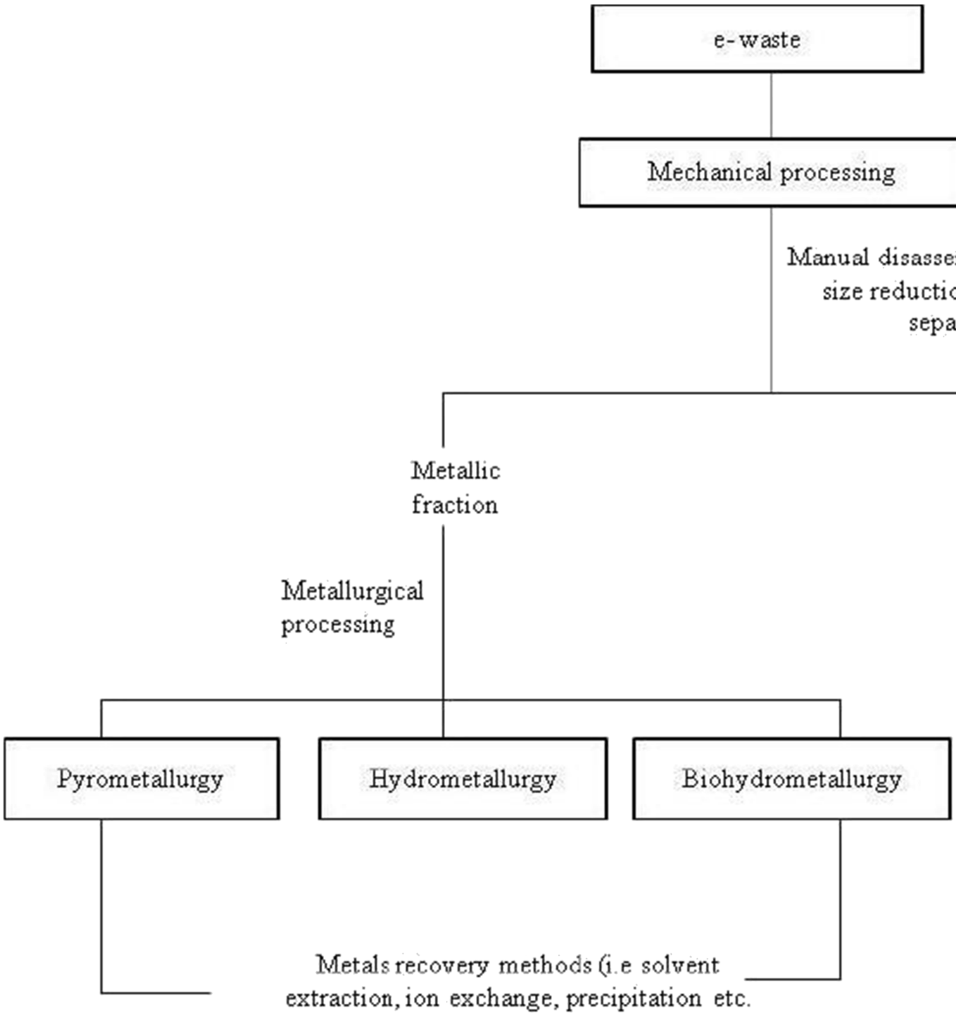

Fig. 2 Processes applied for metals extraction from WEEE

Yoo et al. (2009) processed PCBs through gravity separation method and segregated different particle fractions with 83% Ni and Fe and 92% Cu through two phase magnetic separation process. Separation techniques based on the electric conductivity difference are also predominantly applied to segregate WEEE as electric conductivity of different metallic constituents is the major property.

Metallurgical methods

Metals recovery from WEEE is a significant aspect in terms of ecological and economical prospective. Pyrometallurgy, hydrometallurgy, and biohydrometallurgy are important metallurgical processes applied for metal recovery from WEEE.

Pyrometallurgy process

Incineration, smelting, and roasting are most widely used pyrometallurgical methods. Aurubis smelter, Noranda smelter, Ronnskar smelter, and Umicore are some of the thermal plants, available globally for formal processing through pyrometallurgical approaches. During smelting process, WEEE with high copper concentration is melted at high temperature (i.e., 1250 °C), followed by a conversion process to obtain 99.1% pure copper (Cu) blisters. Several precious metals remains left over after the completion of this process, which can be recovered through different methods, such as electrorefining and electrowinning. Therefore smelting process is considered as one of the finest methods for pure metals recovery. Presence of alloys and pure metals mixture makes the upgradation of final metals a challenging task, and it requires more energy (Chauhan et al. 2015, Chauhan et al. 2018). Xie et al. (2009) recovered Cu and Fe through new pyrometallurgy processes supported with cleaner ultrasonic technique, this showed higher efficiency rate and less toxic by-products generation. Polybrominated dibenzodioxins (PBDDs), phenol, dibenzo-p-dioxin, biphenyl, anthracene, dibromobenzene, naphthalene, and polybrominated dibenzofurans (PBDFs), are some of the toxic compounds emitted through pyrometallurgical treatment, which are highly hazardous and may lead to serious environmental issues (Tue et al. 2013). Toxic byproducts and complexity in pure metals recovery are some of the additional problems, which limit the use of pyrometallurgical methods (Kuyucak and Ata 2013).

Hydrometallurgical process

Limitations of a pyrometallurgical process necessitate the use of hydrometallurgical methods. A release of less amount of toxic gases, less energy demand, easy accomplishment in the laboratory environment, and lower operating costs together with higher efficiency rate are some of the advantages, which attract the use of hydrometallurgical processes (Chauhan et al. 2015; Ni et al. 2013). Significant recovery of base metals by hydrometallurgical treatment ensures fortification of some precious metals recovery and improves leaching efficiency rate through conventional methods such as ion-exchange and electrowinning (Coman et al. 2013). In hydrometallurgical method, cyanide and noncyanide chemical reagents act as strong lixiviants. Cyanide is a strong lixiviant, predominantly applied for precious metals recovery. At pH greater than10.5, cyanide loss is limited as most of the free cyanide exists in ionic form (CN^-), so, at higher pH, cyanide is considered as an efficient and environmental friendly lixiviant (Kuyucak and Ata 2013; De et al. 2009). During cyanidation reaction, first cyanide is oxidized to cyanate which further reacts to form complex with metals (Chauhan et al. 2018).

$$4Au + 8(K/Na)CN + O_2 + 2H_2O \longrightarrow 4(K/Na)[Au(CN)_2] + 4(K/Na)OH \tag{1}$$

$$\text{Overall: } 4Au + 8CN^- + O_2 + 2H_2O \longrightarrow 4Au(CN)^-_2 + 4OH^- \tag{2}$$

Temperature, pH, surface areas of WEEE sample, and presence of other ions are the predominant parameters which affect the rate of cyanidation process. Some of the researchers recommend pretreatment with acids like sulfuric acid or nitric acid along with some oxidizing agent to improve precious metals leaching by minimizing the simultaneous dissolution of copper metal. In spite of adequate information about the toxicity and safe handling of cyanide, environmental issues are still of serious concern and limit its use in an industrial practice (Chauhan et al. 2015). Therefore, researchers have been moved towards less toxic chemical lixiviants such as thiourea, ferrous sulfate, and halide. Thiosulfate is another lixiviant primarily used for the solubilization of gold metal. Copper ion and ammonia along with thiosulfate solution improve gold metal recovery, and act as catalyst too (Aylmore and David 2001). The optimization of ammonia to thiosulfate ratio is considered as an important parameter for efficient recovery of metals. Equations (3) and (4) represent the chemical reaction for thiosulfate leaching.

$$2Cu(NH_3)_4^{2+} + 8S_2O_3^{2-} \longrightarrow 2Cu(S_2O_3)_3^{5-} + S_4O_6^{2-} + 8NH_3 \tag{3}$$

$$2Cu(S_2O_3)_3^{5-} + 8NH_3 + 0.5O_2 + H_2O \longrightarrow 2Cu(NH_3)_4^{2+} + 6S_2O_3^{2-} + 2OH^- \tag{4}$$

Xi et al. (2015) and Altansukh et al. (2016) recovered precious metals from mobile PCBs such as gold and silver by utilizing iodide as lixiviant. They claimed that iodine (I_2) to iodide (I^-) ratio affects the leaching efficiency. Low iodine concentration leads to less iodide formation and weak metal complexation ability, thus, precipitation of metal iodide complex takes place. Whereas higher iodine (I_2) concentration, simultaneously forms complex with base metals and create hindrance for precious metals leaching.

Hydrochloric acid (HCl), sulfuric acid (H_2SO_4), and mixture of sulfuric and nitric acids (HNO_3) are some of the strong inorganic acids which have also been employed as lixiviant for metals dissolution. Behnamfard et al. (2013) utilized sulfuric acid with hydrogen peroxide for copper leaching from waste PCBs. Then followed the treatment with thiourea reagent along with ferric ion, and resulting into high gold and silver recovery (85.76%, 71.36%, respectively). Use of strong chemical reagents may result into release of large amounts of toxic byproducts associated with risk of environmental impact and hence reduce the applicability of hydrometallurgy.(Shah et al. 2014)

Biohydrometallurgical process

Biometallurgy or biohydrometallurgy is an ecofriendly, economical way of e-waste treatment. Biomining, bioextraction, biorecovery, and bioleaching are the alternative terms used for biohydrometallurgical process, which utilize metal solubilization potential of microorganisms from solid residues followed by recovery from solution. Brandl (2001) explained three principles of bioleaching process (1) acidolysis, (2) complexolysis, and (3) oxidoreduction reaction. During the last few decades, researchers have shown keen interest towards the exploration and characterization of microorganisms which shows natural mechanism for mineral leaching in natural environment. Table 5 enlisted microorganisms which have been used for bioleaching. Many studies reported that moderate thermophiles exhibit higher bioleaching potential than mesophilic and extreme thermophiles, therefore many *Thiobacilli* bacteria and thermophilic fungi (e.g., *A. niger* and *P. simplicissimum*) have been used extensively to retrieve metals from e-waste or low grade metal reservoirs (Brandl et al. 2001; Jadhav et al. 2016).

Chemolithoautotrophic bacteria such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* has been the most extensively used microorganisms, which have strong affinity to solubilize base metals (Cu, Ni, Al) (Brandl et al. 2001; Valix and Hong 2014). In 2013, Hu et al. involved bioinformatics approach to understand the metal resistance mechanism of *A. ferrooxidans.* They assessed differential gene expression of copper resistant gene *afe*_1073 in *A. ferrooxidans* DY26 and A. *ferrooxidans* DC strains through reverse transcription polymerase chain reaction followed by bioinformatics analysis of its P1b1-ATPase protein. They obtained lesser gene expression in strain DY26 than strain DC, although strain DY26 exhibited higher toxicity tolerance at same copper concentration. Due to differential expression of afe_1073, the transmembrane protein P1b1-ATPase played better role in pumping out Cu^{2+} ions from the cell and could reduce the harm of metal at lower afe_1073 expression. Further bioinformatics approach was utilized to predict the gene function regulation by quorum sensing involved in cell and substrate attachment. Banderas and Guiliani (2013) used Hidden Markov Models to predict the palindromic and nonpalindromic region used for binding sites. They identified 75 target genes out of which 34 showed high confidence levels. They also confirmed the presence of afeR-1073 gene, directly involved in polysaccharide production and enhance attachment of cell with substrate during bioleaching reaction. Other than acidophilic, cyanogens such as *C. violaceum* and *P. aeruginosa* have also been explored for the solubilization of precious metals (Au, Ag) (Ilyas et al. 2013). In 2001, Brandl et al. exploited the potential of acidophilic bacteria (*A. thiooxidans*, *A. ferrooxidans*) and fungi (*A. niger*, *P. simplicissimum*) to mobilize metals from e-waste. They obtained 65 % solubilization of Cu and Sn and 95% of Al, Ni, Pb, and Zn by fungal strains whereas bacterial strains solubilize 90 % Cu, Zn, Al, and Ni from 100 g/l e-waste sample. They suggested that high concentration of metals inhibit bacterial growth, therefore, reduce the production of lixiviants. Hence they proposed two step bioleaching processes where bacterial culture is first allowed to produce maximum lixiviant in the absence of e-waste followed by leaching process.

Table 5 Microorganisms capable to produce leaching lixiviants for metal extraction

Domain	Organism	Leaching agent
Archaea	*Sulfolobus* sp.	Ferric iron and sulfuric acid
	Acidianus sp.	Sulfuric acid
	Thermoplasma acidophilum	-
Bacteria	*Acetobacter methanolicus*	Gluconate
	Acidiphilum cryptum	Organic acid
	Bacillus sp.	-
	Bacillus megaterium	Citrate
	Chromobacterium violaceum	Cyanide
	Leptosprillium ferrooxidans	Ferric iron
	Pseudomonas putida	Citrate, gluconate
	Thiobacillus sp.	Ferric iron and sulfuric acid
Fungi	*Aspergillus* sp.	Citrate, oxalate
	Aspergillus niger	Oxalate, citrate, malate, succinate
	Penicillium funiculosum	Citrate
	Penicillium simplicissimum	Oxalate, gluconate, citrate
Yeast	*Candida lipolytica*	-
	Saccharomyces cerevisiae	-

Thereafter, many researchers selected two step bioleaching to reduce toxic effect of higher pulp density, to enhance leaching rate researchers utilized bacterial consortium and investigated synergistic effect of bacterial consortium. In 2009, Xin et al. commenced the use of mixed acidophilic bacteria for solubilization of Co and Li from spent lithium-ion batteries. Their results indicated that maximum Li solubilization occurred at lowest pH with sulfur as energy source whereas Co solubilization was influenced by higher pH medium supplemented with FeS_2 + S as energy source. Xiang et al. (2010) used bacterial consortium of genera *Acidithiobacillus*, *Gallionella*, and *Leptospirillum* and optimized the condition to favor best bacterial growth and maximum solubilization of Cu metal. They accomplished 95% Cu leaching with initial pH 1.5, 9-g/l Fe^{2+} concentration and 20-g/l pulp density. They demonstrated that leaching time can be reduced to 5 days by using consortium as compared to use of single culture (i.e., 12 days). They specified that two step bioleaching is necessary for bacterial growth and maximum lixiviant (Fe^{2+}) production; therefore, e-waste should be added to suitable time period. Pardhan and Kumar (2012) explored the bioleaching capability of cynogenic bacteria (*C. violaceum*, *P. aeruginosa*, and *P. fluorescens*) with a two-step bioleaching. They assessed the leaching rate of single as well as combined bacterial cultures of these three cyanide producing bacteria. They reported *C. violaceum* with highest leaching efficiency for maximum metals. 79.3% copper, 69.3% gold, 46.1% zinc, 9.8% iron, and 7.8% silver were recovered with *C. violaceum* among single cultures. Whereas, mixed culture of *P. aeruginosa* and *C. violaceum* showed maximum leaching for copper (83.46%) followed by gold (73.17%), zinc (49.11%), iron (13.98%), and silver (8.42%). Copper is the predominant metal present in e-waste, so its higher concentration causes hindrance for recovery of other valuable metals. Therefore some hybrid methods were developed to increase the bacterial leaching rate (Shah et al. 2015). Sahni et al. (2016) developed a chemobiohydrometallurgy hybrid technology to improve metal dissolution rate. They recovered 72% of copper through pretreatment of SIM waste with nitric acid; remaining copper was recovered from pretreated SIM waste by *C. violaceum* within 4 days. Khatri et al. (2018) and Ilyas et al. (2014) suggested ferrous sulfate and ferric sulfate as chemical lixiviant to improve the microbial leaching efficiency for base metals. Biohydrometallurgy process has also motivated researchers towards the genetic engineering approach to develop recombinant strains to enhance lixiviant production for improved bioleaching process. Natarajan et al. (2015) examined genetically engineered *C. violaceum* strains for gold recovery. They have demonstrated that cyanide production can be enhanced by engineering lixiviant metabolism in *C. violaceum.* Cyanide production increased up to 34.5 mg/L than the wild-type (20 mg/L) when additional hcnABC operon was induced. The genetically engineered strain showed 30% Au recovery at 0.5% w/v pulp density, compared with 11% recovery by wild-type *C. violaceum*. In contribution of bioleaching process, Kumar et al. (2018) had seek out new isolate (*Pseudomonas balearica* SAE1) from e-waste recycling habituate, which shows higher toxicity tolerance (325.7 g/L of the e-waste pulp density) therefore exhibit increased leaching efficiency. For maximum metals solubilization, they optimized different parameters such as pulp density, glycine concentration, pH, and temperature. Their parameters optimization study resulted in 68.5 and 33.8% of Au and Ag solubilization, respectively, at pH 9.0, pulp density 10 g/L, temperature 30 °C, and a glycine concentration of 5 g/L. Biohydrometallurgical processes have found to be (advantageous) as directed towards the reduced environment footprint: cleaner alternative lixiviant, improve the methods for maximum lixiviant products, and decreased the production of hazardous material. Therefore Ilyas and Lee (2014) investigated the effect of different parameters such as aeration, O_2, CO_2 agitation, and pulp density and concentration of sulfur for *Sulfobacillus thermosulfidooxidans* in stirred tank reactors (2 L baffled glass reactor) to scale up the bioleaching process for industrial applicability. They obtained 91% Al, 95% Cu, 96% Zn, and 94% Ni in the presence of 2.5% sulfur containing medium enriched with 25% O_2+ 0.03% CO_2 at a 10% pulp density.

Sensitivity of microorganisms for different parameters, time requirement, selectivity, toxicity of elements, and possibility of contamination are some of the major issues which oppose commercial application of bioleaching process (Pollman et al. 2018). Sensitivity of microorganisms towards high toxic metal ions causes specificity; therefore, microorganisms cannot recover metals quantitatively. Toxicity of e-waste also has a direct influence on applied pulp density for large scale productivity. The exploration of indigenous bacteria habituating to high metals concentration as an alternative of recognized microbial strains from lab collections may overcome these limitations. Genetic manipulation of potential microorganisms and hybrid techniques may also empower biohydrometallurgical process. Thus, this technique requires interdisciplinary contribution from microbiology, biotechnology, engineering, and chemistry as well (Boxall et al. 2018). However, it is predictable that future development can overcome some of these limitations to progress biohydrometallurgical technique towards commercialization.

Comparative evaluation of metallurgical processes

The selection of an appropriate metallurgical technique is subjected to criteria such as capacity to metal extraction, workforce, time and energy requirement, and compatibility with environment. So, environmental consequence, resources involved, labor, expertise, and metal grade are some of the parameters must be considered prior to utilization of metallurgical technique (Chatterjee 2012). Pyrometallurgical treatment involves high-speed thermal

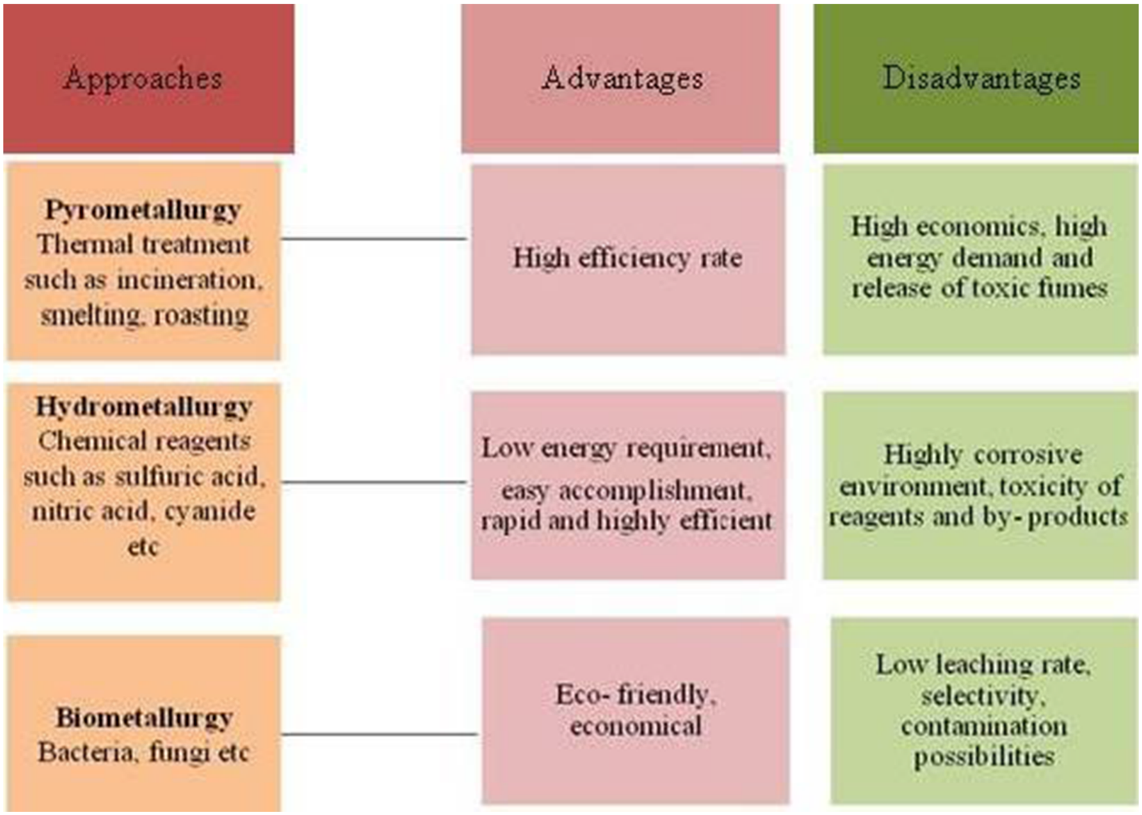

Fig. 3 Advantages and disadvantages of different metallurgical approaches

treatment which is overwhelming other techniques at industrial scale management. Due to high grade feed requirement, pyrometallurgy is not found to be suitable for low grade WEEE (Xia et al. 2018). Figure 3 represents the comparative assessment of all metallurgical processes. Loss of metals into slag or dust form and emission of poisonous gases are also a limitation accompanied with pyrometallurgy technique. Whereas hydrometallurgical process releases lesser poisonous gases in comparison with pyrometallurgy but high risks of acid solutions, and toxic fumes are major limitations associated with hydrometallurgy. Hydrometallurgy and biohydrometallurgy achieved high recuperation rate as compared with pyrometallurgy (Anshu and Hait 2017). Energy requirement, operation, maintenance cost, and environmental consequences are some other potential factors associated with pyrometallurgical and hydrometallurgical methods hence may not prove to be profitable. Biohydrometallurgy is green and economical alternate to the traditional metallurgical processes. In 2002, a US National Research Council committee states “the application of biohydrometallurgy as an important hydrometallurgical processing tool” (Rahman 2016). Therefore now, mining industries also recognize this technique as promising technology showing great potential for economic metals recovery from mineral ores as well as secondary resources.

Next-generation sequencing can also play an important role to raise bioleaching application at industrial scale. This technique is accelerating progress in genomic studies of bioleaching microbes from acid mining conditions. By 2016, 157 genomic analyses of acidophilic bacteria was done through next-generation sequencing which quantified genomic expression level under different conditions and produced their genetic and metabolic models to propose ecophysiological interaction mechanism during bioleaching reaction (Zhou et al. 2018). Therefore, this technique can be a powerful tool to enhance bioleaching rate by deepen our understanding towards functional elements of the genome and revealing molecular mechanism involved in bioleaching process.

Mechanical processing is the prior requirement of hydrometallurgy and biohydrometallurgy techniques to recover metals. Currently, industries use high capacity range (200–500 kg/h) granulators to produce different particle size material and recover plastic, nonferrous and ferrous metallic components (Chauhan et al. 2018). Due to high efficiency rate, pyrometallurgy and hydrometallurgy are still suitable techniques opted at industrial scale for leaching different metallic components. However, there are environmental risks and concerns associated with these techniques.

Chelation, membrane filtration, photocatalysis, and green adsorption are some of the novel alternative techniques which have been recently considered for metals extraction from contaminated sites. Chelation technology involves stable metal-ligand complex formation. DTPA (diethylene triamine pentaacetate), oxalate, tartrate, and EDTA are some of the ecofriendly chelating agents used for extraction of some hazardous metals from contaminated soil. Reusability and ecofriendly behavior of chelating agents are key factors which provide attention to this technology in the field of medicine and environmental remediation. Photocatalysis is a recently developed process which utilizes photons from UV range and degrades pollutants. However, utilization of this process for metal recovery is still at infancy stage. Green adsorption is another green and economical technology which utilizes agriculture residues as metal adsorbents. Membrane filtration is costly and energy demanding as it requires membrane regeneration (Chauhan et al. 2018). None of these alternatives have been implemented for metals extraction from e-waste. Thus future attempt should be prompted to explore applicability of these novel technologies for metal extraction from e-waste. Computational tools may also be helpful to identify capable biodegradable chelating agents and adsorbents for metals recovery from WEEE.

Conclusions

Rapid improvements and technological innovation in EEE have increased the WEEE generation globally. Hence, e-waste recycling not only provides the opportunity for point treatment but also helps us in conserving our natural resources by recovering the precious metals. Current review encourages the need for extensive research to develop innovative, economical and ecofriendly approaches to extract metals from WEEE. Taking into account the 3Rs (reduce, reuse, and recycle) policy, high importance is given on reusing and recycling the electronic waste to decrease e-waste quantity ending up at dumping area. So far, various approaches like hydrometallurgical, pyrometallurgical, and biohydrometallurgical are being utilized for metals recovery from WEEE, but not a single technique can serve as a complete approach because of certain limitations associated with it, so there is need for the exploration of clean technology to recover metal from WEEE. It is evident that biohydrometallurgy probably to be hotspot in the near future. Effort to explore new relevant strains and their interaction with mineral surfaces in mixed species culture, as well as development of biomarkers to study associated microbial biodiversity may enhance knowledge for future bioleaching process. Further, use of some hybrid methods and sophisticated process design can enhance bioleaching efficiencies for industrial scale application.

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REVIEW

Evaluation of e-waste status, management strategies, and legislations

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Abstract

E-waste is the globally mounting solid waste stream which is increasing due to domestic as well as a result of transboundary exportation. This solid waste stream is considered as an amalgam of valuable and toxic material which is concern of environmental and human health risks. To accomplish successful management of electronic equipment after post-consumer stage, numerous lucid initiatives have been offered globally. The ever-increasing quantum of e-waste has enforced environmental organizations of many developing world such as India and China to invent, and implement environmentally favorable opportunities and strategies for mitigation and control of environmental and human threat. Many developed countries have given topmost priorities for e-waste management, so setup appropriate facilities to treat maximum of the generated e-waste, but in low economic nation like India, economic conditions, infrastructure scarcity, and inappropriate legislations are some of the problematic issues which dodge complete replication of the WEEEs management system. Our study represents an evaluation and valuation, of e-waste generation and cross-border movement to the management strategies followed in highly industrialized as well as developing nations. These findings would expand into regulated and non-regulated countries to put more emphasize on e-waste management.

Keywords WEEEs · E-waste · Transboundary export · Waste management · Strategies · Legislations

Introduction

The market demand of electric and electronic equipment (EEE) is uninterruptedly mounting, which enforces electronic industry to provide updated products with shorter life span. This technological revolution results in obsolescence of electronic products, generally characterized as waste electric and electronic equipment (WEEE) or e-waste (Chauhan et al. 2018; Awasthi et al. 2018). The e-waste or WEEEs includes any type of electronic and electrical equipment such as computers, TVs, laptops, mobiles, refrigerators, etc. which are discarded by the user due to their end-of-life or technological upgradation (Chauhan et al. 2018; Sahni et al. 2016). As every country define e-waste as their own way, but the most satisfactory definition is given by WEEE Directive of European Union EU, 2003a which describe e-waste as all the EEE including the components and subassemblies which are of no use are covered under e-waste (Wath et al. 2011). Global E-waste Monitor 2017 revealed that, European Union (EU) account 8% of e-waste solid stream to all of the municipal waste, whereas in developing countries, this waste presents 2% of total municipal waste generation. In the view of global scenario, in 2016 United Nation (UN) reported that 44.7 million ton of e-waste was formed worldwide, which currently reached to 50.2 million ton (Julander et al. 2014; Awasthi et al. 2016; Kumar et al. 2018a). Such projections emphasize on persistent need to address e-waste matter not only in developed countries but in developing world like India and China as well. This new kind of solid waste stream comprises not only loads of valuable material as gold (Au), silver (Ag), but also a heap of toxic constituents such as lead, mercury and brominated flame retardant (Pb, Hg, BRFs, respectively, etc.) which raised up the serious apprehensions about their offensive disposal all over the world (Chatterjee and Abraham 2017; Kumar et al. 2018b). The high value metals from this economical waste are mostly extracted through untrained and unsafe techniques such as burning, acid leaching and roasting, not only in developing but in developed countries as

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well, which is concern of not only environment pollution but for human health as well. In addition, landfilling of residues and informal processing results in carbon emission and cause global warming (Sthiannopkao and Wong 2013). Further, it is estimated that in 2016, the potential raw materials in e-waste were worth approximately 55 billion euro, which means proper processing of this economical waste would not only be an environment and human health protection endeavor but also a possible commercial prospect (Balde et al. 2017; Patil and Ramakrishna 2020).

Developing nations, like India and China, are combating a twofold problem of e-waste due to massive domestic production as well as illegitimate importation from developed countries. The availability of low-priced workers and underprivileged environmental strategies of these countries are the major cause behind the cross-border transportation of e-waste which aim to cheap disposal of e-waste by neglecting their negative effects (Tansel 2017). Therefore, nowadays governments and public facing a significant challenge in regards of the safe e-waste management and processing. However, the peculiar practices utilized for valuable metals extractions are associated with environmental pollution as well as human health threats, which make e-waste management and regulation a point of concern. To tackle the issue of fast growth and to take opportunities from this high value waste, a series of regulations and legislations in regards to transboundary movement, low utilization of hazardous components and formal processing of generated e-waste have been enacted in many countries. According to the Global E-waste Monitor 2017, The United Nation's University (UNU) reported that approximately 66% global community are roofed under e-waste legislation (Patil and Ramakrishna 2020). The Basel Convention is one of the major initiative treaties intended to regulate transboundary movements and disposal of e-waste hazardous constituents among the international borders (Zeng et al. 2017).

Our main objective is to get acquainted with this highly increased solid waste flow, its environmental and human health impact and to conversant with laws and policies which have been initiated and enacted to manage WEEEs. So, in this review article, we are intendingly assess the domestic e-waste generation as well as its cross-border movement, major hazardous constituents of WEEEs and their influence on human health. We intended to evaluated the initiatives and legislative polices enforced for striving e-waste issue. The main motive is to comprehensively prevail initiatives concerned for e-waste management by unfolding the strategies, organizations and legislations followed in highly economic as well as underprivileged countries. However, e-waste legislature is not holistic, as each nation has framed laws in order to address country's own particular problem leading to different management issues. The various strategies and initiative have been listed and enframed to considerate the status quo.

E-waste composition and their impact

WEEEs are an amalgam of organic material, metals and ceramics with more than 1000 substances of "hazardous" and "non- hazardous" material. Majorly, WEEEs comprises ferrous, plastic and non-ferrous metals in the ratio of 50%, 21% and 13%, respectively, along with some amount of glass, wood and ceramic etc. (Vats and Singh 2014; Pant et al. 2012). Base and precious metals like copper, zinc, aluminum and gold, platinum, palladium, respectively, are categorized as non-ferrous metals. However, determination of precise composition is difficult as metallic content of e-waste varies significantly with the type of equipment (Chauhan et al. 2018; Pardhan and Kumar 2014). But typical composition of metallic and non-metallic constituents present in waste printed circuit board is illustrated in Fig. 1. Other than non-hazardous metals, high concentration of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants (BFRs), dioxins and heavy metals like lead, mercury, cadmium, selenium, attribute toxicity and hazardous to environment and human health upon disposal (Uddin 2012; Chauhan et al. 2018; Awasthi and Li 2017). According to Duan et al. (2016), during land filling disposal of e-waste; lead gets reached into ground water then become bioaccumulated and biomagnified in food chain and to pose serious threat to ecosystem. Priya and Hait (2017) also claimed the deposition of these hazardous substances into the soil surrounding the e-waste processing area (Abdelbasir et al. 2018; Priya and Hait 2017). Few hazardous and secondary substances existed in e-waste and their plausible human effects are summarized in Table 1. The enormous generation, complexity, and heterogeneity of e-waste demands its management and disposal in highly organized way in the mean of resources conservation as well as to avert environmental pollution.

E-waste production and transboundary movement

E-waste production is anticipated to excel continuously with an average of 20–50 million tons production every year. Presently, worldwide e-waste production is ascending approximately 53.6 million tons with 4000 tons generation per hour, which was 49.8 million tons in 2017 as shown in

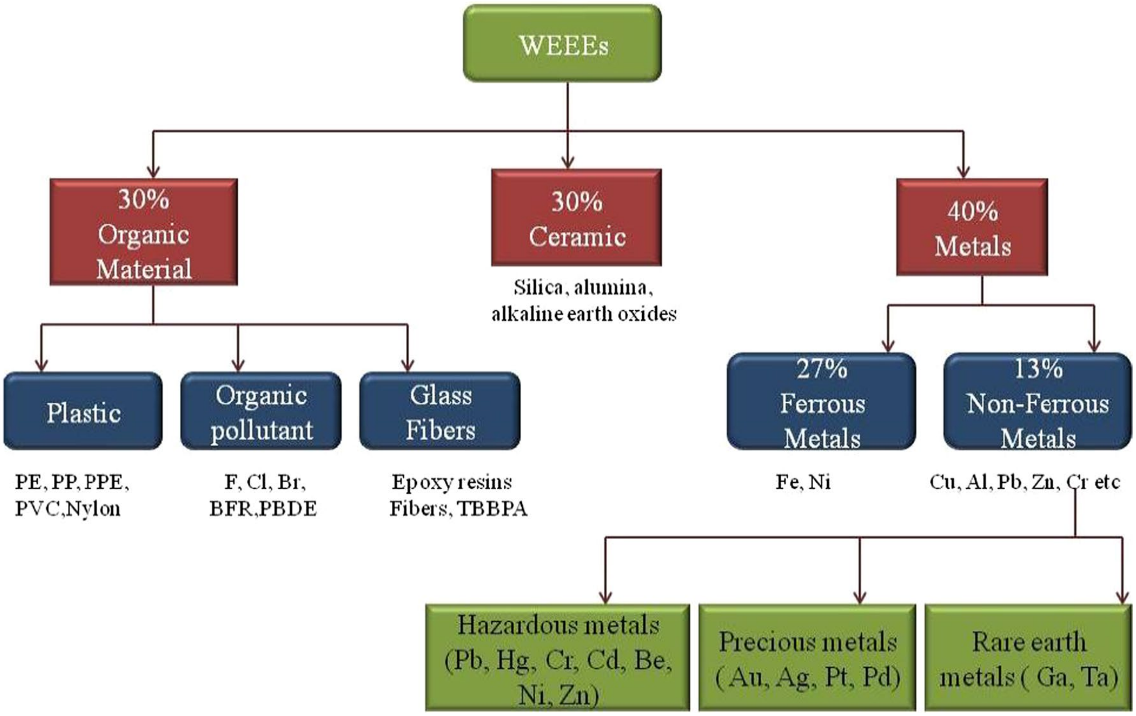

Fig. 1 Constituents of electric and electronic equipment in end- of- life (Chauhan et al. 2018)

Table1 Components consisted in e-waste and their human health influences

Material	E-waste source	Human health impact
Arsenic (As)	Cathode ray tubes screens, diodes and printed circuit boards	Cause various cancers and skin diseases Affects respiratory system, cardiovascular system, and impair reproductive system
Brominated flame retardant (BFR)	Flame retardant for electronic equipment	Causes endocrine and reproductive system, thyroid and liver problem Also include impaired nervous system in children
Copper (Cu)	Printed circuit boards	Causes diarrhea and liver disorders
Chromium (Cr)	Disks, computer housing, cathode ray tubes and batteries	Responsible to cause brain, neurovascular, respiratory and, kidney damage
Lithium (Li)	Batteries	Affect nervous system and intestinal system
Mercury (Hg)	Lamps, batteries and switches	Affect central nervous system and kidney
Nickel (Ni)	Batteries and cathode ray tubes	Responsible for skin and, lungs diseases, Also cause different kind of cancers
Cadmium (Cd)	Printed circuit board, switches, chips in semiconductors	Extremely carcinogenic, Affect bones, kidney and respiratory system
Polychlorinated dibenzodioxins (PCDDs)	Combustion byproducts	Disrupt nervous system, and causes different cancer
Polyaromatic hydrocarbons (PAH)	Released as combustion byproduct	Occupational exposure may lead to cause various types of cancer Responsible for reproductive and developmental health effects

Source: (Perkin et al. 2014)

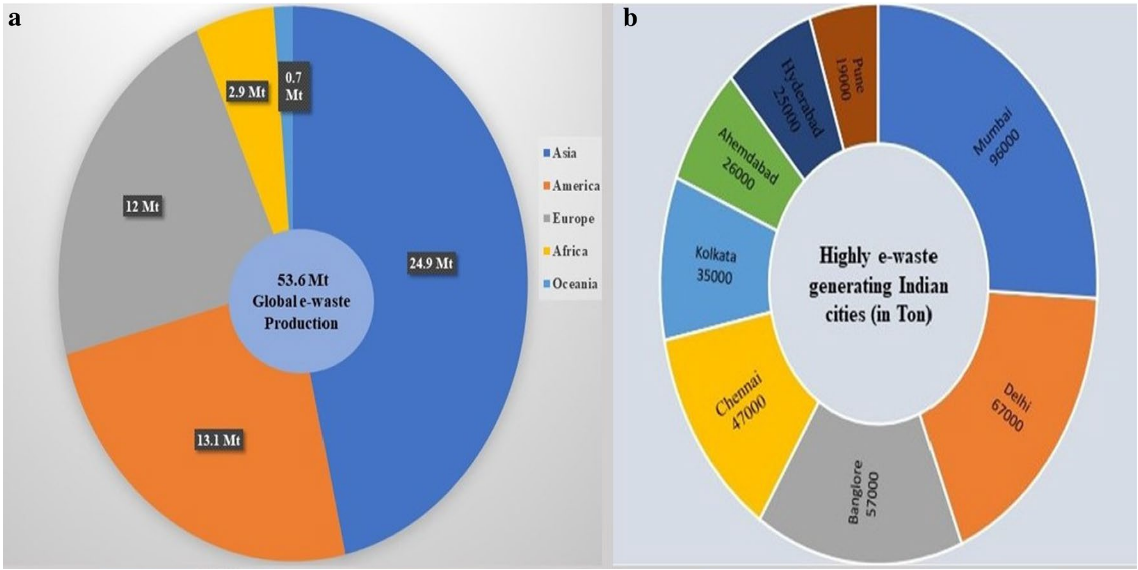

Fig. 2 Statistical contribution of e-waste **a** global e-waste contribution from different continents **b** top Indian cities which contribute majorly in India. (Source: **a** UN's global e-waste monitor 2020. **b** Vats and Singh 2014)

Fig. 2a (Islam et al. 2020; Ari 2016). According to UN's Global e-waste Monitor 2020 Asia is leading internationally with total 24.9-million-ton contribution, whereas America offers 13.1 million ton followed by Europe with 12 million ton, Africa 2.9 million ton and Oceania 0.7 million ton (Chauhan et al. 2018; Thakur and Kumar 2020).

According to the annual estimation of 2016, China positioned at the top with total 7.2 million metric tons contribution. According to EPA statement United State alone contributes 3.19 million tons of e-waste and placed at second rank after China (Julander et al. 2014; Pradhan and Kumar 2014). Besides, the domestic production, illegal exportation from other countries also upsurge the total waste proportion of China (Hopson and Puckett 2016). Ilankoon et al. (2018) stated that 80% exported e-waste from US are received by Asian countries out of which 90% is directed to China only. Above all, Ghana and Nigeria, are considered to be uppermost e-waste center among all the continents. Although low inhabitant of the countries demands less and produce not much of the waste domestically but the massive amount of importation from the developed nations excels their e-waste content (Chatterjee and Abraham 2017; Ilankoon et al. 2018).

In Asian region, India is considered to be second e-waste counting country with 4,00,000 ton annual generation (Kumar et al. 2018a, b). 60% contribution is considered from 65 cities only (Vats and Singh 2014). Maharashtra is the topmost e-waste producing state followed by Tamil Nadu, Andhra Pradesh, Uttar Pradesh and West Bengal, Delhi, Karnataka, Gujarat, Madhya Pradesh and Punjab for total 70% of e-wastes production (Wath et al. 2011; Ari 2016). In view of top most metropolitan cities Mumbai ranked first with total 96,000-ton e-waste generation as shown in Fig. 2b, followed by Delhi with 67,000 ton, Bangalore (57,000 ton) (Vats and Singh 2014). This contribution is exceeding constantly. According to the United Nation Environment Programme (UNEP) statement India is now generating eighteen times much more discarded mobile phones than 2007 which will increase the rate of total e-waste generation (Vats and Singh 2014).

Developed countries with highly saturated electric and electronic demands are seemed to be major e-waste originating core. In the view of developing and under-developed countries loopholes in regulation permits the cross-border transport as of "hidden flow" which is untraced and undocumented (Hopson and Puckett 2016). Investigation of the cross-border distribution trends indicates that huge amount of e-waste is moved internationally mostly from developed or low populated countries to extremely inhabited countries which are existed with low-priced workforce (Perkin et al. 2014). Priya and Hait (2017) have stated that India and China are imperiled to 50–80% illegal trans-boundary e-waste exportation by few developed countries. The quantification assessment of the exported e-waste amount is a key issue as of illegal transportation. But the Basal Action Network (BAN) is key alternative which works to assess the transboundary flow of e-waste material (Lee et al. 2018). According to Global E-waste Monitor 2017, One of BAN's key verdicts disclosed that there are almost 205 tracker deployments out of which 34% moved through coastline, to developing nations, with 93% exportation of the total 50 million tons to Asia and Africa, where no appropriate recycling facilities are existed, whereas 7% moved to countries such as Mexico and Canada (Duan et al. 2016). The European Environment Agency estimates that EU exports approximately1.3 million ton of e-waste annually to Africa and Asia (Perkins et al. 2014). One of the studies revealed that 80% e-waste of US have been ended up in Asian countries which is tracked by the 200 GPS trackers (Lee et al.2018). In 2007 it was found that India generated 3,82,979 tons e-waste itself whereas an additional 50,000 tons was unscrupulously imported from US and EU countries in the ratio of 80 to 20, respectively, whereas additional 20% is exported in "official" way from US to India and China as of comprehend donation for formal recycling for short term economic benefits (Zeng et al. 2017; Awasthi et al. 2016). Another study by Perkins et al. (2014) claimed that, in 2003, India and Africa were exposed with almost 23,000 metric tons of illegal transportation from the United Kingdom.

Waste management initiatives, strategies and legislations

To concern this ever-growing e-waste and to take advantage of this valuable secondary resource many countries have taken initiatives and levied many legislations. Currently, more than 2000 sections of legislation with over 90 jurisdictions has been in force worldwide to control the detrimental consequences of WEEEs (Ilankoon et al. 2018). Earlier, most of the regulations and strategies have been impelled and aimed to environmental protection, but at present most of management strategies have been implemented with human health concerns (Sthiannopkao and Wong 2013; Hopson and Puckett 2016). There are numerous numbers of international organizations and initiatives shown in Table 2 which have been step forward for suitable monitoring and recycling. These initiatives and organizations are coordinately making efforts to alert consumers and explore key solutions for e-waste management (Perkin et al. 2014; Patil

Table 2 Initiatives and legislation addressing e-waste issues

Initiatives	Key features
Basal convention	Endorsed in 1992 to avoid exportation of hazardous waste from producer countries. 172 nations stand by the agreement but US does not ratify the treaty
Bamako Convention	Aimed to restrain the import of e-waste more stringently than Basel Convention. Applied in African Union nations from 1998
EU WEEE Directive	In 2007 all the EU members adopted the system with initiation of take-back approach for 10 groups of electrical things
Restriction of Hazardous Substances Directive (RoSH)	Enforced along with EU WEEE, particularly aimed to restrain the use of hazardous substances. also validated by various nations, counting China and India as well
Solving the E-waste Problem (StEP)	Initiated in 2007 by UN agencies to promote reusability of the recycled components to limits the waste generation
3Rs (Reduce, Reuse, Recycle)	Initiated by Japan. Work to prevent e-waste generation. Allows exportation to other countries for remanufacture and recycling. Conflicting the goal of Basel Convention treaty
US State laws and the Responsible Electronic Recycling Act (HR2284)	25 states of US, imposed with the law. HR2284 is anticipated to control e-waste exportation. This law enforces assembly and reprocessing of e-waste via stipulating deposits from consumers
US NGOs—Basel Action Network (BAN), Silicon Valley Toxic Coalition (SVTC), Electronics Take-Back Coalition (ETBC)	These three acts work to promote the "Basel Ban" amendment for restrictive transborder exportation. Enhance general e-waste assortment and reusing programs
National Strategy for Electronics Stewardship (NSES), US	Focus to limits the use of harmful substance Improve the handling and management strategies of e-waste in the US or reduce their harmful impact in other nations
International Environmental Technology Centre (IETC) 7– UNEP	Strengthen utilization of environmentally suitable technologies in developing nations on waste management
Global e-Sustainability Initiative (GeSI)	Focus to engage Information and Communication Technology (ICT) companies, industries and organizations to concern e-waste management

Source: (Sthiannopkao and Wong 2013; Ilankoon et al.2018) and Ramakrishna 2020). Some of the initiated strategies opted for e-waste regulation are as following:

Extended producer responsibility (EPR)

EPR was turned up in academic circle as of environmental policy strategy in early 1990s, which sustain producer's responsibility for total life cycle improvements of product system up to its final disposal. EPR is mainly aimed to prevent and reduce e-waste production with maximum reusability and reduction of consuming natural resources. Currently maximum legislation and strategies refer under the concept of "Extend Producer Responsibility," which are widely executed internationally. This EPR program was originally mandated by Germany's directive on the avoiding of packing waste (as 'Green Dot' Programme), and laid a financial influence of producers for assembling, disposing and recycling (Chaterjee and Abraham 2017; Wath et al. 2011). The Organization for Economic Co-operation and Development (OECD) has defined four broad categories of EPR implementation:

1) Take- Back protocol.

This claims the responsibility of producers or manufacturer to control the waste generation and environmental influences of the products. Producers need to mandate "take- back" or "buy back" approach by providing incentives to customers for product return to authorized retailers (Lu et al. 2015).

2) Economic and market-based tool appears in four forms to provide a financial motivation for EPR implementation.

i) Deposit – refund: Consumers need to take responsibility to restore the consumable product to retailer at the end of their shelf life. To abide by this, consumers need to make initial payment which can be reimbursed after overturn of the product.

ii) Advance disposal fee (ADF): Public and private units can charge estimated cost of collection and treatment of certain product from the consumers, which can be utilized for management of end-of-life product system.

iii) Material tax: To use new, non-recyclable and some toxic material, producer is levied by some fee which can be contributed for collection and handling of products to generate incentives for utilization of recycled and less toxic material. This is aimed to enhance recycling and reusability of material for less waste production (Kiddee et al. 2013).

iv) Upstream Combination Tax/ Subsidy (UCS): Tax is levied on producers or a manufacturer to promote waste treatment whereas subsidy is provided to modify product design and material to support recycling and treatment processes.

3) Regulation and performance standard.

Enforced on producers to encourage take back approach and recycling of post-consumer products. This regulation can be obligatory adapted by industries to reinforce incentive for products redesigning.

4) Instruments based on information create public awareness to promote EPR programs. Measures include customer's awareness about producer responsibilities regarding environmental impact of product system, post- consumer product recycling and management (Patil and Ramakrishna 2020).

The basal convention

In 1989 UN assembled 186 nations to initiate the Basel Convention system with an aim to address the transboundary exportation issue. In 1992 this convention system entered into force as treaty entitled "Transboundary Movement of Hazardous Wastes and Their Disposal" (Wath et al. 2010; Patil and Ramakrishna 2020). Latterly this treaty has been endorsed by 181 countries to prevent cross-boundary transportation of hazardous e-waste. In principle this system is intended to charge nation of hazardous waste generation and its exportation to other countries. This induces the responsibility for the safe destruction of waste within the country and avoids transmission to developing countries except prior notifying agreement from the receiving nation (Uddin 2012). The EU was initiator to enforce legislation within the states which was imposed in most of the countries (Sthiannopkao and Wong 2013). The initiative had not been able to eradicate e-waste exportation totally as in 2012 there had been illicit transportation of 50,000-ton e-waste to India from different developing nations (Wang et al. 2016).

EU's WEEE directive and RoHS

After the existence of Basel treaty, the EU commission introduced the WEEE directive but was printed into law in 2003. Further, in 2012, WEEE directive (2012/19/EU) was passed by commission for uniform regulation of e-waste management in its nation (Ilankoonet al. 2018). This directive sets up 10 categories to direct comprehensive strategies for recovery and reprocessing of all WEEEs to obtain higher turnover of recyclable e-waste fractions. The WEEE Directive counsels the state members to focus on environmentally friendly and recyclable EEE products. The directive has implemented with the EPR system in which the manufacturers are enabled to take the responsibility of product recycling after the consumer usage (Patil and Ramakrishna 2020).

Later on, they added "Restriction of Hazardous Substances" (RoHS) directive 2012/ 95/EC intended to modify product designing and packing to limits the consumption of lead, cadmium, hexavalent chromium, mercury and many other hazardous substances (Nnorom and Osibanjo 2008). This directive aims to increase the recycling rate of equipment over excessive production of the waste. Other countries such as Korea, Australia, Japan, Canada and the US have also been also found to be influenced to determine the legislations by these two stated directives, to increase the recycling rate of domestically produced WEEEs.

The 3Rs and StEP

Japan introduced 3Rs (reduce, recycle and reuse) initiatives domestically and globally at a 2004 G8 summit (Zeng et al. 2017). This initiative was prioritized the prohibition of e-waste generation by promoting reusability of technology, this was furthering goal to remove obstacles to the intercontinental transportation for reprocessing and recovery purpose, which is considered to be contradictory to the aim of the Basel Convention treaty (Ilankoon et al.2018).

On the other hand, Solving the e-waste Problem (StEP) is a United Nation initiative, which emphasize on recovery and reusability of materials all over the world. It consists five concentration groups including strategies, reformation, recovery, reuse and capacity building (Sthiannopkao and Wong 2013). Specified characteristic of the initiatives is to formulate an ease and safely disassemble and recyclable products along with negligible usage of toxic substances.

These strategies have not been followed only in developed nation but also being adopted in developing nations. But apart from these strategies each country has enforced its own rules and legislations to manage this ever-growing solid waste stream. India and China are two main Asian developing countries which are not only ahead in terms of population but in aspect of total e-waste production as well. These two countries incessantly facing the illegal exportation and accelerated production of e-waste. China itself produce approximately 11.7 million ton of e-waste. India is following China in total contribution of e-waste. India's own annual domestic production is 400,000 tons but maximum involvement is through importation from highly developed countries (Vats and Singh 2014; Awasthi et al. 2018). Lu et al. (2015) stated that approximately 57,700 ton of e-waste is illegally exported to China alone from other countries. This continuous donation of e-waste from urban nation has led an important issue related to environmental and health concerns in these Asian countries. (Sthiannopkao and Wong 2013). This increased threat of solid waste stream led to develop management and handling rule in the Indian and China as well (Uddin 2012; Abdelbasir et al. 2018). In India, Ministry of Environment, Forest and Climate Change (MOEFs) took initiative and codified its first specific e-waste Management and Handling Rule in May 2011, and become influential in 2012. The rule is conceptualized on the basis of EPR system to introduce the responsibility of equipment manufacturers for handling e-waste after post-consumer stage (Garlapati 2016). After this, most recent e-waste Management Rule has been imposed since October 2016. The rule has been intended to channelize the e-waste among producer, consumer sales purchase, collection centers, dismantlers and recyclers (Nnorom and Osibanjo 2008; Awasthi and Li 2017).

Prior the e-waste (Management and Handling Rule) 2011, India only focused to maintain hazardous waste and enforced many rules and guidelines to resolve the environmental concern of hazardous waste materials. Garlapati (2016) has introduced the rules which have been implemented in India in concern of hazardous waste management. These rules intended to impose some guidelines for complete management of hazardous waste including e-waste and can be used as a model for initiation of awareness programs, implementation of appropriate waste treatment technologies and confining land-filled disposal. Some of the environmental rules which have been followed in India are as following: (i) The Hazardous Waste Management and Handling Rules, 2003, imposed to categorize e-waste and their components under "hazardous" and "non-hazardous" waste (Chauhan et al. 2018). (ii) The Hazardous waste Management, Handling and Transboundary Movement Rules, 2008. This rule was set up for e-waste recyclers (Kumar et al. 2017). According to rule, e-waste handler, desiring to recover or reprocess hazardous wastes of WEEEs are obligatory to enlisted with the central pollution control board (CPCB) (Wath et al. 2011). (iii) Environmentally sound e-waste management guidelines, 2008. Indian government provided the guidelines for classification of various e-waste resources and their components and was permitted by MoEFs and CPCB (Priya and Hait 2017). These guidelines majorly cover the particulars related to e-waste composition, documentation of possible hazardous contents and ecofriendly recycling, re-use and recovery of economically valuable materials. These guidelines also follow the notion of EPR system (Uddin 2012; Hsu et al. 2019). In spite of all these rules and regulation e-waste management in India is still its infant stage and need more stringent and powerful implementations.

Above all, China government also stands up and steps forward to manage this solid waste stream. In 2002, China ratified the Basel Convention to prohibit importation of hazardous e-waste components but low law enforcement could not stop completely and illegal exportation still persists to China (Duan et al. 2016). Hence China drafted certain laws in regards to manage the domestic e-waste production and environment protection, which are more or less on the same line of thoughts of India (Wei and Liu 2012). In 2005, first management measure had taken to specify the limitations on material similar to EU directive to prevent environment pollution caused by e-waste, which has been named as China's RoSH (Kiddee et al. 2013; Lu et al. 2015). Another Environmental Protection Law of China is drafted very similar to India's Environment Protection Act. The only difference existed is that India seems to have a specific law in that sense which is inclusive of "extended producer's responsibility." Now Amendment has been proposed by China government to entail "EPR" as well as Product and Packaging Recycling" programs for appropriate management of electronic products (Cao et al. 2016). In 2009, new waste disposal law has enforced to regulated safe disposal of e-waste by supporting many recycling facilities. Under this law, producers, venders, and reutilizing corporations are to be responsible for safe handling and management of e-waste (Sthiannopkao and Wong 2013).

Conclusion

The continuous domestic production and cross-border transportation of e-waste among the nation has deleterious effect on the ecosystem. The above comprehended literature and discussion reveals that there is dearth of stringent legislations for WEEEs management not only in developing but in developed countries as well, as each country's has different scenario. But to control this ever- growing solid waste it is necessary to implement a systematic e-waste law and make regular amendments to overcome the drawbacks learned from systematic regular evaluation. The prime rule in any e-waste policy must have been a stringent restriction on global transboundary movement of WEEEs with massive penalization on illegal shipment to allow them to focus on effectual e-waste management.

The "repair and reuse" principle should be applied for effective management of e-waste arising to encourage both reduction and recycling of e-waste. To implement this principal, government should also encourage refurbishment model where consumers can be engrossed with enticements such as lesser tax rates to purchase refurbished articles. In addition to encourage the resources reusability and recycling, producer should be bound to legislations to follow the EPR principle and guidelines must include for minimum use of hazardous substances and virgin raw constituent. There is also a need to reinforce the existing laws and policies through consistent assessments and amendments. As e-waste law is not holistic so implementation of country's own legislation would not be adequate to resolve the e-waste concern at the universal level. There should be an international assembly to oversee and synchronize with e-waste management all around the world. An international council should inclusively frame uniform guidelines of global standards for EEE manufactures as well as e-waste recyclers. In addition, e-waste recycling legislation would be proving to be a driving factor that stimuli the e-waste management by recycling of WEEEs actively in many countries through recovery of certain valuable metals. Pyrometallurgy and hydrometallurgy are two conventional methods which have been regularly indulged in metals recovery form WEEEs. But these methods are again concern of environment pollution as these are associated with harmful fumes and strong chemical lixiviants. Furthermore, accomplished recycling of e-waste depends on its cost and these longstanding methods are labor demanding, energy conserved and infrastructure inclusive therefore opted for recovery of only expensive and barely accessible raw materials. But nowadays biohydrometallurgy, an economical and ecofriendly method has been keenly used to overcome the limitations of these usual methods and attracting the attention of research to uplift this technology for industrial scale application. But prior to the recovery of metals, it is necessary to address recycling challenges such as the assortment of e-waste, storage, disposal, disassembly, and material segregation. Now, a private sectors and various research organizations have been grabbing the opportunities from this economical waste and been looking forward for the environmentally sound recycling purposes. So, to achieve sustainable e-waste recycling, the governments and regulatory authorities should provide facilities and also uplift such originations through funding and inducements. Henceforth future efforts to reduce illegitimate abandonment must include a combination of hostile legislation, new technological solutions and communal accountability through greater education on e-waste. In future, biohydrometallurgy (i.e., bioleaching) approaches can be a significant tool for recovery of metals in eco-friendly and economical way. Thus, tools, including legislative policies tied to recommendations for e-waste management and recycling possibilities such as the EPR approach, can ultimately alleviate most e-waste problems.

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