FACULTY OF SCIENCE AND TECHNOLOGY

MASTER'S THESIS

Study programme/specialisation: Environmental Engineering/Water Science and Technology	The spring semester, 2023 Open access

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Thesis title:

A review of metal recovery from E-waste using current microbial technologies

Credits (ECTS): 30

Keywords: Metal recovery Lithium, Cobalt, Copper Conventional recovery technology Microbial recovery technology Bioleaching Biosorption Lithium-ion batteries (LIBs) Electronic and electrical equipment waste (WEEE)	Pages: 68 Stavanger, *13.06.23*

Abstract

With the increasing demand for metals in many industries, there is a growing need for improved methods of metal recovery. For the management of electronic waste (E-waste), numerous studies have been conducted on extracting metals using technologies such as pyrometallurgy, hydrometallurgy, and biometallurgy. Bioprocessing can help recover metals from secondary sources such as E-waste and lithium-ion batteries (LIBs).

This research review aims to examine methods, focusing mainly on biological based methods for recovering metals from LIBs and E-waste, and to identify research gaps and areas for further research. This thesis contains a comprehensive overview of the metal recovery technologies from E-waste and LIBs, highlighting their benefits and drawbacks. A scoping literature review based on published articles and reviews using different keywords in Scopus database has been provided to give a complete overview of metal recovery from E-waste and LIBs using green technologies such as bioleaching and biosorption.

Implementation of biotechnology is essential in achieving the goal of minimising waste, conserving valuable metals, and mitigating the negative environmental impact of metal extraction. Improving bioprocessing methods can provide the industry with an eco-friendly technology to address the challenges of the increasing lithium (Li) demand and waste from LIBs in the future. Despite the increased research effort regarding the use of biotechnological methods for metal recovery, the research is only in the early stages. Biotechnological based methods have a promising future. However, further large-scale research and pilot studies on microbial technology for metal recovery are needed to facilitate industrial upscaling.

Acknowledgements

I would like to give my sincere gratitude to my supervisor Krista Michelle Kaster for giving me the opportunity to work on such an exciting topic. Thank you for your valuable guidance, high availability and input during the last year. I am thankful for your contributions and constant push for me to improve and learn more. Your help and support have been a great encouragement. I would also like to thank Kari Østerli Frafjord at the University library for helpful guidance regarding my literature review.

Further, I would thank my fellow students at environmental technology for a good and thriving environment. A special thanks to Marit Henriksen for valuable conversations and inputs, especially this last semester.

I would also like to express my gratitude to my family. Your unwavering support, motivation, and help throughout my studies have been invaluable. I could not have achieved this without you!

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List of Abbreviations

CE	Circular economy
CRMs	Critical raw materials
EC	The European Commission
EEEs	Electronic and electrical equipment
E-waste	Electronic waste
IOB	Iron-oxidising bacteria
IRB	Iron-reducing bacteria
LIBs	Lithium-ion batteries
MICP	Microbe-induced carbonate precipitation
NCM	Layered oxide $LiNi_xCo_yMn_zO_2$
PCBs	Printed circuit boards
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
SLR	Systematic literature review
SDS	Sustainable development scenario
SOBs	Sulphur-oxidising bacteria
STEPS	Stated policies scenario
WEO	World energy outlook

1. Introduction

Since the 1950s, the world has seen immense growth in both population and technology. With the evolution of new technologies and increasing wealth standards, the use of non-renewable resources has increased. Recently, there has been a shift towards exploring renewable energies, leading to changes in energy geopolitics (Lee, 2011). Critical raw materials (CRMs) are vital in developing high-tech applications and sustainable materials, particularly in producing renewable energy, electric vehicles and equipment (Işıldar et al., 2019). The world must shift towards a green transition. The planet needs to become more economically sustainable by shifting towards an economy that does not rely on fossil fuels or consume natural resources excessively. The green transition will require the use of various metals and minerals (Heldal et al., 2019). Metals and minerals are essential components in manufacturing various industrial products, crafts, and processes, and their availability plays a crucial role in their production. (Dominguez-Benetton et al., 2018). However, the sustainable supply of CRMs is a significant concern (Işıldar et al., 2019).

Achieving a low-carbon future relies heavily on electrification. The world's economic model has long been linear, following the "take-make-waste" principle, where materials are extracted, used, and discarded. Presently, industries are experiencing a rapid surge in the production of electrical equipment (Dave, 2018). The demand for electrical equipment and secondary batteries has steadily expanded since electronic appliances such as mobile phones and computers became portable. A consequence is the increased amount of waste the surge generates (Shin et al., 2005). Electronic waste (E-waste) is a term that refers to disposed of devices (all items of electrical and electronic equipment) at the end of their economic use that consumers cannot utilise anymore. It is an important secondary source of CRMs and valuable metals (Işıldar et al., 2019). The problem is that most electronic wastes are neither treated nor recycled properly (Dave, 2018). 1.7 billion tons of solid waste is generated annually worldwide and is estimated to increase to 2.7 billion tons in 2024 (Wu et al., 2022). Recycling and recovering metals from electronic and electric equipment (EEE) have become significant concerns regarding environmental and socio-economic aspects. Considering the overall demand for valuable metals for producing new EEEs, E-waste has been considered a highly important secondary source (Dutta et al., 2023). Electric transportation, especially electric vehicles, is rising in popularity and largely depends on lithium-ion batteries (LIBs) (Dave, 2018). LIBs have been extensively utilised in electronic vehicles and portable devices since the 1990s. The production of LIBs has significantly increased in recent years because of the fast-paced updates in consumer electronics (Zheng et al., 2018). Spent LIBs consist of critical metals such as cobalt (Co), nickel (Ni), lithium (Li), copper (Cu) and aluminium (Al) and environmental pollutants in case of their disposal (G. Mishra et al., 2022). Inappropriate disposal of E-waste, including used LIBs, directly into the environment causes the generation of toxic gases such as hydrogen fluoride (HF) into the atmosphere and hazardous components comprising heavy metals, such as lead, chromium, mercury, and polychlorinated biphenyls, among others, increasing the need for recycling (Dutta et al., 2023). Used LIBs possess considerable economic worth as they contain a substantial quantity of precious metals, surpassing the metal concentration in mineral ores (Zheng et al., 2018).

Research has been conducted worldwide on the recycling of LIBs. However, the technology and processes for recycling them are still limited to laboratory settings due to the intricate structure of LIBs. Pyrometallurgy processes are the most widely used technology for industrial metal recovery (Zheng et al., 2018). The existing metal recovery processes have high capital costs and chemical processes that increase the pollution of toxic gases and liquid waste (Dutta et al., 2023). Conventional recovery and recycling methods are successful, but greener technologies have developed, focusing on evolving efficient, economical, and environmentally friendly processes (Roy et al., 2021). Biotechnology is a promising alternative to the best available industrial technologies (Işıldar et al., 2019).

The first objective of this thesis is to give an overview of the current literature regarding metal recovery for lithium battery waste and other electronic waste using biotechnology, identifying any knowledge gaps regarding the subject. The first objective is a part of the research aim one. A scoping review (SR) is chosen as the preferred methodology for this particular aim. The secondary objectives and research aim two are to discuss the need for metal recovery and identify different technologies for extracting and recovering metals based on relevant research literature. This thesis focuses mainly on recovering metals such as lithium, cobalt, and copper and technologies primarily involving bacteria.

2. Methodology

This section provides an overview of the literature review (research aim one). The method describes the research methodology, the strategy, and the implementation of a literature review. This method will be conducted throughout the thesis "A review of metal recovery from E-waste using current microbial technologies".

2.1 Introduction

Literature reviews are performed for several reasons, including providing a theoretical background of the following research, learning the research on the topic of study, or answering questions by understanding the existing research literature. As such, a literature review is often written as the introduction of an article/study. However, another literature review establishes an original and valued research work. According to Fink (2005), a literature review requires a more systematic approach (Okoli & Schabram, 2010). An adaption of Fink's (2005) Definition of a research literature review defines a systematic literature review (SLR) as *"a systematic, explicit, comprehensive and reproducible method for identifying, evaluating and synthesising the existing body of completed and recorded work produced by researchers, scholars, and practitioners"* (Okoli & Schabram, 2010). A systematic review is often chosen because it will provide a comprehensive overview of the current knowledge. SLR can also identify research gaps in the field of study and be a valuable tool to emphasise potential methodological concerns in the studies part of the particular study (Peričić & Tanveer, 2019).

Literature reviews can vary in type based on the methodology employed for the review. This thesis uses a scoping review to answer research aim one. Performing a scoping review enables an accurate assessment of the available research literature's size and scope. The primary objective is to identify the nature and extent of research evidence; therefore, formal quality assessment is not required for this type of review (Grant & Booth, 2009). There is limited guidance when choosing between a systematic review and a scoping review. A SR aims to provide evidence in a given field, clarify key concepts and definitions, examine research practices in a specific topic, and analyse knowledge gaps. Scoping reviews can complement systematic reviews by confirming inclusion criteria (Munn et al., 2018). Since the first research aim is to identify knowledge gaps and the extent of relevant literature without providing a formal quality assessment, a scoping review is an optimal choice.

2.1.1 Database

The database Scopus was used for the scoping review. Scopus is the largest abstract and citation database of peer-reviewed literature, scientific journals, books, and conference proceedings. The database delivers a complete overview of the world's research output in science, technology, medicine, social sciences, arts, and humanities (Elsevier, 2022).

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) is an evidence-based minimum for systematic reviews and meta-analyses reporting. PRISMA primarily focus on reporting studies evaluating interventions' effects or writing systematic reviews with other objectives (Page et al., 2021). PRISMA provides flow diagrams for better visualisation of a systematic review. The flow diagram represents the flow of information through various phases of a systematic review (Landford, 2022). The PRISMA extension for scoping reviews was published in 2018.

This thesis is inspired by the PRISMA framework, but only the first step in the flow chart (Figure 2) is used for all searches in this study to answer research aim one. A more detailed PRISMA chart is provided for two of the searches (Figure 4 & Figure 5).

2.1.2 Data sources and search history

An SLR aims to find as many studies related to the research questions as possible (Sheuly, 2013). A search strategy is applied to proceed with the systematic review process, shown in Figure 1 below.

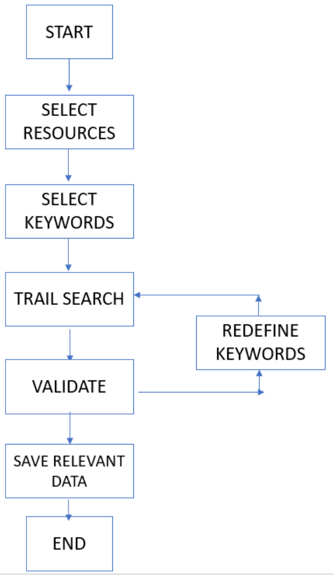


Figure 1: Search strategy, adapted by Sheuly. S. (2013)

This search strategy was applied using the Scopus database. The search results are affected by the database and the keyword used in the searches. An initial search is completed to get an overall idea about the number of studies. This search was moderated by introducing more relevant keywords and searching again. The search term combinations were used to get an overview of the literature including bioleaching of the metals copper, cobalt and lithium, biosorption of lithium and metal recovery from secondary sources such as lithium-ion batteries and printed circuit boards. Scopus provides an overview of the search history, which can easily be extracted to reference applications such as Endnote, Zotero or Rayaan. The search history shows which keywords you have searched for, the combination of keywords and how many hits each search has returned. Table 1 below shows the primary search without any exclusion criteria.

Table 1: Overview of searched keywords on Scopus with results.

Search #	Date	Search term // combination	Results
1	14/12/2022	“Bioleaching AND Cobalt”	313
2	14/12/2022	“Bioleaching AND Lithium”	106
3	16/12/2022	“Bioleaching AND Copper”	2033
4	13/01/2023	“Biosorption” AND “Lithium”	31
5	26/02/2023	“Metal reclaiming”	3
6	15/05/2023	“Metal recovery” AND “Lithium-ion batteries”	585
7	15/05/2023	“Metal recovery” AND “Printed circuit boards”	698
			Total: 3769

2.1.3 Study inclusion criteria

The following inclusion criteria were chosen; 1) Peer-reviewed studies related to the research questions 2) The article’s full text is available 3) Article was published in an indexed journal. 4) Written in the English language.

The following exclusion criteria were chosen: 1) Articles in a language besides English. 2) Documents other than articles and reviews. 3) Duplicates.

Table 2 provides an overview of the published studies based on keywords with exclusion and inclusion criteria.

Table 2: Overview of searched keywords with exclusion and inclusion criteria

Search #	Date	Search term // combination	Results
1	17/12/2022	"Bioleaching AND Cobalt"	238
2	17/12/2022	"Bioleaching AND Lithium"	89
3	17/12/2022	"Bioleaching AND Copper"	1429
4	13/01/2023	"Biosorption" AND "Lithium"	27
5	26/02/2023	"Metal reclaiming"	2
6	15/05/2023	"Metal recovery" AND "Lithium-ion batteries"	513
7	15/05/2023	"Metal recovery" AND "Printed circuit boards"	646
			Total: 2944

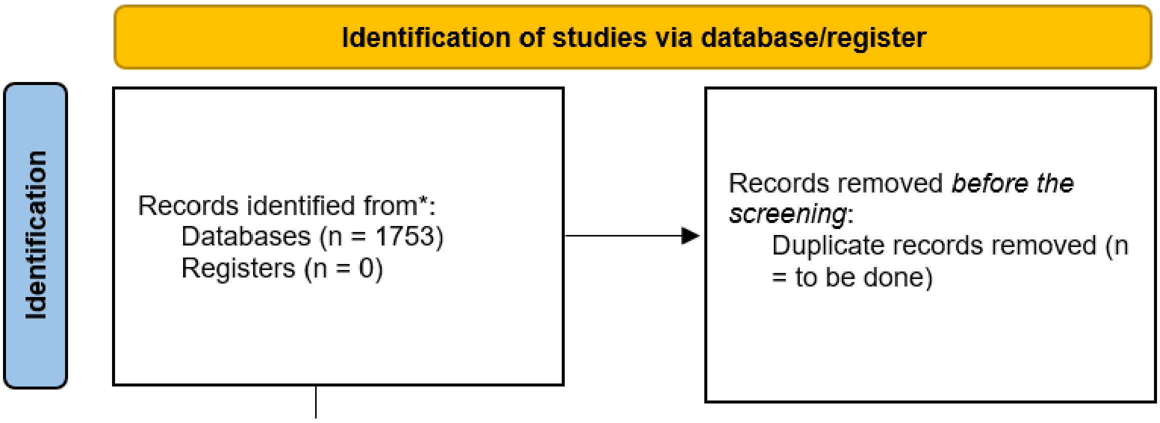


Figure 2: Flow chart inspired by the PRISMA Flow Diagram 2020 (Page et al., 2021).

Figure 2 illustrates the first step in the PRISMA flow diagram of the findings from the number of records identified from the Scopus database. This identification step was done for all seven searches in Table 2. The visual representation in Figure 3 displays the records identified from Scopus for the seven initial searches (Table 1), along with the definitive studies that were included after removing irrelevant records. To further investigate, a systematic literature review could provide a more thorough understanding of the literature. By employing the PRISMA checklist, an evaluation of each article would be assessed to ensure its quality and relevance to the subject matter. This would also provide more accurate quality assurance of included records (Page et al., 2021). However, this type of review was not performed in this thesis.

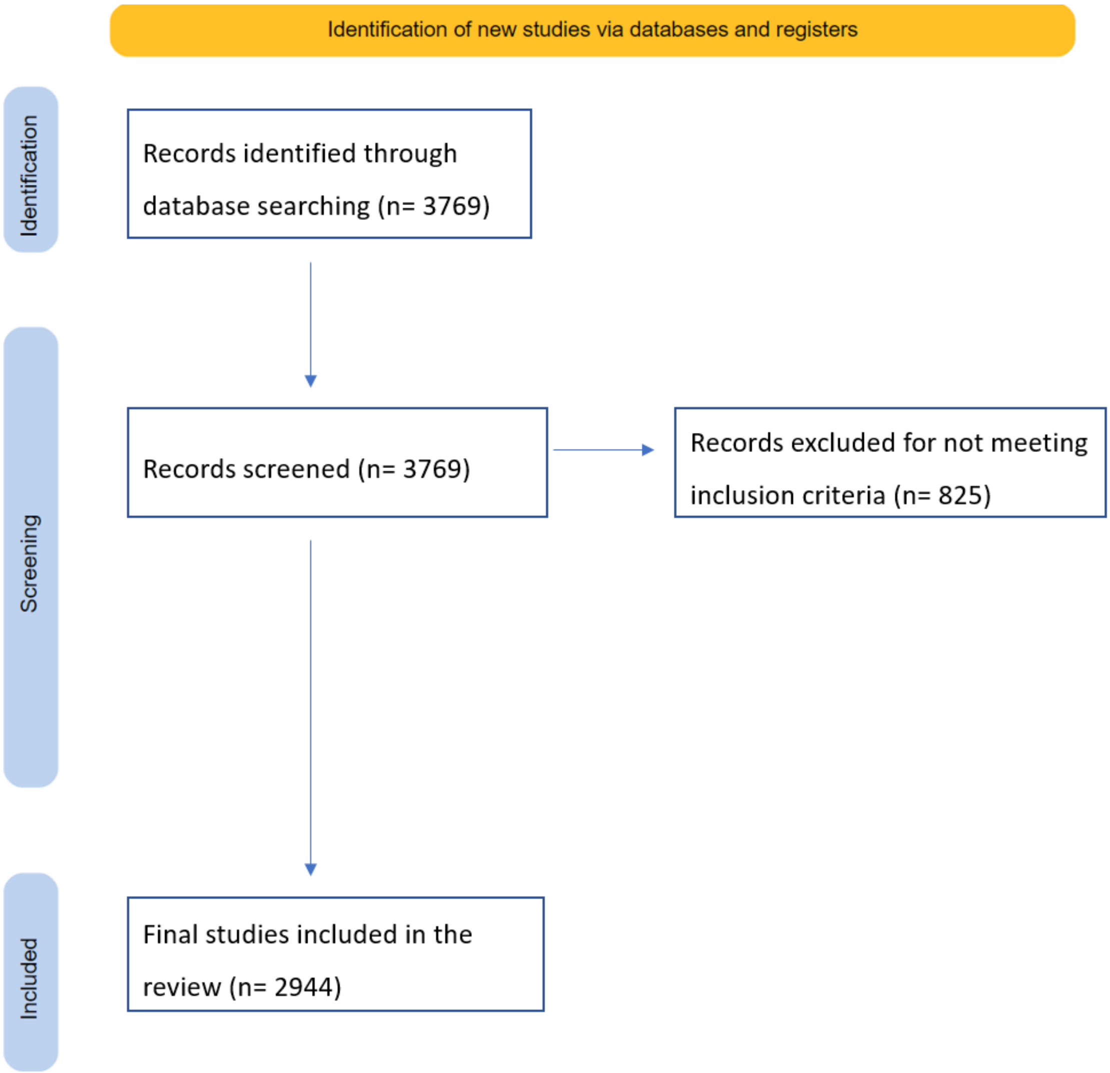


Figure 3: Inspired by the PRISMA flow diagram, a representation of initial records identified and final studies included in the review.

In order to delve deeper into research aim one, a PRISMA flow diagram was produced for a scoping review of two searches conducted in the Scopus database. The initial search combination selected was "bioleaching *and* lithium" due to bioleaching being a prevalent biotechnological method for metal retrieval. The goal of the research aim was to understand the existing literature on lithium recovery from lithium-ion batteries, and identify any knowledge gaps in this field of study.

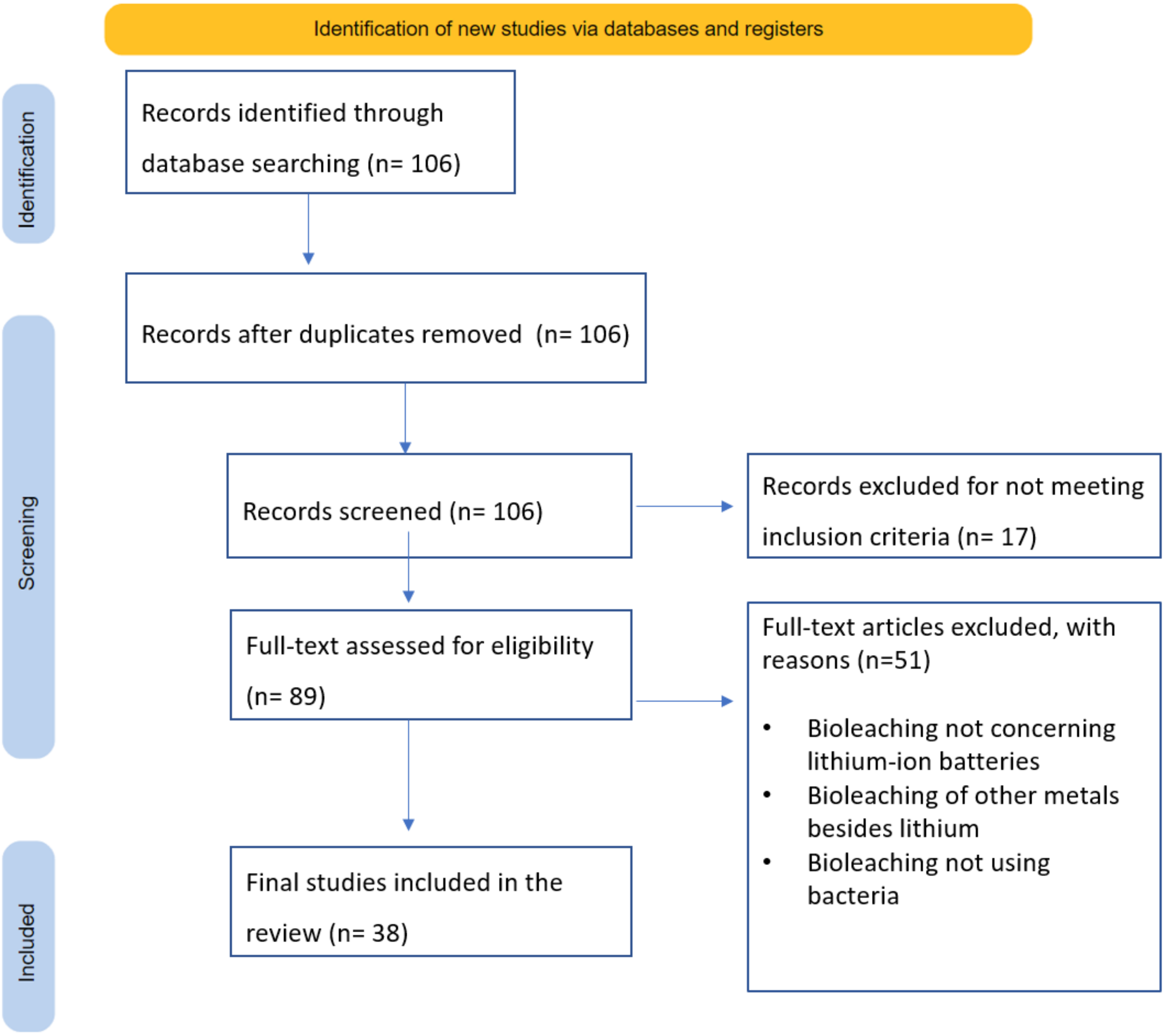


Figure 4: PRISMA flow diagram for the scoping review process on bioleaching and lithium.

Figure 4 illustrates the flow diagram for the scoping review process of the search term combination "bioleaching *and* lithium". Initially, 106 studies were found, and after the screening, 38 were included in this thesis by excluding records that did not meet the inclusion and other relevant criteria. The articles and reviews included in the final studies focus only on the biotechnology method of bioleaching to extract Li from LIBs.

Another biotechnology method investigated in this review was biosorption. The second search combination was, therefore "biosorption *and* lithium". The evaluation of this search is illustrated in Figure 5 below.

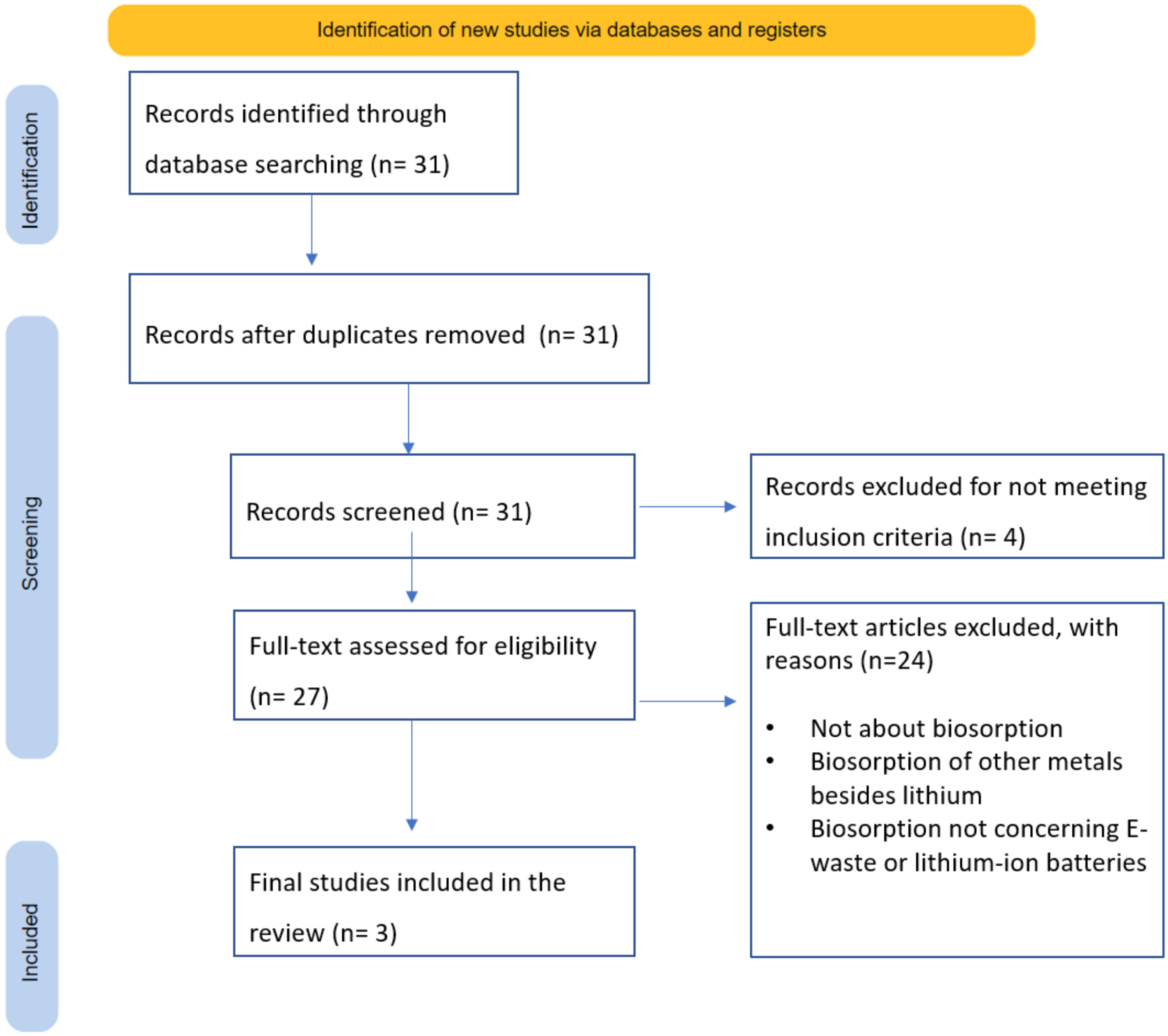


Figure 5: PRISMA flow diagram for the scoping review process for biosorption and lithium.

After an extensive search on Scopus, a total of 31 records were discovered. However, only three studies met the criteria after careful screening. By utilising a PRISMA chart, there is an apparent disparity in available literature between the two different types of microbial technologies for metal recovery. Moreover, since the investigation only included three reviews on metal recovery of lithium from LIBs, this finding underscores the urgent need for additional research in this area.

2.1.4 Published studies of bioleaching per year

The number of studies published on the bioleaching of lithium has increased from 2008 to 2022. Since the first studies on bioleaching of lithium in 2008, where there were only 2-3 per year, the number has doubled since 2017 and continues to increase towards 2022, as shown in Figure 6.

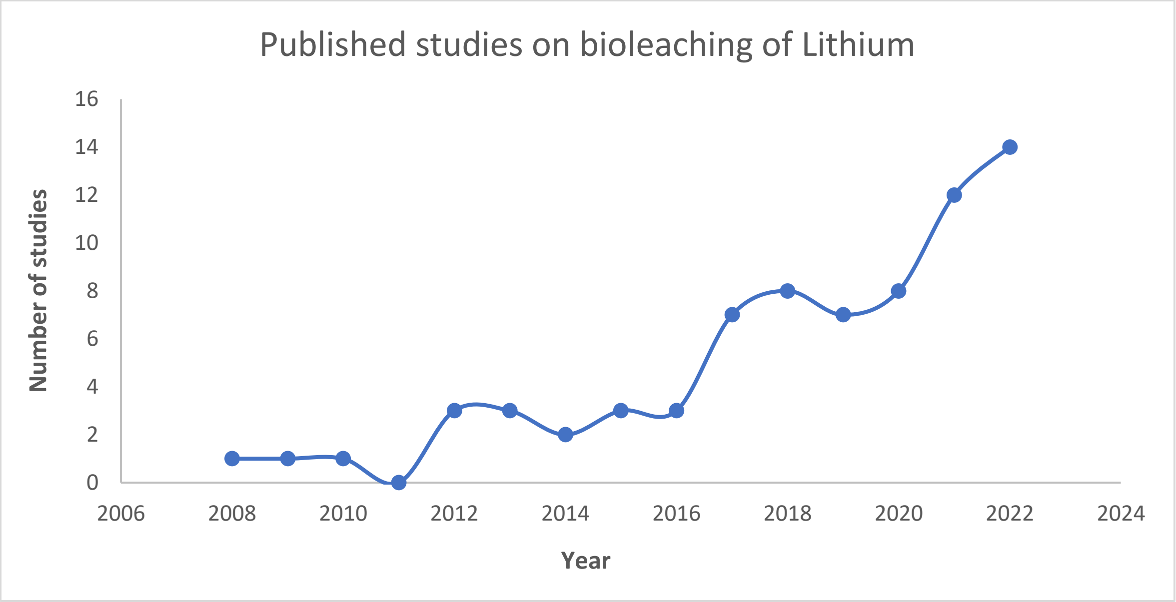


Figure 6: Published studies on bioleaching of Lithium per year. Source: Scopus

As with the bioleaching of Li, the number of articles published on cobalt bioleaching and copper bioleaching has also increased in recent years (Figures 7 and 8). The number of publications on cobalt bioleaching has increased significantly between 2018-2022.

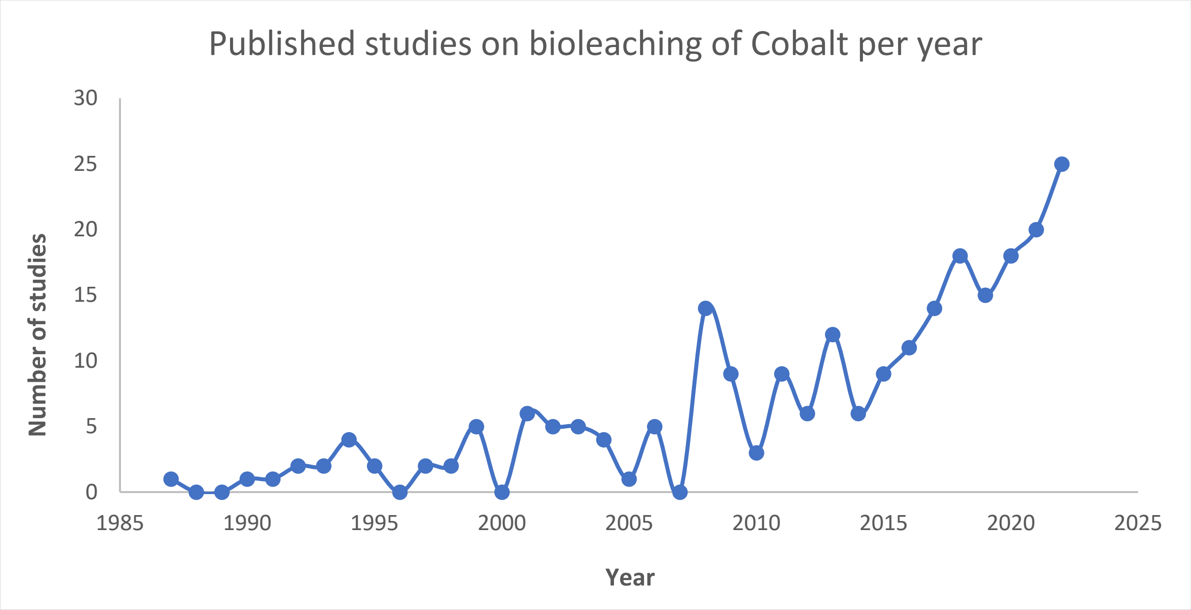


Figure 7: Published studies on bioleaching of Cobalt per year. Source: Scopus

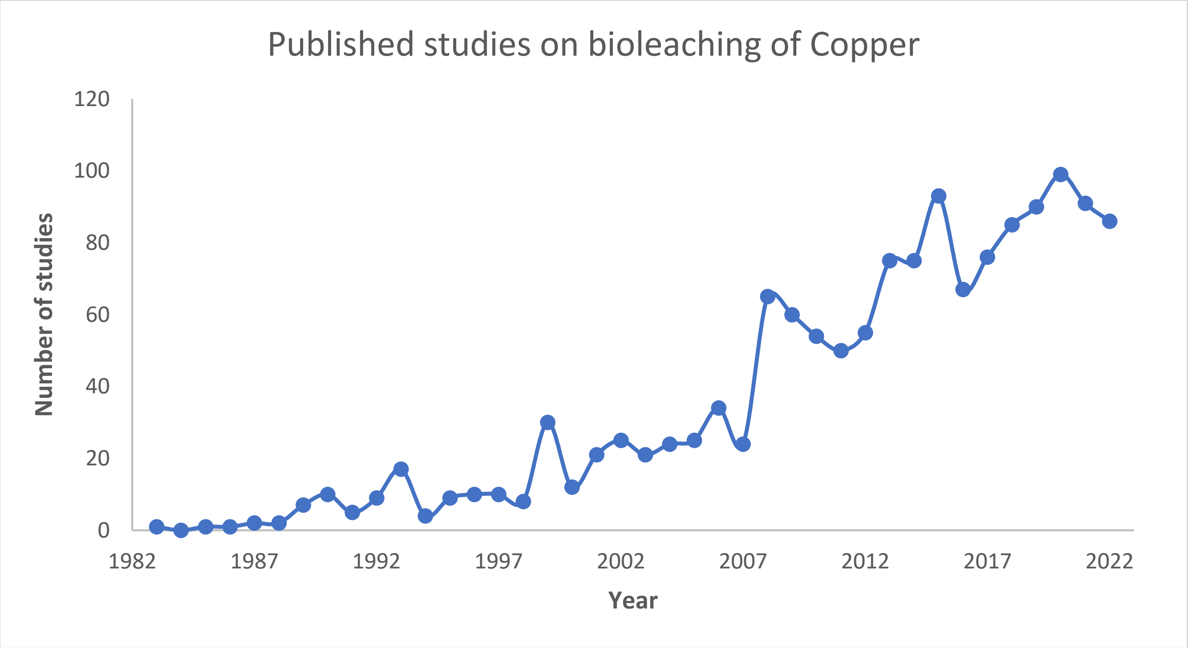


Figure 8: Published studies on bioleaching of Copper per year. Source: Scopus

Based on the primary search of bioleaching and the three metals, Copper has the most published studies, with 1429 articles. It should be noted that the search term combination "bioleaching *and* *type of metal*" includes bioleaching of both primary and secondary sources.

2.1.5 Additional research studies used in this thesis

Additional studies was identified and used through other sources such as Web of Science (ISI), Google Scholar and the digital library Oria of University of Stavanger. The search terms to obtain the literature for research aim two included among others; conventional recovery methods (“pyrometallurgy” and “hydrometallurgy” and “pretreatment”), metal recovery (“metal recovery of copper” and “metal recovery of lithium” and “metal recovery from E-waste”, “metal recovery from lithium-ion batteries”), green technology transition (“sustainable metal recycling” or “circular economy of metals” or “green transisiton regarding waste disposal”). Titles and abstracts of the articles and reviews were screened, followed by reading the relevant ones. Only publications in English were included.

3. Minerals and metals

The shift towards a more environmentally-friendly metal recovery approach is accelerating, encompassing climate, energy, and technology production with minimal intervention and consumption. Nevertheless, producing clean energy technologies with minimal pollution relies heavily on various minerals and metals, commonly called "green minerals". The demand for these materials is expected to rise in the coming years. In 2011, the European Commission (EC) created a list of critical materials, including several that are crucial for the production of electronic and electrical equipment (EEEs) (Table 3) (Işıldar et al., 2019).

Table 3: Overview of common metals with the following chemical symbols

Metal	**Chemical symbol**
Copper	Cu
Cobalt	Co
Lithium	Li
Nickel	Ni
Zinc	Zn
Chromium	Cr
Lead	Pb
Graphite	Graphitic C
Aluminum	Al
Manganese	Mn
Neodymium	Nd
Silver	Ag

Some non-renewable resources, like minerals and metals, can become renewable through recycling in the future. Heavy metals are not chemically nor biologically degraded, thus making them difficult to remove from the environment (Gan et al., 2016). However, this makes the metals available for extraction and recycling (Heldal et al., 2019). A scenario showing the need for specific metals in 2040 is illustrated in Figure 9 below. IEAs analysis is based on two scenarios, one is drawn from the *World Energy Outlook (WEO*) *sustainable development scenario* (SDS), and the other from the *stated policies scenario (STEPS)*. Comparison between the two scenarios indicates the range of possible scenarios in the future (IEA, 2021).

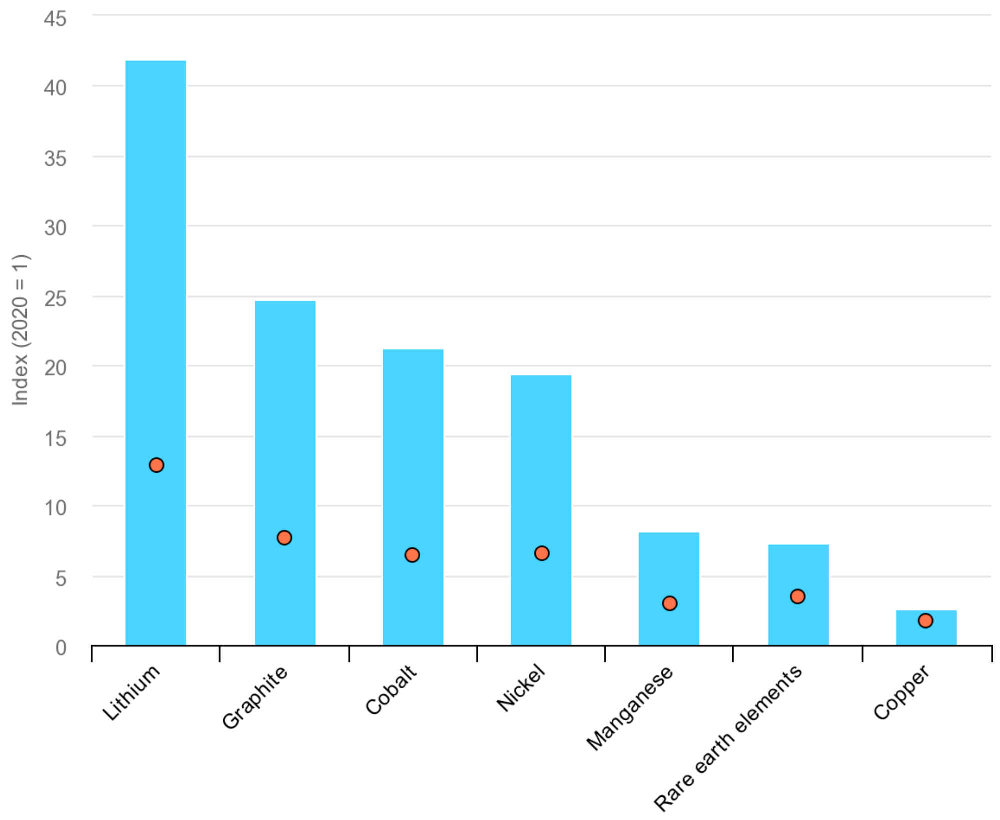


Figure 9: Growth demand for selected minerals by 2040. Blue represents the sustainable development scenario, while red represents the stated policies scenario (IEA, 2021).

Many metals and minerals with unique properties are essential in the electrification of the transport sector and are required in even more substantial quantities (IEA, 2021). EEEs contain a heterogeneous mixture of metals such as lithium (Li), copper (Cu) and cobalt (Co), summarised in Table 4 ((Dutta et al., 2023).

Table 4: Metals present in electric and electronic equipment, adapted from (Dutta et al., 2023)

E-waste	Metals
Printed circuit boards	Cu, Pb, Al, Zn, Ni, Fe, Sn, Sb, Au, Ag, Pd, Co
Lithium-ion batteries	Cu, Al, Co, Ni, Li, Ni, Mn
Telephones	Cu, Fe, Al, Ni, Pb, Ag, Au, Pd, In, Nd
Liquid crystal displays	Al, Zn, Cu, As, Mo, In, Sn, Fe
Lighting phosphorus	Sc, La, Ce, Nd, Pr, Y
Turbine	Y, Pr, Nd, Dy
Permanent magnets	Pr, Nd, Dy, Gd, Tb
Electric vehicles	Li, graphitic C, Co, Ni, V, Cu, Ti, Al, Mn, Ce, La, Nd, Y

The usage of mineral resources varies depending on the technology. Lithium, nickel, cobalt, manganese, and graphite enhance battery performance and durability. Copper is extensively utilised in all electricity-related technologies (IEA, 2021). Recycling LIBs has become a global concern because around 35% of Li and 25% of Co produced worldwide are utilised in producing LIBs (G. Mishra et al., 2022). Lithium-ion batteries are the most commonly used rechargeable batteries, and their demand is rapidly increasing due to the growth in the electric vehicle market (Kang et al., 2013; Roy et al., 2021). LIBs are essential in manufacturing electric vehicles and portable electronic devices (Rahimi, 2021). According to Swain (2017), by 2025, the utilisation of Li in LIBs for vehicles will account for 66% of the overall Li production. However, the present recycling rate of waste LIBs is merely 3%, with the recovery of Li being below 1%. IEA Sustainable Development Scenario has developed a scenario that meets the Paris Agreement, showing a 90% increase in demand for lithium, 60-70% for nickel and cobalt, and 40% for copper and rare earth elements over the next two decades. This is critical because electric cars use six times as many minerals as conventional cars (Figure 10) (IEA, 2021).

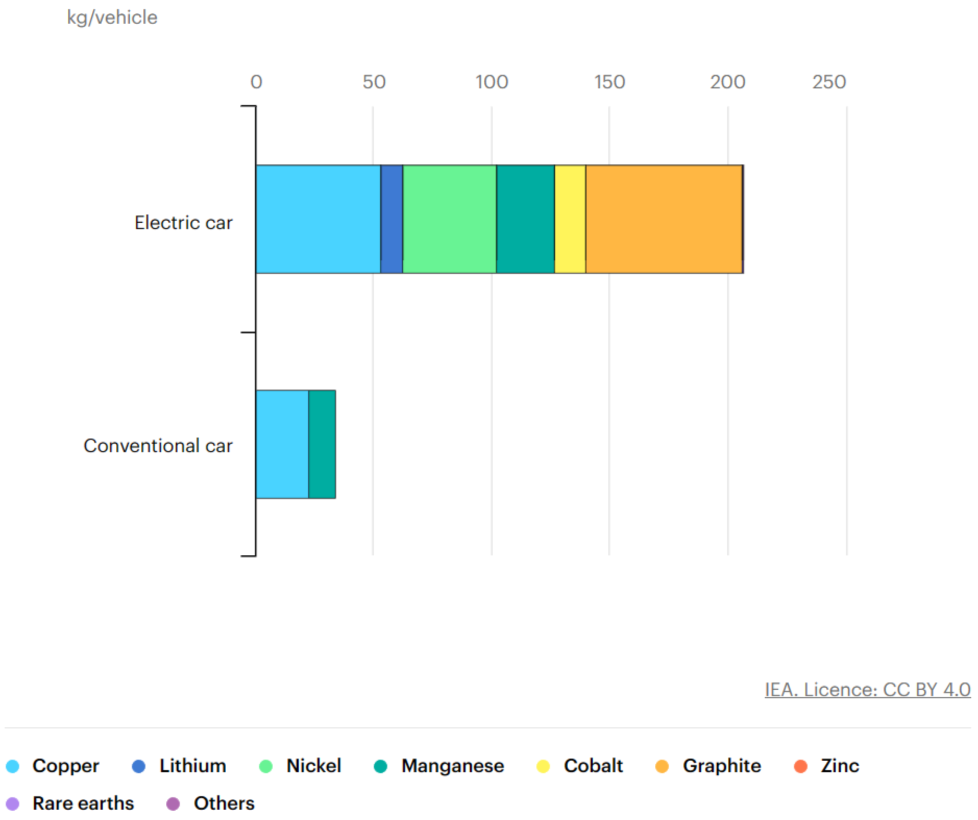


Figure 10: Minerals used in electric vs conventional cars (IEA, 2021).

The metals required to produce LIBs are summarised in Table 4, and the components of LIBs are illustrated in Figure 11 below (G. Mishra et al., 2022).

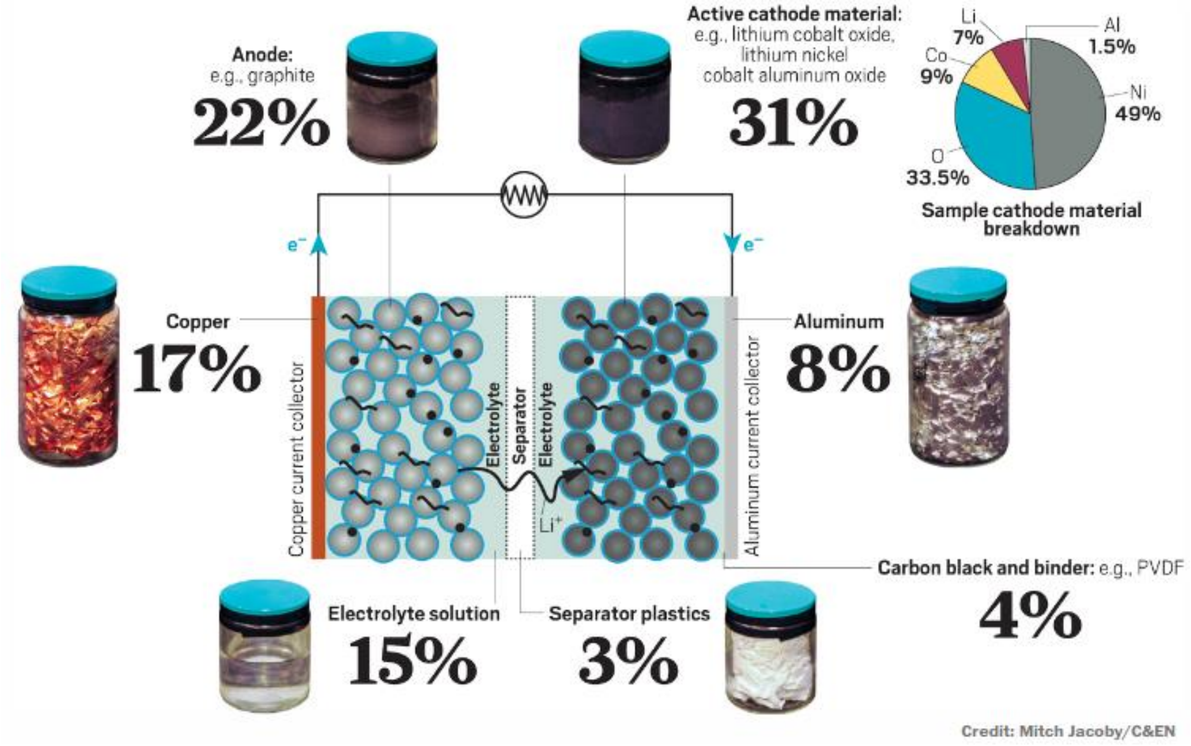


Figure 11: Main components of lithium-ion batteries (G. Mishra et al., 2022).

LIBs often end up in landfills where critical metals go to waste, and the chance of toxic materials leaching and contaminating water supplies and ecosystems increases (Anderson, 2020). Batteries that undergo recycling are usually melted down to extract their metals, which is carried out in extensive commercial facilities. Unfortunately, this process consumes a significant amount of energy and generates a considerable amount of CO_2 emissions. Despite the high cost of operating these facilities, they often fail to recover all the valuable materials within the batteries (Sebastian Farnaud, 2021).

The industry relies on battery wastes for recycled lithium (G. Mishra et al., 2022). Pyro- and hydrometallurgy is established methods for recovering critical metals like copper and cobalt, but lithium is often lost in the slag, as noted by Moazzam et al. (2021). However, these conventional metal recovery techniques could be more environmentally friendly. The solution could lie in biotechnology, which offers advantages in terms of cost-effectiveness and lower environmental impact (Işıldar et al., 2019). For a sustainable battery industry, economically viable technology is essential, with metal recovery playing a critical role (Roy et al., 2021). Nonetheless, there is a need for more information on the economic perspective of battery recycling (Yang et al., 2021).

3.1 Lithium

Lithium (Li) is a chemical element with the symbol Li and atomic number 3. It is a soft, silvery-white metal belonging to the alkali metal elements. Li is the lightest metal and the lightest solid element, with a density of only about half that of water. The chemical characteristics of Li are shown in Table 5 (Emsley, 2011). Lithium is not found freely in nature but in natural minerals, clays, brine, and seawater. 59% of global lithium resources are in mineral springs. Extracting lithium from brines is complicated and, together with minerals, considered a non-renewable primary resource that is slowly depleted through long-term exploitation (Moazzam et al., 2021). Li is essential for several reasons. One of its most common uses is rechargeable batteries used in telephones, computers and vehicles. In addition to its use in batteries, Li is also used in producing ceramics, glass and aluminium alloys (Emsley, 2011). Back in 2011, the usage of Li was primarily for ceramics and glass, accounting for 30% of its consumption. However, in the present time, about 60% of Li consumption is linked to the production of batteries (Bae & Kim, 2021).

Table 5: Chemical characteristics of lithium (Emsley, 2011).

Chemical characteristics	
Chemical symbol	Li
Atomic number	3
Atomic weight	6.941
Melting point	181℃
Boiling point	1,347℃
Density	0.53 g/cm^3
Oxide	Li_2O

3.2 Copper

Copper is a chemical element with the symbol Cu and atomic number 29. Table 6 defines the chemical characteristics of this element. It is a relatively soft and ductile metal used by humans for thousands of years. Unlike Li, which is a rare metal, Cu is the 26th most abundant element in the environment. Copper has a characteristic golden-orange colour and belongs to metal group 11, coinage metals. Cu is resistant to air and water but may be attacked by acids. Two well-known oxidation states of Cu are +1 as in Cu_2Cl_2 and +2 as in $CuCl_2$, where the latter is the most stable (Emsley, 2011). Due to its excellent electrical conductivity, one of its most well-known applications is in electrical wiring and conductors. Around 60% of Cu is used in electrical equipment and the production of various other products, such as pans, kettles, and coins. It is usually found in mineral ores such as chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4) and malachite ($Cu_2CO_3(OH)_2$), and is often extracted through smelting or leaching processes. Two main types of copper ore exist; copper sulphide and copper oxide (IEA, 2021).

Today, Cu is mined in more than 50 countries around the world. The largest producers of copper are Chile, Peru and China. Electricity production heavily relies on copper, which is considered a fundamental component of all electricity-related technologies (IEA, 2021).

Table 6: Chemical characteristics of copper (Emsley, 2011).

Chemical characteristics	
Chemical symbol	Cu
Atomic number	29
Atomic weight	63.546
Melting point	1.084℃
Boiling point	2.567℃
Density	9.0 g/cm^3
Oxide	Cu_2O and CuO

3.3 Cobalt

Cobalt has the chemical symbol Co, atomic number 27 and is a member of group 9 in the periodic table. Table 7Table 7 shows information on the chemical characteristics of cobalt. Most of the element is located in the Earth's core, but it can also be found in the crust and natural waters, where it has precipitated as cobalt sulfide (CoS). Cobalt is silvery-blue and is considered a relatively hard, ferromagnetic metal. It is stable in air and is not affected by water. Co is used to produce magnets, ceramics, catalysts, and paint. Another well-known application of Co is in the production of aircraft engines and gas turbines (Emsley, 2011). The production of electric vehicles requires Co, which makes up to 20% of the weight of the cathode in LIBs. Unfortunately, cobalt is considered the most risky material in the supply chain due to its availability (Office of Energy Efficiency & Renewable Energy, 2021). This is another reason why improved recovery methods are essential.

Table 7: Chemical characteristics of cobalt (Emsley, 2011).

Chemical characteristics	
Chemical symbol	Co
Atomic number	27
Atomic weight	58.93
Melting point	1.495℃
Boiling point	2.870℃
Density	8.9 g/cm^3
Oxide	CoO, Co_2O_3 and Co_3O_4

4. Conventional technologies for metal recovery

Recycling and recovering critical metals are a focus of the EU circular economy (CE) mission. Recovery of critical metals from electronic and electrical waste presents an opportunity to guarantee adequate supply levels, while reducing the negative environmental footprint. Many thermo-chemical and bio-chemical processes have been tested in pilot and laboratory scales for metal recovery from E-waste (Debnath et al., 2018). High-temperature pyrometallurgy processes are well established for the recovery of critical metals from EEEs, and other technologies, such as hydrometallurgy, have also been proven successful (Işıldar et al., 2019). Extensive research and many review papers regarding the different recycling technologies have been published. Unfortunately, few of these publications comprise all the E-waste technologies, their advantages, disadvantages and economic aspects (Dutta et al., 2023). In pursuing new methods, biotechnology shows excellent potential as an alternative to established practices. An example of this is bioleaching, which is a recognized technique for extracting metals from mineral ores. This method could become crucial in the future of urban mining for CRMs and valuable metals.

There are various conventional techniques for extracting metals from E-waste, such as pyrometallurgy, hydrometallurgy, and electrometallurgy. A hybrid of these methods has also been introduced to achieve maximum efficiency and save time. Pre-treatment is a crucial process that must be performed before selecting any combination of these techniques to ensure the highest metal recovery from E-waste (Dutta et al., 2023). Table 8 summarise recovery technologies and the metals they recycle.

Table 8: Overview of metal recovery technologies and the metals they recover

Metal recovery technology	Metals recovered	References
Pyrometallurgy	Au, Cu, Ag, Platinum group metals (PGMs), Ga, Base metals, Se, In, Pd, Ni, Zn, Pb, Cd, Ge	(Dutta et al., 2023)
	Li	(Bae & Kim, 2021)
	Cu, Co	(Moazzam et al., 2021)
Chlorinationmetallurgy	Pb, Cu, Zn, Au, Ag, Li, La, Nd, Ce, Ni	(Ge et al., 2022)
Hydrometallurgy	Pb. Cu, Co, Li, Ni, Sn, Au, Fe, Al, Zn, Ag, Y, Eu	(Dutta et al., 2023)
	Li	(Bae & Kim, 2021)
	Cu, Au, Zn, Ni	(Yaashikaa et al., 2022)
	Cu, Co	(Moazzam et al., 2021)
Pre-treatment	Cu	(Debnath et al., 2018)
Biometallurgy	Au, Ag, As, Co, Cu, Mn, Mo, Ni, U, V, W, Zn	(Işıldar et al., 2019)
	Cu, Ni, Zn, Cr (from PCBs)	(Yaashikaa et al., 2022)

4.1 Pre-Treatment

Pre-treatment is an important step for maximising the metal recovery from E-waste. Before the leaching process, enhancing the dissolution efficiency and reducing energy consumption is crucial (Dutta et al., 2023). The pre-treatment involves physical or mechanical techniques such as chopping, shredding, crushing etc. Following this, metal separation can be achieved through ferromagnetic or density separation processes.

The non-metals can be recovered using a combination of electrostatic and magnetic separation techniques (Debnath et al., 2018). The pre-treatment process for separating waste lithium-ion batteries is crucial for ensuring their safe discharge. After depleting the charge of lithium batteries, the battery may still have some power. LIBs contain various materials, so treating the batteries directly could be more efficient. Mechanical, solution- and calcination separation, can be used to pre-treat spent LIBs, as illustrated in Figure 12. Each has advantages and drawbacks, and a more scalable approach must be developed for commercial use (Bae & Kim, 2021).

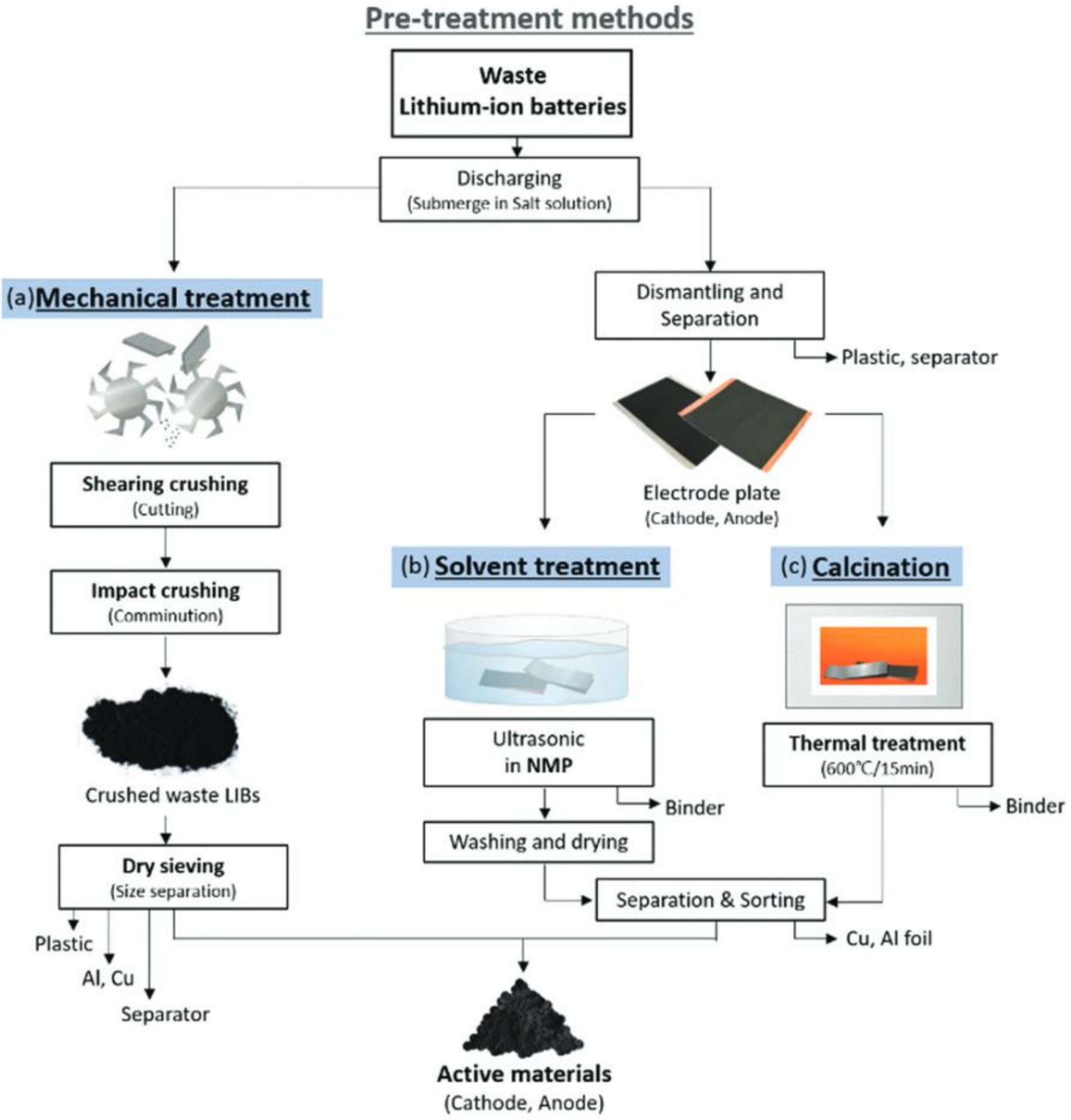


Figure 12: Pre-treatment methods of waste LIBs (Bae & Kim, 2021).

Printed circuit boards (PCBs) contain several valuable metals, and figuring out how to properly recycle the metals promotes the growth of a secondary resource economy. Conventional recovery methods such as hydrometallurgy have already successfully recovered metals from PCBs. However, the processes involve high capital and energy cost. A study by Moyo et al. (2020) investigates pre-treatment methods options to promote the leaching of metals. Shredding, milling and separation are typically used to extract the metals from the PCBs. Physical treatment methods are preferred over chemical methods due to their environmental advantages. Physical pre-treatment does not produce chemical changes in the components and does not generate liquid effluents. The study concludes that the metals can be effectively extracted with minimal environmental impacts by integrating pre-treatment technology, preferably physical methods. It should be noted, that at when the study was conducted, in 2020, there were no available studies in terms of energy costs, material loss and environmental impact reviews of these pre-treatment methods. Hence it is considered, that while some process technologies show promising results, there is a need for more research before concluding what type of pre-treatment is better (Moyo et al., 2020).

4.2 Pyrometallurgy

Pyrometallurgy involves heating E-waste in a combustion chamber at high temperatures ranging from 600-1200 °C. The process involves several steps including roasting, smelting, and chlorination. Valuable non-ferrous metals can be extracted from E-waste through a process called leaching, which allows for the recovery of pure desired metals using pyrometallurgy. This process can be challenging as it involves high temperatures to melt the desired metal before it can be condensed and recovered in the final step. Additional processing, such as hydrometallurgy, is sometimes necessary to obtain pure metals from E-waste (Dutta et al., 2023). Pyrometallurgy can be used as an extraction method to produce usable lithium compounds such as Li_2CO_3 and Li_3PO_4. When exposed to high temperatures, the cathode and anode undergo reactions that result in the solubility of lithium in water, allowing for the possibility of recycling. Specifically, temperatures exceeding 700 °C lead to the formation of Li_2CO_3 and metal oxides through the reaction of lithium metal oxide in the cathode and anode. The complete process is shown in Figure 13. To produce a Li_2CO_3 solution, the active material is first heated through calcination. After that, it undergoes water leaching, and then filtration. The last step involves evaporation, which results in obtaining Li_2CO_3 (Bae & Kim, 2021).

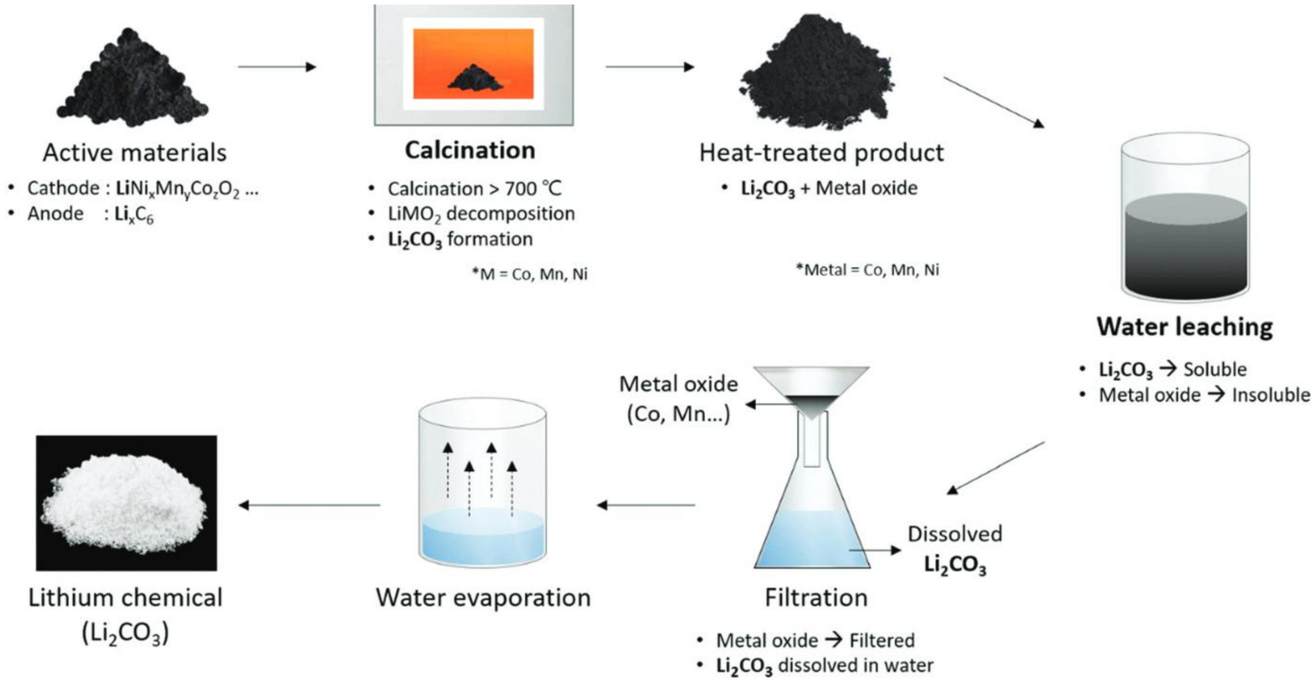


Figure 13: Lithium recycling from spent LIBs using pyrometallurgy (Bae & Kim, 2021).

One disadvantage of using pyrometallurgy for lithium recycling is that further steps are required after calcination. The process commonly involves dissolving the product in water or solvent and afterwards, the metal still needs to be separated. Due to the low solubility of Li_2CO_3, pyrometallurgy requires a considerable amount of solvent for the dissolution of Li. Another drawback is that the pyrometallurgy process uses complicated calcination equipment that may lead to the emission of harmful gases (Bae & Kim, 2021).

Chlorination metallurgy is also used in pyrometallurgy (Bae & Kim, 2021). Chlorination roasting is a method that utilises the characteristics of low melting and boiling point, high volatility and solubility of metal chlorine products for metal recovery. This method can be used to recover metals from various types of waste, including electronics. Huang et al. (2021) concluded that a leaching rate of Li (94%) may be obtained from waste batteries using the chlorination roasting method (Ge et al., 2022). Layered oxide $LiNi_xCo_yMn_zO_2$ (NCM) is a component in LIBs. Spent NCMs consist of metals such as lithium, cobalt, nickel, manganese, iron and copper, where lithium content may be as high as 5-7 wt.%. The chemical structure of NCMs is complex, making the recycling process more difficult. Studies of chlorination processes show great promise for Li recovery under optimal conditions. However, a drawback of this method is that the high content of ammonia produced in the process ultimately increases the environmental impact. The study by Huang et al. (2021) investigates whether chlorination can selectively extract Li without adding acid. In conclusion, the study was successful and may provide an alternative option for the sustainable recycling of spent NMC materials (Huang et al., 2021).

4.3 Hydrometallurgy

Hydrometallurgy involves using aqueous solutions with organic and inorganic acids for metal recovery. The steps often involve the leaching of metals in the solvent, followed by purification steps. There are several methods used in hydrometallurgy, such as ion exchange, solvent extraction, precipitation, cementation, absorption, and electrowinning. Hydrometallurgy is a cost-effective and efficient way of recovering metals. It consumes less energy, yields high metal purity, and is simpler to use compared to pyrometallurgy. However, one of its weaknesses is the use of acidic, alkaline, and flammable solvents, which can pose a challenge for proper disposal (Dutta et al., 2023).

Hydrometallurgy is recognised as one of the primary methods for recycling Li from LIBs (Ghassa et al., 2020). The pre-activated material contains Li ionised with acids and bases, followed by leaching to obtain Li^+ solutions from which Li can be removed. To increase the efficiency of leaching heat or redox reactions, H_2SO_3, NH_2OH and H_2O_2 are applied. The latter is often preferred for its affordability and lack of toxicity. There are certain disadvantages associated with using an acid with a low pH level, as it could release harmful gases like Cl_2 and NOx. Lithium compounds can be created using acids and bases for leaching, followed by precipitation, solvent extraction or selective adsorption, summarised in Figure 14 (Bae & Kim, 2021).

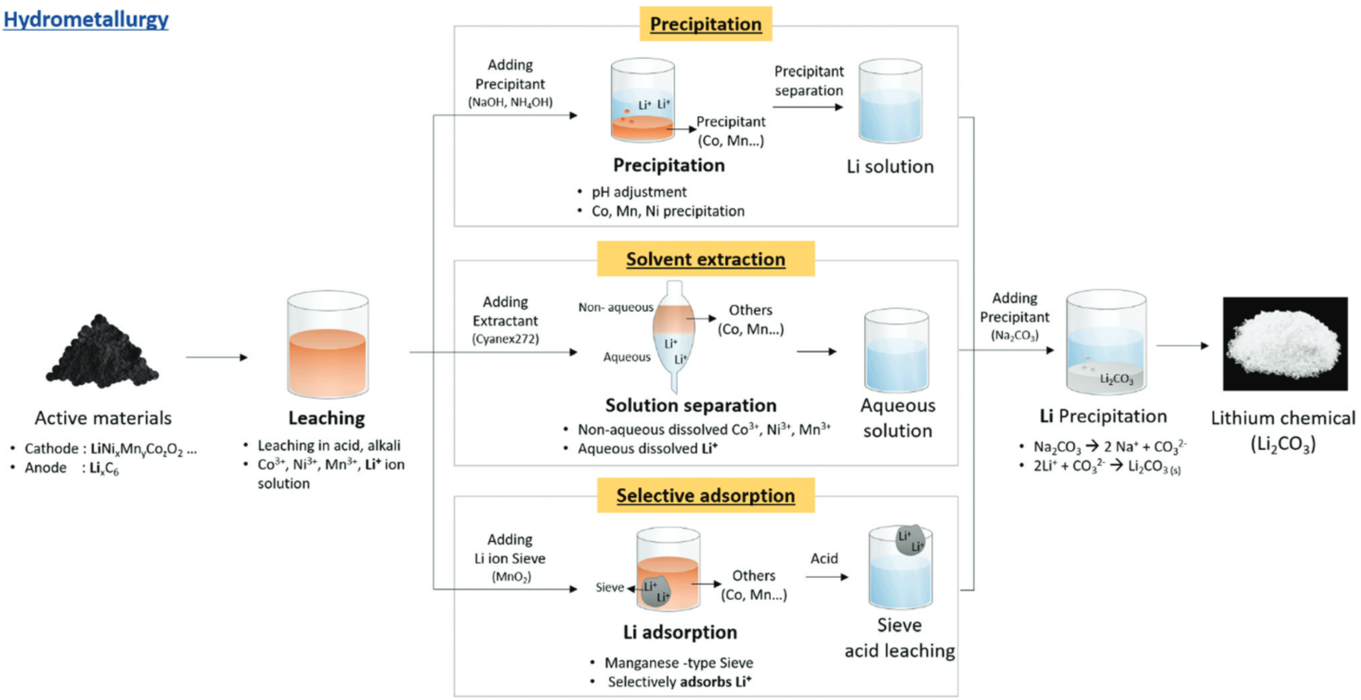


Figure 14: Lithium extraction from spent LIBs components using hydrometallurgy (Bae & Kim, 2021).

Conventional recovery technologies lack selectivity towards valuable metals, whereas biotechnology does not (Işıldar et al., 2019). How to selectively recycle lithium from spent LIBs is becoming an important area of research with increasing interest in this area, but there is still room for improvement. Even though hydrometallurgy is a recognised method for metal recycling, metal selectivity within this method is low. The purification process is often complex and involves many chemicals (Xiao et al., 2021).

The conventional technologies discussed above are not considered to be green technologies. The traditional physical and chemical techniques used to recycle E-waste have several limitations, such as producing harmful gases and toxic by-products. Therefore, other methods are required (Kaur et al., 2022).

5. Microbial technologies for the recovery of metals

Biological methods may represent a greener alternative to conventional method, which are not optimal, as discussed in the sections above. Utilising green biological technology can help address the drawbacks of conventional methods (Kaur et al., 2022).

The process of recovering metals through biological treatment technologies involves the conversion of metals from insoluble to soluble forms with the help of bacteria, archaea, fungi, or a combination of these microorganisms. This is then followed by the purification of the dissolved metal (Dutta et al., 2023). Microorganisms and their metabolites are essential in metal extraction (Dave, 2018). Microorganisms can interact with metals using many different mechanisms, such as bioleaching, biosorption and biomineralisation, some of which may be used as the basis of potential bioremediation strategies. Bacteria can oxidise or use the inorganic and organic substrate to convert the metal into its soluble form to extract it (Dutta et al., 2023). Through the solubilisation of metals, microorganisms can recycle and extract metals from tailings, ores, concentrates and E-waste. Microbial technologies work under mild conditions at ambient temperature and pressure, making them easier to regulate and maintain. Knowledge regarding metal extraction using biometallurgy technology originally came from the mining industry. Microorganisms were used in sulphide mines to recover the metal from low-grade sulphide ores (Dave, 2018).

Today there is increased academic and industrial interest in using bioprocessing for metal recovery, not only from mineral ores but also from secondary sources such as electrical equipment. There are several reasons behind the increased interest in bioprocessing. Using biotechnology for metal recovery offers several benefits, such as creating a cleaner environment, being a more affordable option, providing a more straightforward operation, and increasing the selectivity of metals. Microorganisms can be modified to target particular metals, resulting in highly effective extraction. By being selective, extensive downstream processing is reduced and the environmental impact of conventional recovery methods is minimised (Işıldar et al., 2019). Table 9 presents a comprehensive summary to microbial recovery technologies along with the metals they can extract. The table covers the most prevalent types of metals extracted by various microbial technologies. However, it's worth noting that there may be other metals that aren't included in the table.

Table 9: An overview of biological technology and metals recovered by them.

Microbial metal recovery technology	**Metals recovered**	**References**
Bioleaching	Cu, Co, Au	(Dave, 2018)
	Li	(Bae & Kim, 2021)
	Cu, Co	(Moazzam et al., 2021)
	Ni, Zn, U	(Rohwerder et al., 2003)
	Ag, Cd	(Yaashikaa et al., 2022)
	Co, Ni, Li	(Ghassa et al., 2020)
	Fe	(Kaur et al., 2022)
Heterotrophic bioleaching	Au, Ag, Pt, Pd, Rh, Ru, Go, Ga, Ge, Li, Sb, W	(Işıldar et al., 2019)
Acidolysis (bioleaching)	Al, Cu, Ni, Zn, Pb	(Dutta et al., 2023)
Redoxlysis (bioleaching)	Al, Cu, Ni, Zn, Pb	(Dutta et al., 2023)
Complexolysis (bioleaching)	Au, Ag, Pt	(Desmarais et al., 2020)
	Cu, Fe, Zn, Mg, Pd	(Dutta et al., 2023)
	Co, Li	(Sethurajan & Gaydardzhiev, 2021)
Biosorption	Cu, Fe	(Kaur et al., 2022)
	Pb, Ni, Cu	(Vijayaraghavan & Yun, 2008)
Biomineralisation	Pb	(Zhang et al., 2022)

There are various biological treatment technologies available, such as bioleaching, biosorption, and biomineralization. These technologies are illustrated in Figure 15 as the primary types of biological treatment options (blowes & Philp, 2005, pp. 293–317) & (Dutta et al., 2023).

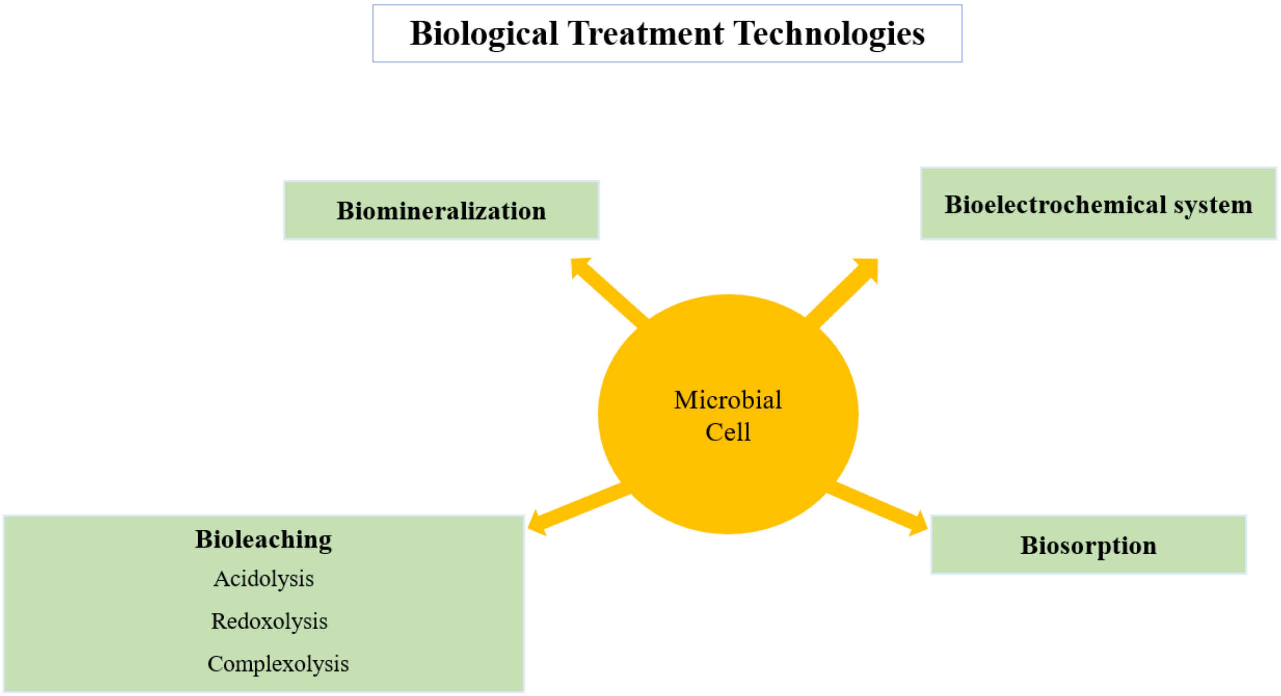


Figure 15: Mechanisms of metal-microbe interactions, adapted from (Dutta et al., 2023) and (Atlas & Philp, 2005).

5.1 Bioleaching

Bioleaching, a biometallurgy technique, is a method where microorganisms can transform solid compounds into soluble and extractable elements to be recovered (Srichandan et al., 2019). Bacterial metal leaching is not a newly discovered technology but a naturally occurring process known for hundreds of years (Krebs et al., 1997). Bioleaching was originally used for extracting metals from minerals and ores. It has been in use as a metal recovery technology for the last 20 to 30 years. Research on bioleaching from E-waste has recently increased, but the use for bioleaching in regards to E-waste has not been commercialised yet (Roy et al., 2021).

In this thesis, the first research aim showed that bioleaching is the most researched method within microbial metal recovery, with the most published studies on bioleaching copper. 1429 studies were found on the search term combination of "bioleaching *and* copper," while "bioleaching *and* cobalt" ranked second with 238 studies, and "bioleaching *and* lithium" had only 89. It is important to note that these studies encompass primary sources, such as ores, sewage, and wastewater, and secondary sources, like E-waste, LIBs, and PCBs.

Three different mechanisms have been reported for extracting valuable metals from ores and E-waste: acidolysis, redoxlysis and complexolysis (Dutta et al., 2023). Figure 16 summarises and illustrates the pathways involved in bioleaching from E-waste and low-grade ores (Sethurajan & Gaydardzhiev, 2021).

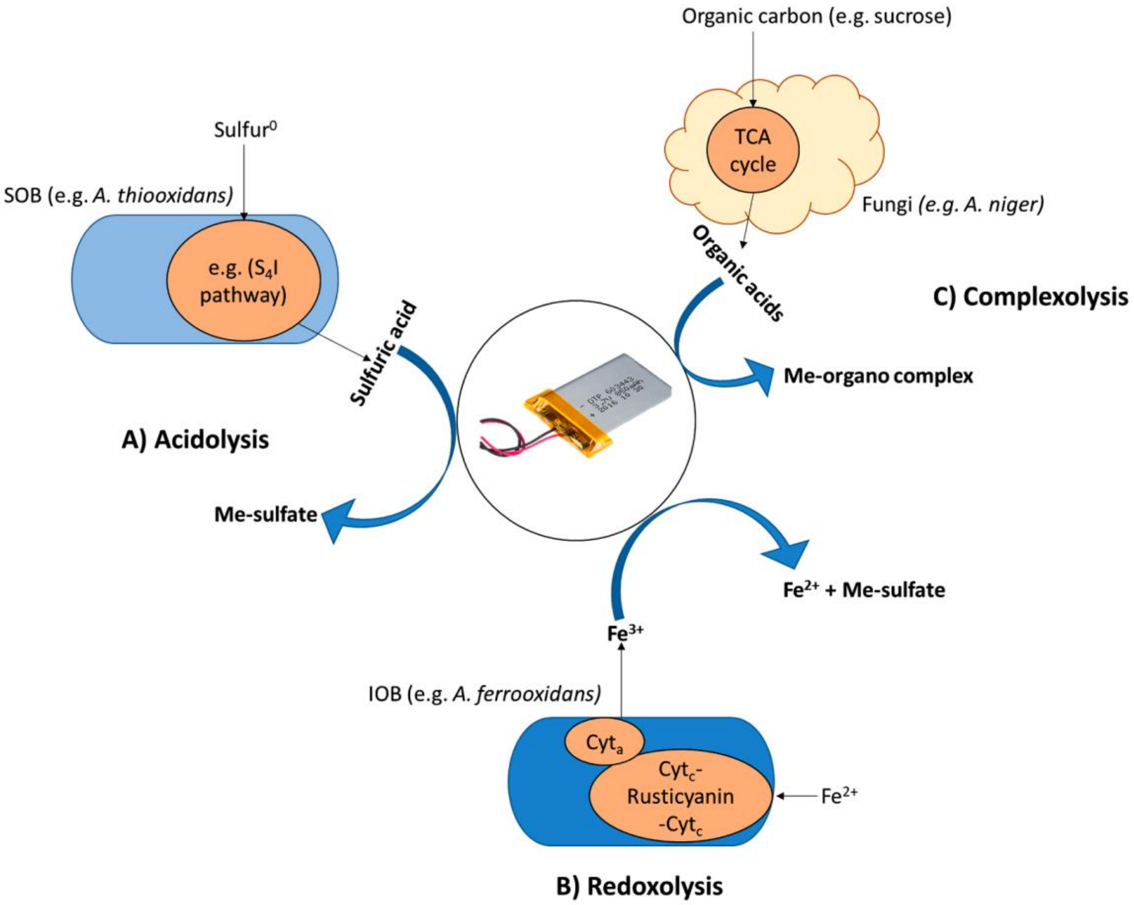


Figure 16: Three mechanisms involved in the bioleaching of metals from electronic and electric equipment waste and mineral ores (Sethurajan & Gaydardzhiev, 2021).

5.1.1 Acidolysis

Acidolysis is a pathway in bioleaching used to extract metals from ores and E-waste. In the process of acidolysis, metal is displaced from the source surface after the oxygen atoms are protonated (Moazzam et al., 2021). In ores, oxygen is present in the metal oxide leading to increased solubilisation. In this process, the metal sulphide ore is treated with an acid solution, generally sulphuric acid, formic acid, citric acid or pyruvic acid, leading to the growth of chemolithoautotrophic microorganisms (Desmarais et al., 2020). The acid is generated by the microorganisms, typically *Acidithiobacillus thiooxidans* (Sethurajan & Gaydardzhiev, 2021).

Metal sulphide minerals are oxidised by these bacteria and converted into metal sulphate compounds, which can easily be leached from the ore or E-waste (Dutta et al., 2023). *A. Thiooxidans* has the ability to use elemental sulphur to create biogenic sulphuric acid, which can effectively dissolve metals from target feed stocks (as shown in Figure 16) (Sethurajan & Gaydardzhiev, 2021).

The acidolysis process is particularly effective for extracting copper, zinc, and nickel from sulphide ores. The process can also extract gold and silver from sulphide ores, although the recovery rates for these metals are lower. In the field of bioleaching, acidolysis is a crucial technique with the capability to transform the mining industry. It offers a sustainable and eco-friendly approach to extract metals from ores. One advantage of acidolysis is that metal recovery can be achieved at relatively low temperatures and pressures, making it more energy-efficient and cost-effective than traditional mining techniques. In addition, the process produces fewer harmful by-products and waste materials, making it more environmentally friendly (Dutta et al., 2023). Lithium from spent LIBs is mainly recovered through acidolysis-mediated bioleaching (Sethurajan & Gaydardzhiev, 2021).

The acidolysis mediated bioleaching can be illustrated by the equations (1 & 2) below (Sethurajan & Gaydardzhiev, 2021).

$$2\ S^0 + 3\ O_2 + 2\ H_2O \xrightarrow{At.thiooxidans} 2\ H_2SO_4 \qquad (1)$$

$$2M^0_{(S)} + 2H_2SO_4 + O_2 \rightarrow 2M^{2+}_{(aq)} + 2SO_4^{2-} + 2H_2O \qquad (2)$$

5.1.2 Redoxlysis

Redoxlysis involves microorganisms oxidising and reducing metal ions to facilitate their extraction. The process is based on the principle of redox reactions, where one chemical species is oxidised while another is reduced (Dutta et al., 2023). Redox reactions of this nature typically take place in an acidic environment and their rate of mobilisation is contingent upon the metal's oxidation state and type (Moazzam et al., 2021)

In redoxlysis, microorganisms such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans and Sulfobacillus thermosulfooxidans* are used to oxidise the metal sulphide minerals present in the ores (Dutta et al., 2023). Bacteria reduce the metal ions produced due to the oxidation reaction. Through the use of extracellular polymers (EPS) and biofilm formation, bacteria are able to attach to mineral surfaces. This process facilitates the transfer of electrons from solid feedstock to microorganisms, resulting in the dissolution of metals, as illustrated in Figure 16. Redoxlysis can be achieved through the oxidation of ferrous (Fe^{2+}) to ferric ions (Fe^{3+}) (as shown in equation 3), which results in metal solubilisation (Sethurajan & Gaydardzhiev, 2021).

$$2\ Fe^{2+} + 0.5\ O_2 + 2\ H^+ \xrightarrow{At.\ ferrooxidans} 2\ Fe^{3+} + H_2O \qquad (3)$$

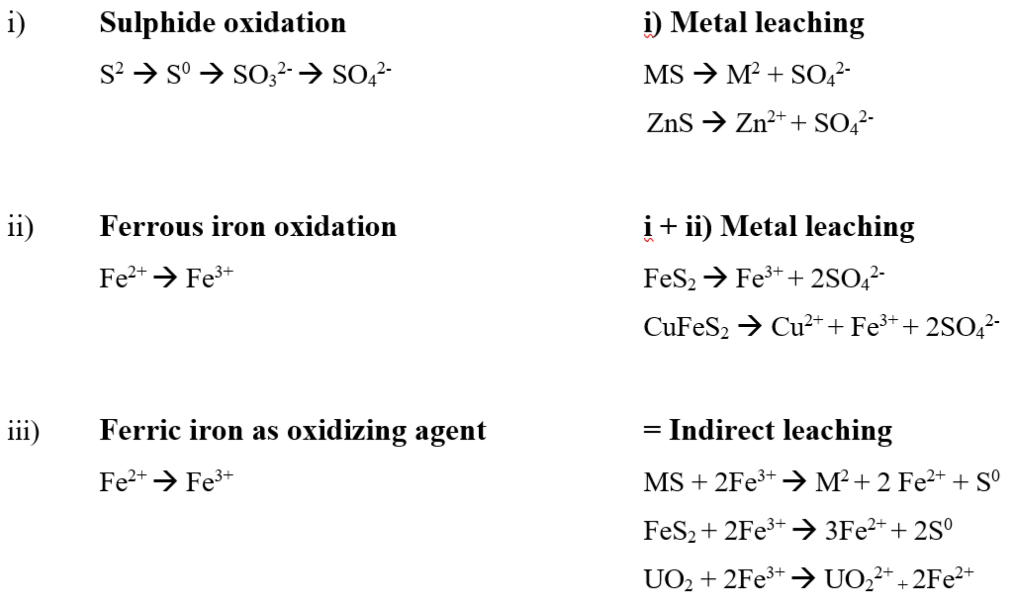
Microorganisms in mineral ores can utilise electrons produced during oxidation to reduce metal ions to their metallic form, enabling their extraction. This process also provides energy for the microorganisms to grow. Redoxlysis is particularly effective for extracting copper, zinc, and iron from sulphide ores (Desmarais et al., 2020). Redoxlysis bioleaching generally drives Co solubilisation from lithium-ion batteries ((Wu et al. 2019): Sethurajan & Gaydardzhiev, 2021)

5.1.3 Complexolysis

The complexolysis process involves forming a complex between organic acid and metal ions. The metabolites produced from protein catabolism, specifically amino acids, can facilitate this process. Alkaline leaching is the term used to describe this type of solubilisation, which occurs at higher pH values (Moazzam et al., 2021). Complexolysis, also known as “ligand-induced solubilisation, " involves processes where metal-ligand complexes and chelates forms the release of metal ions in the solution. Molecules like siderophores, long-side organic acids, and cyanides are specific ligands responsible for solubilisation. After acidolysis, complexolysis stabilises the metal ions. Microorganisms involved in metal extraction through complexolysis include *Bacillus megaterium, Chromobacterium violaceum, Pseudomonas fluorescens,* and *Pseudomonas aeruginosa* (Dutta et al., 2023). Specific metals extracted through this process are described in Table 9. Studies show that Co and Li can be bioleached from LIBs using complexolysis-based fungal bioleaching. Figure 16 illustrates the production of a soluble metal-organic complex through chelation reaction between metal ions and secondary metabolites created by microorganisms (Sethurajan & Gaydardzhiev, 2021).

5.1.4 Bioleaching from mineral ores compared to electronic and electrical waste

Bioleaching is commonly used in mining to extract valuable minerals present in ores. Mineral solubilisation is typically accomplished by bacterial oxidation as mentioned above. Microorganisms can convert metals by redox processes (e.g., Fe and Mn) or alkylation (e.g., Hg). Bacteria can also take up and accumulate metals by metabolism-dependent or independent methods. Both processes may occur in the same organism (Ledin, 2000). The chemical reactions involved in metal oxidation from ores are shown in Figure 17 (Suzuki, 2001).



i) **Sulphide oxidation**

$S^2 \rightarrow S^0 \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$

i) Metal leaching

$MS \rightarrow M^2 + SO_4^{2-}$

$ZnS \rightarrow Zn^{2+} + SO_4^{2-}$

ii) **Ferrous iron oxidation**

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

i + ii) Metal leaching

$FeS_2 \rightarrow Fe^{3+} + 2SO_4^{2-}$

$CuFeS_2 \rightarrow Cu^{2+} + Fe^{3+} + 2SO_4^{2-}$

iii) **Ferric iron as oxidizing agent**

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

= Indirect leaching

$MS + 2Fe^{3+} \rightarrow M^2 + 2\,Fe^{2+} + S^0$

$FeS_2 + 2Fe^{3+} \rightarrow 3Fe^{2+} + 2S^0$

$UO_2 + 2Fe^{3+} \rightarrow UO_2^{2+} + 2Fe^{2+}$

Figure 17: Chemical reactions by bacterial oxidation of metals (Suzuki, 2001).

Bioleaching of metals may be accomplished by large, diverse groups of microorganisms comprised of three main groups i) chemolithotrophic prokaryotes like *A. ferrooxidans*, *A. thiooxidans*, *L. ferrooxidans* and *S. thermosulfidooxidans*, ii) heterotrophic bacteria such as *C. violaceum*, *P. flourescens* and *P. aeruginosa* and iii) fungi (Işıldar et al., 2019).

Acidophilic sulphur-oxidising and iron-oxidising bacteria are the most effective microorganisms for extracting metals (Krebs et al., 1997). Iron-oxidising *Acidithiobacillus ferrooxidans* and sulphur-oxidising *Acidithiobacillus thiooxidans* are the two most common species to extract metals. The bacteria produce ferric iron and sulphuric acid, which are required for bioleaching reactions (Rawlings, 2005). Chemolithotrophic and acidophilic bacteria solubilise metals in sulphides and oxides in ores or secondary resources into a leaching medium (water) as metal cations. Processes such as solvent extraction, adsorption, ion exchange and membrane separation can purify the metal cations (Srichandan et al., 2019).

Bacteria grow attached to the surface of mineral sulphides. How bacteria can detect the attachment sites on mineral surfaces is still unknown to scientists and will require more focus in the future (Vera et al., 2022). The electrochemical processes at the interface between the cell wall and the sulphide surface dissolve sulphide minerals (Xin et al., 2009).

Solid wastes of secondary resources include slag, sludge-containing metals, fly ash, electronic waste, and refinery catalyst, among others. These heavy minerals, like molybdenum, nickel, copper, cobalt, and lead, are environmentally hazardous. Some heavy metals are toxic, even in small quantities. Thus, treating these wastes is required to avoid damage to the environment. Bioleaching is an alternative to address the problem of metals in industrial waste management (Srichandan et al., 2019).

Research has been conducted on extracting metals from E-waste using autotrophic microorganisms such as sulphur and iron-oxidisers and heterotrophic microorganisms that produce cyanide. On the other hand, primary sulphidic ores have different metal chemistry and require different leaching mechanisms, as illustrated in Figure 18 (Işıldar et al., 2019).

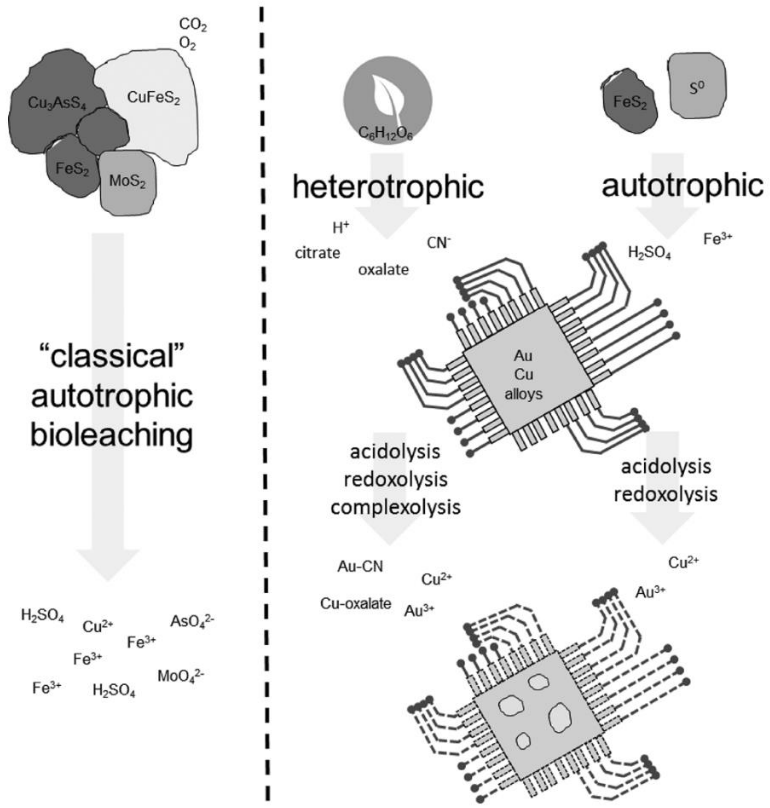


Figure 18: Classical bioleaching of primary ores versus bioleaching of secondary raw materials (Işıldar et al., 2019.)

Autotrophic bioleaching of secondary raw materials may be confusing because autotrophs do not grow directly via oxidation/dissolution of the EEEs matrix. However, when mixed with the E-waste substrate, sulphide minerals such as pyrite can provide energy for autotrophic growth. Mining activities are associated with a major environmental concern known as acid mine drainage. This problem is especially prevalent in areas where sulphide minerals, such as pyrite

FeS_2, are exposed to air and water during mining operations. The contact between these minerals and oxygen and water leads to the formation of sulphuric acid (chan et al., 2003). Like natural autotrophic leaching, microorganisms facilitate the oxidation of sulphide minerals, producing acid and ferric ions that aid in the solubilisation of metals from waste. Where autotrophic bioleaching from sulphur ores eventually will eventually lead to the matrix's dissolution, the non-metallic fraction of EEE will not dissolve. Due to different chemistry, the methods behind autotrophic bioleaching cannot be directly applied to WEEEs bioleaching. This is an important difference between classical bioleaching and bioleaching of secondary sources, such as E-waste. Research on chemolithotrophic autotrophic bioleaching from E-waste using acids produced by iron- and sulphur oxidisers is limited (Işıldar et al., 2019).

Organic acids are produced by heterotrophic bacteria, which extract metals from solids by altering their acidity level. This bioleaching technique is referred to as heterotrophic bioleaching. The term “heterotrophic bioleaching” can be misleading because the microorganisms are classified as heterotrophs. However, it is important to note that heterotrophic organisms do not solely drive bioleaching. A more accurate description would be “acid bioleaching by heterotrophs” (Vera et al., 2022). Heterotrophic bioleaching of metals from E-waste has primarily used cyanide and organic acid-generating microorganisms. Acidophilic bioleaching involves acidolysis and redoxlysis pathways, while the heterotrophic leaching of metals from LIBs is enhanced by acidolysis and complexolysis (Sethurajan & Gaydardzhiev, 2021). Cyanogenic bacteria like *Chromobacterium violeaceum, Pseudomonas fluorescens- and aeruginosa* are used to recover metals such as Au, Ag, Pt, Pd, Ti and Mo from E-waste through heterotrophic bioleaching processes. These bacteria can produce hydrocyanic acid (HCN) / cyanide ion (CN^-) as their secondary metabolite, which is essential for the dissolution of solid metals (Magoda & Mekuto, 2022). Rare earth element (REE) waste typically does not include metal sulphides, making heterotrophic microorganisms more suitable for bioleaching. In addition, they tolerate higher pH conditions and complex metals in the solution (Işıldar et al., 2019). Bioleaching kinetics depends on how the bacteria promote redox reactions, the metabolites produced by bacteria that are complex with metals and the bacteria’s ability to bind to metal substrates. The exact mechanisms of microbial metal extraction from ores and E-waste have still not been determined (Roy et al., 2021).

5.1.5 Bioleaching of metals from used lithium-ion batteries

Bioleaching has recovered valuable materials from electronic waste to protect the environment and achieve economic benefits. The Bioleaching Research Group at Coventry University discovered that all metals in electric vehicle batteries could be recovered using bioleaching (Sebastian Farnaud, 2021). Lithium-ion batteries are considered one of the most important categories of urban waste. Besides being used in electric vehicles, LIBs are widely used in other electronic devices, such as laptops and telephones. If not managed correctly, LIBs can severely impact the environment by releasing heavy metals and poisonous organic compounds (Ghassa et al., 2020). Bioleaching of LIBs intends to separate the metal components of the batteries into discrete fractions to reuse them (Roy et al., 2021). It is essential to implement additional measures to ensure the successful bioleaching of Li in LIBs. One crucial parameter that affects bioprocessing efficiency is particle size. Therefore, reducing particle size through physical pretreatment is a vital step. This reduction lowers the shear stress and increases mass transfer area, resulting in more efficient leaching. Typically, the average particle size of LIBs ranges from 75 µm to 130 µm (Sethurajan & Gaydardzhiev, 2021).

The literature review identified 38 relevant studies regarding this topic. Some relevant results from these studies are; The first research on using bioleaching to extract Li was conducted in 2008 by (D. Mishra et al., 2008). The investigation focused on the capacity of *Acidithiobacillus ferrooxidans*, a bacterium that oxidises iron and sulphur, to extract Co and Li from lithium-ion batteries. The findings demonstrated that this bacterium could potentially extract metals from these batteries. *Acidithiobacillus ferrooxidans* create sulphuric acid to indirectly leach metals from the LIBs. The outcomes showed that cobalt leaching occurred at a faster rate than lithium. Moreover, the presence of energy sources such as elemental sulphur and Fe(II) also increased the leaching process. The presence of bacteria increased cobalt leaching from 41 % to 65% (D. Mishra et al., 2008). Figure 19 below overviews essential chemical reactions during lithium bioleaching (Moazzam et al., 2021).

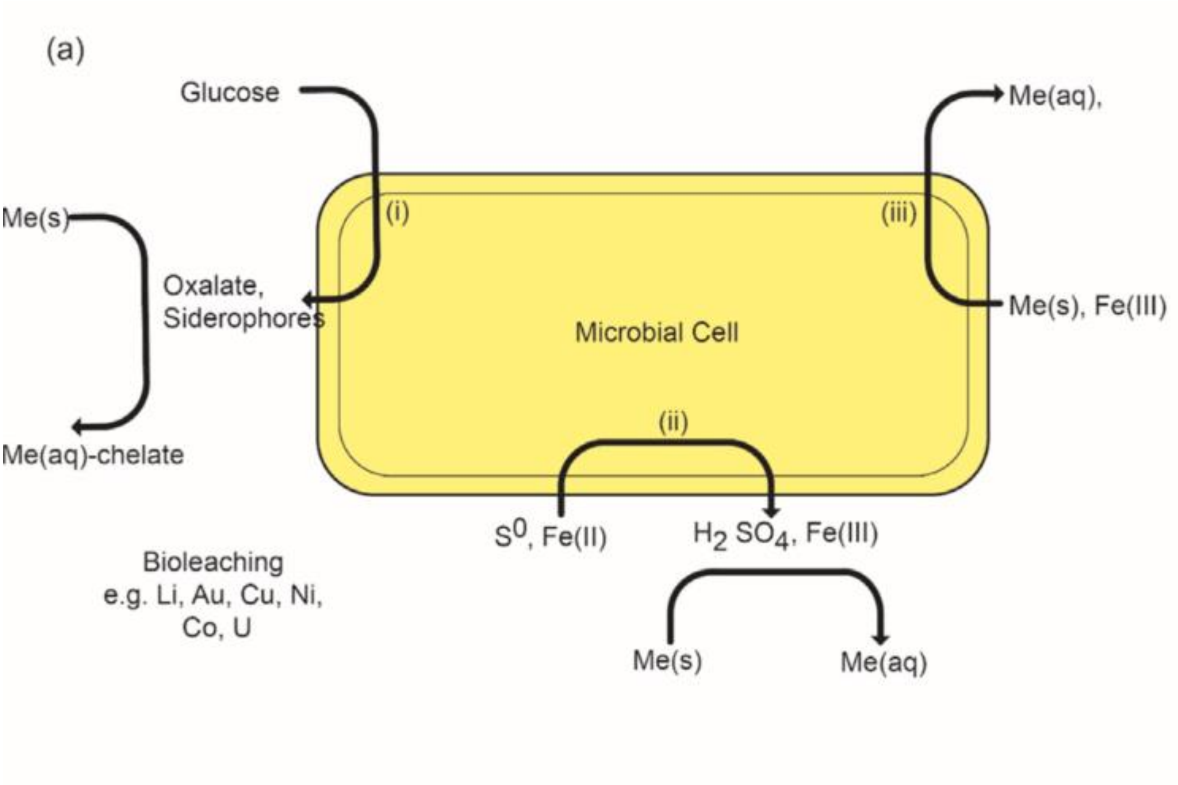


Figure 19: Bioleaching mechanisms involving chemical oxidation/reduction reactions of lithium etc. (Moazzam et al., 2021)

Bacterial leaching is classified into direct and indirect leaching, also known as contact and non-contact, as shown in Figure 20 (Moazzam et al., 2021). The classification is based on whether there is contact between the microorganisms and the waste (or mineral ores) (Sethurajan & Gaydardzhiev, 2021).

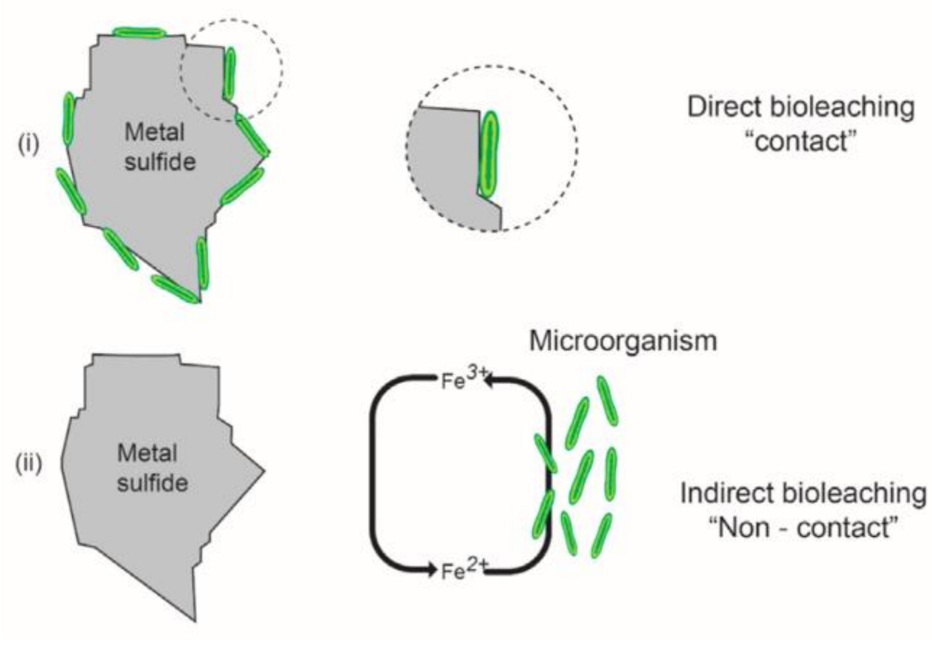


Figure 20: Direct and indirect bioleaching (Moazzam et al., 2021)

Direct leaching involves microbial oxidation of sulphide or reduced metals and transferring electrons to the cell attached to the mineral surface. Produced biochemical metabolites allow for indirect bioleaching by microorganisms, such as organic acids (Moazzam et al., 2021). Metal-oxides and hydroxides characterise batteries. Therefore, bioleaching mechanisms may differ from that with sulphides (Xin et al., 2009). In mineral ores, Fe^{3+} amplify local ferric iron concentration, which attacks the reduced form of metals and sulphur as an oxidising agent. In metal recycling from spent LIBs, Fe^{2+} plays a vital role in a similar process (Moazzam et al., 2021). The microbes can attach to the mineral surface in the batteries to oxidise ferrous ions to ferric ions to facilitate the leaching. The concentration of Fe^{2+} ions plays a crucial role in enhancing the bioleaching efficiency of cobalt from lithium-ion batteries (Sethurajan & Gaydardzhiev, 2021). A study of sulphur content in lithium recycling showed that Li leaching is higher when the amount of sulphur as the energy source is high in the medium (G. Mishra et al., 2022).

In biohydrometallurgy, some research indicates that direct bioleaching and attachment are critical in recycling metals from LIBs and PCBs. In research conducted by Silvia et al. (2015), they analysed the influence of microbe attachment to PCBs and LIBs. The study showed that the bioleaching efficiency of indirect bioleaching was 25% lower than a bioleaching system that allows for bacterial contact. Indirect bioleaching occurs when there is no contact between the bacteria and the waste. It should be noted that the bioleaching decreased for the following metals; Co, Ni and Mn, whereas Li bioleaching efficiency was not affected by the absence of bacterial contact (Sethurajan & Gaydardzhiev, 2021). Xin et al. (2009) examined the relationship between non-contact and contact bioleaching of Li and Co from waste mobile phones containing Li batteries. Bioleaching of Li from LIBs found in telephones and laptops is primarily based on non-contact mechanisms, while leaching of Co can be accomplished by contact and non-contact bioleaching. According to the findings, the bioleaching of Co and Li was unaffected by bacterial contact with the battery. This contradicts the discovery made by Silvia et al. (2015) concerning Co leaching, indicating the need for further research to better comprehend the relationship between bacterial contact and bioleaching efficiency. Regarding bioleaching, acidophiles utilise both acidolysis and redoxolysis, while heterotrophic bioleaching of LIBs is enhanced by acidolysis and complexolysis. More detailed research is needed to explain the difference in bioleaching mechanism and efficiency (Xin et al., 2009) (Sethurajan & Gaydardzhiev, 2021).

5.2 Biosorption

Biosorption removes pollutants that are not easily biodegradable from aquatic environments, such as metals and dyes. Bacteria, fungi and algae can bind to these pollutants, acting as biosorbents (Vijayaraghavan & Yun, 2008). Bacteria are effective biosorbents (Priya et al., 2022). Effective metal biosorbents bacteria include; *Bacillus, Pseudomonas and Streptomyces* (Vijayaraghavan & Yun, 2008). An overview of metal biosorption by various microorgansims is listed in Table 11.

The term biosorption describes the metabolism-independent sorption of metals to biomass. It encompasses adsorption, absorption and the accumulation of substances on a surface or interface (Atlas & Philp, 2005, pp. 293–317). The mechanisms behind biosorption can be one or a combination of the following ion exchange, complexation, coordination, absorption, adsorption, electrostatic interaction, chelation and microprecipitation (Vijayaraghavan & Yun, 2008). Both living and dead biomass is capable of biosorption, and ligands involved in metal binding include carboxyl, amine, hydroxyl, phosphate, and sulfhydryl groups (Atlas & Philp, 2005, pp. 293–317). Since biosorption regularly utilises dead biomass, this reduces the requirement for nutrient addition, and biosorption using dead biomass can be utilised in highly toxic environments (Das, 2010). There are advantages to using live biosorbents, such as their ability to transfer adsorbed heavy metals into cells, reduce toxicity, and remove heavy metals at a low concentration. However, there is a need for further research to compare the effectiveness of live and dead biosorbents (Hu et al., 2020).

Heavy metal sorption by microorganisms occurs in different steps. Depending on the type of microorganism, at least two-step mechanisms are proposed for metal accumulation. The first step involves the electrostatic interaction of metal ions with reactive groups that are accessible on the surface of the bacteria (Ledin, 2000). The bacterial cell wall is the first area where metal ions encounter the bacteria. Solutes can be deposited on the surface or within the cell wall structure. The chemical groups within the cell wall play a vital role in biosorption as the mode of uptake by cells is extracellular (Vijayaraghavan & Yun, 2008). The second step involves nucleating heavy metals and counter ions deposition to make the aggregate grow. The hydrated mineral aggregates may approach the mass of the bacterial cell and are usually shapeless due to the high degree of hydration. After a while, the water will deplete, becoming crystalline mineral phases over time. The cells further retain macromolecules produced by bacteria outside the cell wall that are known to bind metals (Ledin, 2000). Microbial biomass is a sink for metals and is thus helpful for metal recovery. The properties which make biomass useful for metal recovery are its ability of cell walls to bind metals and precipitate metals in and around the cell. Chemical groups such as OH, COOH, NH_2 etc., are attached to the metal ions during biosorption (Dutta et al., 2023). The surface of microorganisms differs from mineral surfaces due to its multiple reactive layers, each with a distinct structure and chemical composition. The ability of these biomaterials to absorb heavy metals is due to the presence of proteins, carbohydrates, and compounds containing chemical groups such as OH, COOH, and NH_2. Therefore, it is essential to identify the functional groups responsible for metal binding (Choi & Yun, 2006).

The biosorption process is primarily passive, as the biosorbent absorbs metals through electrostatic attachment, without requiring any energy costs (Sethurajan & Gaydardzhiev, 2021). Biosorption depends on the chemical composition of the biomass, solution chemistry and external physiochemical factors. Important biosorption factors affecting solution includes pH, temperature, ionic strength, biosorbents dosage, and agitation rate. Of these factors, pH is extremely important in the biosorption process as it affects the solution chemistry of metals and the activity of the functional groups in the biomass. Research has demonstrated that weakly acidic pH conditions result in maximum biosorption for most metal ions. This is because the carboxyl and other acidic groups bind metal cations through various mechanisms such as electrostatic attraction (Vijayaraghavan & Yun, 2008). Studies have shown that as the pH level rises to 5.0, there is an increase in the exposure of negatively charged functional groups. This results in the increase of attraction sites, allowing for the absorption of positively charged ions and thus, enhancing the biosorption capacity (Hu et al., 2020). A study by Golab and Breitenbach (1995) suggested that the carboxyl groups of the cell wall of *Streptomyces pilosus* were behind the binding of copper. On the contrary, forming metal hydroxide and other metal-ligand complexes reduces the biosorption of metal ions at high pH. Higher pH levels allow for precipitation, which may complicate the process. Biosorption of metal ions by bacterial biomass is most optimal at pH values between 3 and 6 (Vijayaraghavan & Yun, 2008).

The biosorption process includes both adsorption and absorption as mentioned above, which can be easily confused (Michalak et al., 2013). Adsorption refers to the surface process where molecules or particles are transferred from a fluid bulk to a solid surface without being absorbed into it. Usually this process is reversible (Artioli, 2008). The molecules are attracted to the material’s surface and stay on the solid surface due to chemical and physical bonding forces. These forces are called Van der Waals forces (Metcalf & Eddy, 2014). Adsorption is a vital mechanism in biosorption processes. The extent of adsorption depends on the characteristics of the biomass, such as age, surface area, porosity, solubility, and the type and number of functional groups (Derco et al., 2018).

Absorption is a process where the molecules or particles are taken up or dissolved within another substance. Absorption is not a commonly used mechanism in biosorption processes, as biosorption typically refers to a subcategory of adsorption (Michalak et al., 2013). However, in some cases, absorption may play a role in the overall biosorption process. In the context of biosorption, absorption can refer to the uptake of a substance into the interior of a biological material, such as the cytoplasm of a bacterial cell, by microbial biomass. Microbial cells have the ability to concentrate chemicals from the aquatic environment. This absorption type would be an active transport process, which requires energy to move contaminants across the biological membranes or cell well. Thus, making the mechanisms and kinetics behind biosorption necessary and important to understand. The mechanisms behind the absorption of the contaminant vary depending on the type of applied biomass (Derco et al., 2018). In summary, it is important to note that absorption is not typically the primary mechanism in the biosorption process. Adsorption is often favoured over absorption in biosorption processes because it is a more straightforward process that does not require energy input and can be more efficient at removing contaminants from the solution.

Biosorption is getting more and more attention in the field of recovery of metals from electronic waste. This modern biological method is known for a high recovery rate for metals in low concentrations, fast kinetics and no generation of secondary residues (Ambaye, 2020). Recent studies have investigated the possibility for the extraction rare earth metals from electronic waste using bacteria as biosorption material. The studies mainly focus on lanthanum from E-waste using bacteria. As discussed above, bacterial cell walls contain essential functional groups required for the sequestering metal ions present in E-waste (Kaur et al., 2022). Printed circuit boards (PCBs) are the base of many electronic industries and contain valuable metals (S. Abdelbasir & Kamel, 2018). A study by Sheel & Pant, (2018) showed that the bacteria *Lactobacillus acidophilus* was able to extract 85% of gold from electronic waste. Another recent study by Kaur et al., (2022), on the biosorption from PCBs showed that bacteria could extract metals such as copper and iron through mechanisms like ion exchange and complexation. Dolker & Pant, (2019) conducted a study where they examined a chemical-biological hybrid method to extract metals from LIBs. The bacterium *Lysinibacillus* sp., together with citric acid, was able to provide a pathway where Li and Co could be extracted from the batteries. This method increased Li leaching by 25% and Co biosorption by 98%. In a recent study, researchers utilised *Arthrospira platensis* biomass to remove Li ions from batch solutions. The findings demonstrate that this type of biomass is a cost-effective and effective sorbent for removing Li from wastewater. The research further suggests that functional groups play a crucial role in binding Li ions. The primary mechanism of Li biosorption by *A. platensis* is proposed to be metal binding to functional groups and ion-exchange (Liliana et al., 2021).

The existing research shows that biosorption has promising future opportunities for metal recovery from E-waste. However, the research done is currently limited to a laboratory scale studies (Kaur et al., 2022). Thus, it is extremely important for upscaling in larger pilot-scale and full-scale studies with the most promising technologies in the future.

5.3 Biomineralisation

Biomineralisation refers to the process in which harmful metal ions binds to an ion or ligands produced by microorganisms to form precipitation. There are several microorganisms that can be used in the biosorption of toxic metals from E-waste (Table 11) (Marappa et al., 2017). Biomineralisation is a critical process controlling the biogeochemical cycling, fate and impacts of heavy metals. Some believe that biomineralisation is the result of the interaction between extruded metabolites and extraneous metal ions in the environment. Studies show that *Citrobacter* creates hydrogen phosphate ions through phosphorylation and forms minerals on the bacteria's surface to remove heavy metal ions from aqueous solutions. Current studies indicate that the cell is used as the nucleation site and is encapsulated by metal minerals until the cell is inactivated (Zhang et al., 2022). Microorganisms can precipitate metals as carbonates and hydroxides via plasmid-borne resistance mechanisms, whereby proton influx counter-current to metal efflux results in localised alkalinisation on the cell surface. Metals can precipitate with enzymatically generated ligands, e.g. sulphide or phosphate (Atlas & Philp, 2005, pp. 293–317). *Bacillus* is a good microbe for biomineralisation in the construction industry because it can effectively adsorb metal ions and crystallise them into minerals. The mechanism of biomineralisation of Pb^{2+} is widely recognised (Zhang et al., 2022). Microbial-induced carbonate precipitation (MICP) is a method within biomineralisation used in soil remediation.

In recent years, microbial-induced carbonate precipitation (MICP) has shown potential to be used for the removal of heavy metals from wastewater. The principle behind this method is to use urease microorganisms to breakdown urea to produce carbonate and which through metal precipitation lead to the formation of cadium carbonate ($CdCO_3$) for example. Urease microorganisms are an excellent choice of bacteria because they offer several advantages, including minimal energy consumption, species richness, and eco-friendliness (Shan et al., 2021). A study by Zeng et al. showed that *Sporosarcina pasteurii* can remove 99% of Cd from sewage (Song et al., 2022). In Figure 21, the process of biomineralisation of heavy metals through microbial-induced carbonate precipitation (MICP) is illustrated. This method involves a) the formation of calcium carbonate deposits in close proximity to the migration pathway of heavy metal ions. B) Additionally, heavy metal ions undergo exchange with Ca^{2+} during the mineralisation process, leading to the formation of carbonate deposits (Shan et al., 2021).

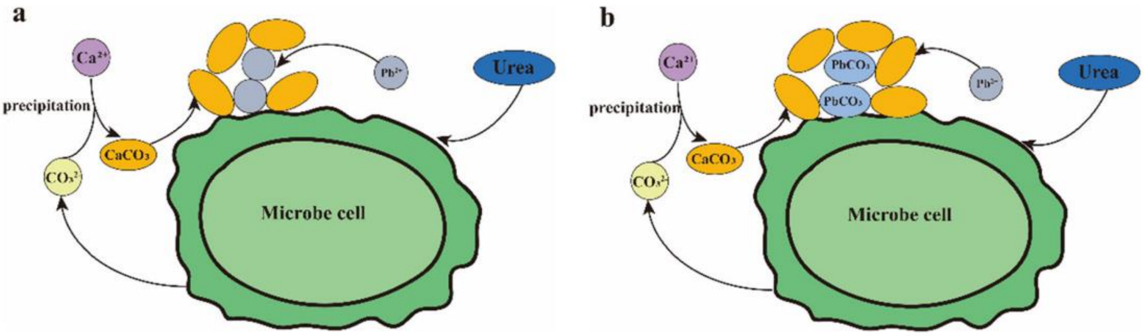


Figure 21: Biomineralisation of heavy metals through microbe-induced carbonate precipitation (Shan et al., 2021)

Overall, biomineralisation offers a promising avenue for the recycling of metals. Still, further research is needed to better understand the mechanism by which biomineralisation can be harnessed for metal recovery from E-waste, and to identfify the most efficient microorganisms and optimal conditions for metal recycling purposes.

6. Microorganisms involved in microbial technologies

6.1 Chemolithotrophy

Chemolithotrophs are a group of organisms that obtain energy through the oxidation of organic and inorganic compounds. The majority of these bacteria are considered autotrophs. Autotrophic bacteria are any microorganisms that can grow with CO_2 as its only source of carbon. Some chemotrophs are however, considered to be heterotrophs. Heterotrophic bacteria are microorgansims that requires organic carbon as their carbon source, also known as a chemoorganotroph (Madigan et al., 2015). Chemolithoautotrophic bacteria, possess the ability to extract metals from mineral ores, but also secondary sources like E-waste. They can utilise various inorganic donors such as hydrogen, sulphur, nitrite, phosphite, ammonia and iron (II) to meet their carbon, electron, and energy needs (Dutta et al., 2023). The amount of energy gained by the oxidation of these donors varies. Inorganic donors contribute electrons to electron transport chains, creating a proton motive force which drives ATP synthesis through ATPases (Madigan et al., 2015). While oxidising sulphidic minerals, they also fix atmospheric CO_2, resulting in the generation of ferric ions (Fe^{3+}) responsible for bioleaching. These organisms play a significant role in generating lixiviants for metal solubilisation through acidolysis and recycling Fe^{2+} and Fe^{3+} ions through redoxlysis (Dutta et al., 2023). Chemolithotrophic bacteria have the ability to grow in unfavorable environments, including deep-sea hydrothermal vents, hotsprings, subsurface environments and soil. They play a crucial role in biogeochemical cycles, participating in processes involved in nitrogen, sulphur, and iron cycling (Madigan et al., 2015).

There are four main groups of chemolithotrophic bacteria, determined by the electron donors they use and their carbon source. The first group, known as obligate chemolithotrophs, relies solely on inorganic compounds for energy, and CO_2 for carbon. *Thiomicrospira* and its various species fall into this category. The second group, called facultative chemolithotrophic or mixotroph, can use organic and inorganic compounds for energy and obtain carbon from CO_2 or other organic sources. *Thiosphaera pantotropha* and *Paracoccus denitrificans* are examples of bacteria in this group. The third group, chemolithoheterotrophs, can oxidise inorganic compounds, but cannot fix CO_2. *Thiobacillus* and *Beggiatoa* are species in this group. Finally, the fourth group, chemoorganoheterotrophs, oxidise reduced organic compounds. *Thiobacterium, Shewanella* and *Thiothrix* belong to this group (Kazemi et al., 2021).

6.2 Sulphur-oxidising bacteria

Sulphur-oxidising bacteria (SOBs) are a group of microorganisms that is considered to be chemolithotrophs. SOBs thrive in environments that contain significant amounts of inorganic sulphur elements (Ranalli et al., 2019). Sulphur bacteria can receive electrons from various sulphur compounds, including hydrogen sulphide (H_2S), elemental sulphur (S^0), and thiosulphate ($S_2O_3^{2-}$) (Madigan et al., 2015). A comparison of the energetics of the oxidations is listed in Table 10 below.

Table 10: Energetics of oxidation of reduced sulphur compounds (Madigan et al., 2015).

Chemolitotrophic reaction	**Electrons**	**Stochiometry**	**Energetics (kJ/electron)**[a]
Sulphide to sulphate	8	$H_2S + 2\ O_2 \rightarrow SO_4^{2-} + 2\ H^+$	$\Delta G^0 = -798.2$ kJ/reaction
Sulphite to sulphate	2	$SO_3^{2-} + ½\ O_2 \rightarrow SO_4^{2-}$	$\Delta G^0 = -258$ kJ/reaction
Thiosulphate to sulphate	8	$S_2O_3^{2-} + H_2O + 2\ O_2 \rightarrow 2\ SO_4^{2-} + 2\ H^+$	$\Delta G^0 = -818.3$ kJ/reaction

During sulphide oxidation, S^0 is produced in the first step. Certain SOBs, such as the bacteria *Beggiatoa*, store S^0 inside their cells as a potential energy source. When the supply of sulphide is exhausted, energy can be obtained by oxidising sulphur (S) into sulphate (SO_4^{2-}). The final oxidation product is typically SO_4^{2-}. Protons are generated as a by-product of reduced sulphur compound oxidation, leading to acidification of the surroundings. Subsequently, many SOBs have adapted to tolerate acidic environments or are even categorised as acidophilic bacteria (Madigan et al., 2015). Sulphur-oxidising bacteria thrive in pH values below 3, preferably in the range of 2.0 – 3.5. One well-known chemolithotrophic bacterium called *Acidithiobacillus thiooxidans (*formerly: *Thiobacillus thiooxidans),* is a species of SOBs belonging to the family *Acidithiobacillaceae*. *A. thiooxidans* is classified as a mesophilic bacteria, with optimal temperatures between 28 to 30 °C (Yang et al., 2019). *A. thiooxidans* are notorious for producing sulphuric acid and therefore, thrives in highly acidic environments. *A. thiooxidans* use atmospheric oxygen as electron acceptor and can convert sulphide and thiosulphate to sulphate, promoting sulphuric acid production and generating protons (H^+) in the process (Ranalli et al., 2019).

This type of bacteria is extensively studied and utilised in the mining industry for its ability to extract metals from sulphide minerals through bioleaching (Dutta et al., 2023). Bioleaching involves the use of acidophilic bacteria to dissolve minerals by metabolising iron and reducing inorganic sulphur compounds, which in turn makes the minerals accessible for extraction. A. *Thiooxidans* is a key sulphur-oxidising bacterium in the bioleaching process because of its autotrophic nature and ability to tolerate heavy metals (Yang et al., 2019). Their particular tolerance to toxic metals such as copper, nickel, zinc, and cadmium is noteworthy (Vera et al., 2022). The possible bioleaching mechanisms include contact and non-contact, as discussed in section 5.1.5. In the contact mechanism, *A. thiooxidans* can adhere to metal sulphide surfaces, through the extracellular polymeric substances (EPS) secreted by the bacteria. The process involves the direct oxidation of metal sulphide using an intracellular specific oxidase system, resulting in the formation of soluble sulphate. In the non-contact mechanism, S^0 or reduced sulphur compounds are oxidised to sulphuric acid by the bacteria. This oxidation reaction reduces the pH and therby dissolves metal sulphide. *A. thiooxidans* could help extract metals from E-waste, especially PCBs (Yang et al., 2019). Table 11 provides an overview of the metals that SOBs can leach.

6.3 Iron-oxidising bacteria versus Iron-reducing bacteria

Iron-oxidising bacteria (IOB) and iron-reducing bacteria (IRB) are two groups of chemolithotrophic microorganisms that play important roles in the biogeochemical cycling of iron (Madigan et al., 2015). Figure 22 illustrates the iron (Fe) redox cycle.

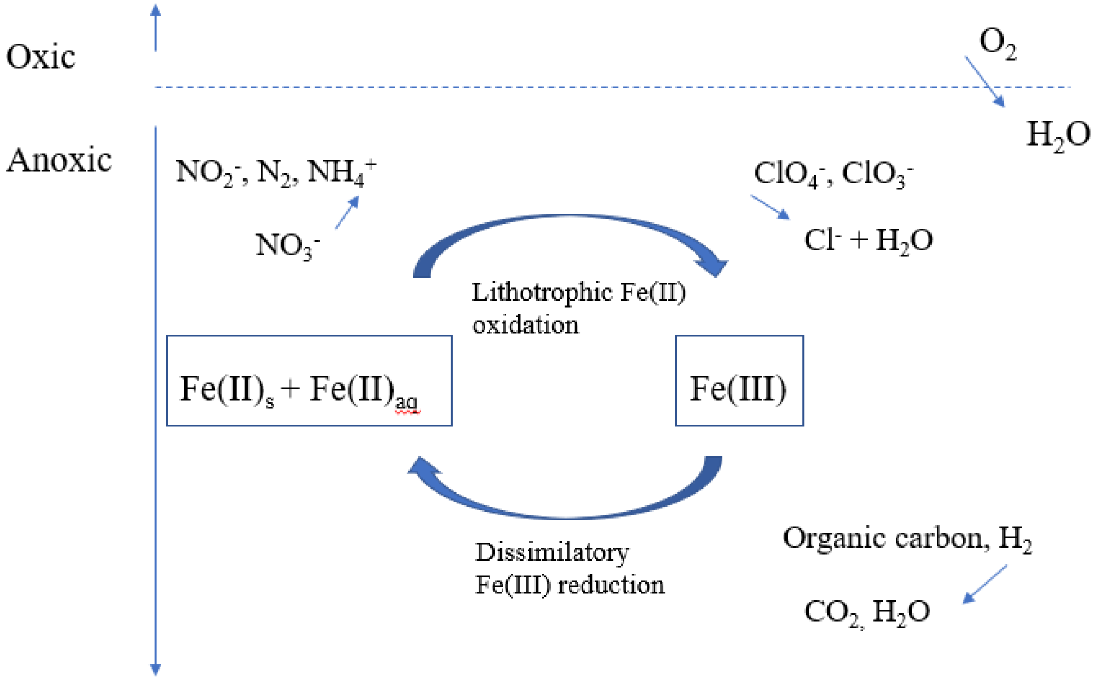


Figure 22: Overview of microbial iron transformations in the environment. Adapted by: (Weber et al., 2006).

The iron redox cycle involves the interaction between IOB and IRB. Fe-reducing bacteria, as the name suggests, have the ability to reduce or remove electrons from iron compounds in the anoxic zone, in the absence of oxygen. They use iron as electron acceptors during their metabolic processes. By oxidising organic matter or other compounds like H_2, these bacteria transfer electrons, converting iron from its oxidised form, Fe (III), to its reduced form, Fe (II). This reduction releases energy that the bacteria can utilise for their growth (Weber et al., 2006). *Geobacter* was initially researched as the bacteria capable of gaining energy through this reaction, but recent studies indicate that other microorganisms are also able to do so (Fredrickson & Gorby, 1996). Fe-oxidising bacteria such as *Acidithiobacillus ferrooxidans* gain energy from the oxidation of Fe (II) to Fe (III), to fix carbon dioxide. The energy released during this oxidation supports the metabolic activities of IOB. The occurrence of the bacteria that is able to obtain energy from the oxidation of Fe (II) to Fe (III) are generally limited by the availability of dissolved Fe. Oxygen availability and pH value have a strong influence on the reaction rate, which explains why at low pH values or low oxygen concentrations, ferrous iron (Fe^{2+}) is stable (Chan et al., 2011). Iron-reducing and iron-oxidising bacteria work together to create a cycle of continuous reduction and oxidation reactions that are crucial for maintaining the balance of iron in different environments.

The two most common iron-oxidsing bacteria are *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*. These microbes grow using Fe^{2+} as an electron source. The process of Fe^{2+} oxidation by *A. ferroxidans* and other IOBs is of great interest due to the electropositive reduction potential of Fe^{3+} to Fe^{2+} (E0 + 0.77 V at pH 2). *A. ferroxidans* utilises the Calvin cycle to support autotrophy. Due to the abundance of electron donors, a significant amount of energy is required for the reverse electron flow reaction to generate the necessary reducing power (NADH) in order to fix CO_2. The reduction of NAD^+ by electrons gained from Fe^{2+} results in the formation of NADH. To face the challenge of low energy yield during the oxidation, *A. ferroxidans* must oxisidise a large amount of Fe^{2+} to produce even a small amount of cell material. IOBs thrive in environments where they generate abundant Fe^{3+} precipitates through their iron oxidation acitivity. The presence of Fe^{3+} precipitates serves as an electron sink, helping acidophilic IOBs maintain their redox balance and meet their energy requirements (Madigan et al., 2015). The ferric iron produced by these bacteria interacts with metal sulphides in ores, facilitating their dissolution and releasing metal ions for recovery.

Iron-oxidising bacteria play a significant role in metal recovery processes, particularly in the context of bioleaching. IOBs have the ability to function in both direct contact leaching and non-contact leaching systems (Sethurajan & Gaydardzhiev, 2021). IOB are used in many applications including metal bioleaching, biomining, and agriculture (Kazemi et al., 2021). *A. ferroxidans* and *Sulphobacillus thermosulfidooxidans* are two commonly used bacteria in mineral ore bioleaching. Their remarkable adaptation to low phosphate environments where Fe precipitation occurs, coupled with their ability to utilise complementary phosphorous sources like phosphonates, is unique (Vera et al., 2022). The oxidation reaction (Fe^{2+} to Fe^{3+}), releases energy that the bacteria utilise for growth and survival. The ferric iron (Fe^{3+}) generated by IOB acts as an oxidising agent, which can react with sulphide minerals present in the ore or waste material. In the precence of sulphide minerals such as pyrite (FeS_2), chalcopyrite ($CuFeS_2$) or sphalerite (ZnS), Fe^{3+} undergoes an oxidative dissolution due to a reaction with sulphide ions (S^{2-}). This reaction converts the sulphide minerals into metal ions such as copper (Cu^{2+}), zinc (Zn^{2+}) or iron (Fe^{2+}) and sulphate ions ($SO_4{}^{2-}$) (Rohwerder et al., 2003). The released metal ions can further be recovered through various techniques such as precipitation or solvent extraction to obtain pure metals (Srichandan et al., 2019). IOB enhance metal recovery by catalysing the oxidation of Fe^{2+}, maintaining a continuous supply of ferric iron in the bioleaching system. This promotes oxidative dissolution of sulphide minerals and facilitates the release of valuable metals. The use of these bacteria in metal recovery processes offers potential advantages, including lower temperature and energy cost, reduced environmental impact and the abilitiy to extract metals from low-grade ores (Johnson, 2014). IOBs also play a significant role in metal recovery from E-waste. *L. ferrooxidans and L. ferriphillum* dominantes over *A. ferrooxidans* bacteria in situations where the Fe^{2+}/Fe^{3+} ratio is high, and play a major role in the oxidation of iron. This results in the production of highly effective lixiviant, rich in ferric content, which is a key part of recovering metals such as Cu from E-waste, particularly from PCBs (Dave, 2018).

Iron-reducing bacteria use the reduction of oxidised metals for cellular growth. It is common for these microorganisms to face the difficulty of utilising an insoluble solid material as an electron acceptor. Bacteria found within this group are *Geobacter*, *Shewanella*, *Bacillus*, *Thiobaccilus and Geothrix* among others. IRB are known to be extremely versatile at anaerobic respiration. Most genra within IRB are obligate anaerobe, however, some are faculative aerobes such as *Shewanella.* Fatty acids, sugar, and alcohols are typically organic compounds used as electron donors. Some species use H_2 as an electron donor, but are then unable to grow autotrophically, requiring a source of organic carbon for microbial growth. *Geobacter* can use a variety of electron donors and acceptors (Madigan et al., 2015). Whereas *Geobacter* is primarly known for its ability to respire on iron minerals, research studies have shown that the bacteria can also respire on other metals, such as cobalt. Despite the toxicity of Co, *Geobacter* species assimilate Co^{II} to synthesise cobamides. *Geobacter* can not only survive cobalt exposure, but effectively form cobalt nanoparticles on its cell surface. These adaptions give *Geobacter* a competitive advantage of growth in metal-rich environments, despite the mobilisation of cobalt. It appears that these species are capable of aiding in the cobalt recycling, maintaining the efficiency of the native microbiomes and contributing to previously unknown reactions of the cobalt cycle (Dulay et al., 2020).

Table 11 provides an extensive overview of diverse microorganisms, the metals they recover, and the mechanisms employed in the recovery process.

Table 11: Metals recovered by different microorganisms in regards to recovery mechanism.

Recovery mechanism	**Name of microorganism**	**Metal(s) recovered**	**References**
Bioleaching	*Acidithiobacillus caldus*	Co, Ni, Li	(Ghassa et al., 2020)
	Acidithiobacillus ferrooxidans	Li, Co	(D. Mishra et al., 2008)
	Leptospirillum ferroxidans *Leptospirillum ferriphillum*	Cu, Zn, Ni, Pb, Cd	(Dave, 2018) (Işıldar et al., 2019)
	Acidithiobacillus thiooxidans	Li, Co	(Ghassa et al., 2020)
		Cr, Ni, Al, V, Mo, Co, Pb, Cd, Zn	(Yang, et al., 2019)
	Sulphobacillus thermosulphidooxidans *Sulpholobus spp.*	Cu, Zn, Ni, Al	(Dave, 2018), (Işıldar et al., 2019)
	C. Violaceum	Cu	(Dutta et al., 2023)
	Chromobacterium violaceum *Pseudomonas aeruginosa* *Pseudomonas florescence*	Au, Ag, Pd, Pt	(Dave, 2018), (Işıldar et al., 2019)
	Pseudomonas biofilm	Ag	(Dutta et al., 2023)
	Marsmius oreades	Au, Ag, Pd, Pt	(Dave, 2018), (Işıldar et al., 2019)
	Aspergillus niger	Ln	(Dutta et al., 2023)
		Cu, Fe	(Kaur et al., 2022)
	Penicillum simplicissimum	Cu, Zn, Ni, Pb, V, Mo, Al, Co, Li	(Dave, 2018), (Işıldar et al., 2019)

	Bacillus foraminous	Ag, Mo, Cu	(Dutta et al., 2023)
	Ferroplasma spp.	Co, Ni, Li	(Ghassa et al., 2020)
	Chromobacterium violeaceum	Au, Ag, Pt, Pd, Ti, Mo	(Magoda & Mekuto, 2022).
	Pseduomonas fluorescens		
	Pseudomonas aeruginosa		
	Bacillus megaterium		
Biosorption	*Acidithiobacillus ferrooxidans*	Cu	(Vijayaraghavan & Yun, 2008)
		Fe, Ln	(Dutta et al., 2023)
	Pseudomonas aeruginosa	Cu, Pb	(Vijayaraghavan & Yun, 2008)
	Pseudomonas cepacia		
	Pseudomonas putida		
	Pseudomonas stutzeri		
	Pseudomonas sp.	La	(Ambaye, 2020)
	Agrobacterium sp.	La	
	Aspergillus niger	Cu, Fe	(Kaur et al., 2022)
	Bacillus thuringiensis	Ni	(Vijayaraghavan & Yun, 2008)
	Bacillus subtilis	Cu	
	B. megaterium	Cu, Au	(Dutta et al., 2023)
	Streptomyces rimosus	Ni	(Vijayaraghavan & Yun, 2008)
	Streptomyces pilosus	Cu	
	Enterobacter sp.	Cu	
	Streptomyces spp.	Fe	(Kaur et al., 2022)
	Lactobacillus acidophilus	Au	(Sheel & Pant, 2018)
	Lysinibacillus ssp.	Co	(Sethurajan & Gaydardzhiev, 2021)
	Bacillus sphaericus	Cr	(Marappa et al., 2017)
	Myxococcus xanthus	U	
	Streptoverticillium cinnamoneum	Pb	
Biomineralisation	*Sporosarcina pasteurii*	Cd	(Song et al., 2022)
	Bacillus fusiformis	Pb	(Marappa et al., 2017)
	Cupriavidus metalidurans	Cd	
	Desulfotomaculum auripigmentum	As	
	Sporosarcina ginsengisoli	As	

7. Discussion

The concept of the circular economy (CE) strives to maximise the utilisation and value of products while promoting the four R's: reduce, reuse, recycle, and recover. E-waste management is essential in achieving this goal and is crucial to the world's economy. The primary objectives are to minimise waste, increase the recovery of valuable materials, and lessen health risks (Dutta et al., 2023). Recycling metals is crucial for minimising environmental harm and preserving critical resources, despite potential costs (Y. Yang et al., 2021).

E-waste generation is estimated to be 20-50 million tons yearly and is continuing to increase (S. M. Abdelbasir et al., 2018). The rise in electronic devices and vehicle usage has caused a substantial growth of LIBs. Consequently, numerous spent batteries are being carelessly discarded into landfills, causing grave harm to our environment (Biswal et al., 2018). The components that make up LIBs are numerous and complex. These components can be broken down into fragments and recycled to re-produce various metals (Moazzam et al., 2021). Toxic compounds such as heavy metal oxides of cobalt ($LiCoO_2$), manganese ($LiMn_2O_4$), and nickel ($LiNiO_2$) are present in batteries, and are categorised as hazardous waste (Biswal et al., 2018). It is important to recycle E-waste and spent LIBs to decrease the release of harmful substances into the environment and preserve essential metals like copper, cobalt, and lithium (Biswal et al., 2018). The nature of electronic equipment is constantly evolving, resulting in challenges when it comes to developing standardised recycling processes that can improve metal recovery (Andrade et al., 2022). Recovering valuable metals from E-waste, especially LIBs is still in the early stages of development. To establish this on a commercial scale, the operation of these technologies must reduce their costs (Y. Yang et al., 2021).

More research is needed on alternative microbial methods beyond bioleaching. In this literature review only 27 relevant studies were found regarding the biosorption of Li, indicating a lack of sufficient knowledge in this area. Out of the 27 studies, only three concerned metal recycling from E-waste. This highlights the necessity for further research on the recovery of metals from E-waste on alternative microbial methods beyond bioleaching. When searching for articles and reviews using the term "metal recovery *and* lithium-ion batteries," only a few published studies involving bacterial methods were discovered out of the 513 retrieved. The limited number of research articles published highlights the need for further studies on this subject. A possible limitation of this thesis is that the literature search used in the scoping review only used the

Scopus database. There are other relevant databases like Web of Science and Google Scholar. A search in these may have provided additional studies not included in this thesis. Reviews vary in methodological quality, which should also be considered. An advantage of using a literature review is that it will provide an overview of the breadth of the research in the field, and it can also identify topics or research domains that require more investigation. Reviews are an important means of summarising science and practice.

Conventional techniques like pyrometallurgy and hydrometallurgy are utilised to retrieve metals from waste (Benzal et al., 2020). Many reviews have been published on the extraction of valuable metals and minerals from ores and E-waste with great success (Roy et al., 2021). However, conventional technologies do not meet the future requirements of the industry due to the high costs and low efficiency (S. M. Abdelbasir et al., 2018). In addition to being expensive, conventional methods emit more carbon and toxic chemicals into the atmosphere and do not recover all the metals (Moazzam et al., 2021). To ensure a sustainable future, it is imperative to adopt green technology. Regrettably, the conventional methods that are in use currently do not fall under this category. Microbial technologies can be an excellent option for traditional recovery methods (Kaur et al., 2022). Biometallurgy is a good option compared to other methods, such as pyrometallurgy or hydrometallurgy, due to its low operational cost, low energy consumption and less use of chemical reagents. Managing secondary waste effluents is a more manageable task during biometallurgical processes (Magoda & Mekuto, 2022).

Table 12 below compares the benefits and drawbacks of various recovery methods. These methods offer various advantages and limitations, and the choice of method depends on factors such as cost, efficiency, environmental impact, and the specific requirements of the recycling process.

Table 12: Advantages and disadvantages of various recovery methods.

Method	**Advantages**	**Disadvantages**	**References**
Pyrometallurgy	No generation of wastewater	Large energy input	(Roy et al., 2021)
	Fewer processing steps	Emission of toxic gasses	
	Metals can be recovered in the form of alloys through direct melting.	Loss of Li during recovery	
	High efficiency rate	High energy demand	(Thakur, 2020)
		Expensive	
	Energy can be utilised in upstream or downstream processes	High operational temperature	(Magoda & Mekuto, 2022)
	Minimal chemical consumption	Produces toxic compounds such as dioxins and furans due to E-waste containing halogenated flame-retardants	
Hydrometallurgy	High sustainability	Complex operation steps	(Roy et al., 2021)
	High extraction efficiency	Generation of acid waste	
	Low energy use	Emission of Cl_2, SO_3 and NO_x	
	Easy accomplishment	Highly corrosive environment	(Thakur, 2020)
	Short process time	A large number of metals in E-waste are required.	(Magoda & Mekuto, 2022)
		High operational cost	
		Generation of effluents that may pollute water resources	
		Costly sulphur conversion technology	
Bioleaching	Low operational costs	Low kinetics	(Roy et al., 2021)
	Less use of chemicals	Electrolytes are toxic to microbes.	
	Higher efficiency at low metal concentrations	High pulp density	
	Less toxic		
		Parts of LIB can be toxic to microbes.	(Moazzam et al., 2021)
	Environmentally friendly	Not easy to control bio-reactions	(Magoda & Mekuto, 2022)
	Easy management	Technology still under development	
	Low energy consumption	Long operational period	
Biosorption	Effective and low operational costs	Early saturation of the active metal binding sites	(Sethurajan & Gaydardzhiev, 2021)
	Low amount of generated sludge	Challenging to alter the valence state of the desired metal	
	Possible regeneration and reuse of biosorbent	Hard to scale up due to biosorbents' size and low density	
	Easy to use	Poor selectivity	(Vijayaraghavan & Yun, 2008)
	Binding sites can accommodate different types of ions.		
	The high degree of uptake		

The process of using bacteria to recover valuable metals is gaining recognition. However, it is crucial to consider the growth characteristics of the microorganisms and their sensitivity to external factors. Factors such as pH levels and oxygen can often pose a challenge when using a biological method to extract metals, especially from solid waste (Wu et al., 2022). In future research on microorganisms, exploring the possibility of introducing magnetic and electric fields to the liquid environment where bacteria reside could be beneficial. This has the potential to stimulate microorganisms and facilitate the isolation and purification of specific elements. Using an appropriate electric current can enhance the enzymatic activity in the bacteria, leading to a higher leaching rate. Additionally, researchers may explore using "ionic liquids" instead of organic extractants and using supercritical fluids to control the extraction of valuable metals (Wu et al., 2022).

Bioleaching is a highly effective microbial technique that aims at extracting and recovering heavy metals from polluted sediment, mineral ores, soil, and sludge and represents a promising technology (Wu et al., 2022). Today this technique is mainly used to recover metals such as copper, cobalt, nickel, zinc, and uranium (Vera et al., 2022). Bioleaching has emerged as a sustainable technology with great potential for recycling E-waste. Numerous studies have been conducted to recover metals from various types of E-waste using this method. In 2013, Johnston et al. investigated recycling REEs from E-waste using bioreduction, acidolysis pathway in bioleaching, heterotrophic bioleaching, and biomineralisation. The study yielded promising results, indicating that it is possible to recover metals using bacteria, ultimately paving the way for the development and understanding of biotechnological processes for metal recovery from electronic waste (Ambaye, 2020).

Various types of equipment fall under the category of E-waste, but one of the most common components found in this category is the printed circuit board (PCB). PCBs comprise numerous precious metals, and several studies have been conducted on retrieving these metals (Joshi et al., 2017). Recently, more reviews have been published on recovering metals from lithium-ion batteries using bioleaching processes (Roy et al., 2021). In 2008, D. Mishra conducted a pioneering study on using *Acidithiobacillus ferrooxidans* bacteria to extract Li and Co from spent LIBs. This study demonstrated that it was possible to use this organism to recover those two metals, showing the feasibility of this process to the industry. Some studies have been carried out to explore methods of enhancing bioleaching in the metal recovery process. According to specific reviews, the presence of sulphur improves the leaching process of Li. Other research indicates that the contact of bacteria with PCBs and LIBs is a crucial element for recycling metals using bioleaching (G. Mishra et al., 2022) (Sethurajan & Gaydardzhiev, 2021). While bioleaching for metal extraction from ores has already been commercialised, it is imperative to note that the process of extracting metals from E-waste and LIBs using this technology is still under development (Roy et al., 2021). Bioleaching is slow to leach metals and is not yet fully industrialised for E-waste (Magoda & Mekuto, 2022). More research is needed on the entire bioleaching process of LIBs, including improving the method's effectiveness, enhancing the process, retrieving metals and restoring electrode material (Roy et al., 2021). Bioleaching of rare and valuable metals remains in the laboratory stage.

Research on screening biological species for their use in bioleaching is gaining interest (Wu et al., 2022). Today microbial processes are time-consuming due to low kinetic energy. One drawback of bioleaching is that parts of lithium-ion batteries can contaminate microorganisms. To overcome this challenge, it is important to optimise factors such as pH, substrate concentration, and pulp density and select bacteria that are more resistant to toxicity (Moazzam et al., 2021). Substrate concentration and pulp density influence to a large extent, the bioleaching efficiency of the critical metals from waste LIBs (Sethurajan & Gaydardzhiev, 2021). Enhancing the extraction process by improving the interaction between used batteries and bacteria can make bioleaching more efficient. The possible area leading advancement in the use of bioleaching for the recovery of metals from LIBs could be; discovering new microbial strains that are tolerant to the toxic material, designing better bioreactors for improved microbial culture, and identifying low-cost nutrient sources for the bacteria (Moazzam et al., 2021). M. Vera (2022) has studied the interactions between microbes and minerals. However, the process of leaching bacteria and their ability to identify and adhere to mineral surfaces remains unclear. According to previous research, cell attachment is not a random occurrence. Studies suggest that microbes prefer to attach themselves to surfaces with visible scratches or defects. Despite this, there is a lack of understanding regarding the molecular-level interactions between cells and minerals, thus, more research in this area to improve our current understanding of this interaction is imperative in order to optimise the bioleaching process.

Research studies on other biological methods, such as biosorption and biomineralisation, yield successful results for the extraction of heavy metals from wastewater. Biosorption is a promising method for metal recovery from E-waste. Still, studies are limited to the laboratory scale (Kaur et al., 2022). There is not enough information on biosorbents to maximise biosorption processes. The appropriate choice of biomass and operational conditions has to be determined for biosorption to become economically viable (Roy et al., 2021).

The costs involved in biometallurgy operations are influenced by various factors, including the cost of bacterial strains, chemicals utilised, and culture conditions. While microbial technologies in E-waste recycling are promising, the technology is still in the infant stages, and further research is required to establish them to be fully functional and sustainable technologies (Debnath et al., 2018). Dolker and Pant conducted a study in 2019, which revealed that a hybrid method utilising citric acid and bacteria could effectively improve the recovery of Li and Co metals from LIBs. This promising combination had not been previously explored or documented, making it a possible breakthrough in the field.

The discussion above highlights the significance of E-waste management and its role in achieving a circular economy. Recycling metals from E-waste, especially LIBs, is crucial to minimise environmental harm and preserve essential resources like Cu, Co, and Li. Conventional methods such as pyrometallurgy and hydrometallurgy are commonly used to recover metals but have certain limitations and disadvantages. Microbial technologies, such as bioleaching, biosorption and biomineralisation, offer a promising and sustainable alternative, however further exploration and optimalisation is needed to make them economically viable at a larger scale. Overall, more research is required to establish microbial technologies as efficient and sustainable methods for metal recovery from E-waste and LIBs.

8. Conclusion

As lithium-ion batteries (LIBs) become more frequently used in electric cars and electronics, hazardous E-waste increases. Therefore, it is crucial to implement effective management techniques to achieve the concept of a circular economy and protect the environment. After considering and comparing the advantages and limitations of conventional and microbial technologies for metal recovery, it can be concluded that biometallurgy has the potential to be a good alternative and/or additional technology for metal recovery from E-waste and LIBs. Bioprocessing aligns with the principles of a circular economy and sustainable resource management. Biotechnology has the possibility to significantly improve the recovery and reuse of metals from secondary sources, thereby contributing to and supplementing sustainable management practices and traditional technologies.

A combination of more than one process or technology can be applied to recover metals from E-waste and LIBs. It is important to note that relying on a single technology may have limitations and may not be able to address all issues due to the intricate nature of the E-waste systema. Thus, research in the area of combined technologies is crucial for effectively recovering metals from E-waste in the future. The low number of published research articles indicates the need for more studies on this topic. Extraction and recovery of lithium and other metals by bacteria must be further tested to determine their applicability at a larger scale. Hence upscaling in more extensive pilot-scale studies is required and of utmost importance.

9. Future perspectives

Electronic and electrical equipment waste (WEEE) provides a substantial source of critical and valuable metals that are indispensable for the transition towards a greener society (Işıldar et al., 2019). Biological methods such as bioleaching for extracting valuable metals from primary and secondary resources can represent an alternative to conventional methods, allowing us to transition into a greener future. However, WEEE differs significantly from primary resources regarding chemical composition, metal content, and complexity. Unfortunately, current biological metal recovery methods from WEEE are insufficient for targeting these critical metals on a larger scale. WEEE contains metals in their native metallic form. Supplementing the microorganisms with an additional energy source to recover metals from E-waste is crucial. This challenge necessitates innovative solutions for extracting valuable metals from WEEE. Thus, expanding this type of biotechnology into full-scale applications requires additional research, including scale-up studies with technological and environmental sustainability analysis. Choosing the right biotechnological strategy for metal recovery is imperative, considering the critical factors mentioned above (Işıldar et al., 2019).

In recent studies, bioleaching has been shown to be a possible method for extracting valuable metals from low-concentration lithium-ion batteries (LIBs) (Moazzam et al., 2021). The process of using biotechnology to extract metals from waste LIBs is still in its infancy stages, requiring additional research to enhance the technology's efficiency and selectivity towards specific metals (Sethurajan & Gaydardzhiev, 2021). Two critical areas for future research are designing LIBs for easy disassembly and modifying and selecting factors for better recycling performance. Redesigning LIBs can make the bioleaching process more affordable, sustainable, eco-friendly, and energy-efficient, with reduced pre-treatment energy consumption. Identifying microbial strains with higher tolerance to toxic substances, enhancing bioreactor microbial culture, and finding cheaper nutrient sources, are vital to improve the extraction process. As long as these two challenges remain unsolved, addressing them is essential to enhance bioleaching efficiency and the future economic value of spent LIBs (Moazzam et al., 2021). Future perspectives should also investigate using *Geobacter* in bioprocessing to reclaim and recycle cobalt from lithium-ion batteries (Dulay et al., 2020).

Efforts in creating a sustainable recycling process for lithium (Li) is vital in order to make it a more eco-friendly and affordable option for emerging technologies. In addition to advancing recycling methods, it is imperative to raise global awareness about the limited supply of Li (Bae

& Kim, 2021). Future studies should concentrate on refining bioleaching parameters to facilitate operations at higher pulp densities and prepare for industrial upscaling in large-scale research and pilot studies. Also, it is essential to establish secure disposal and management protocols for solid residues, process effluents, and possible generated sludge (Sethurajan & Gaydardzhiev, 2021).

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