A review on the recycling of spent lithium-ion batteries (LIBs) by the bioleaching approach

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Abstract

This review discusses the latest trend in recovering valuable metals such as lithium, cobalt, nickel, and manganese from spent lithium-ion batteries (LIBs) to avoid the technological world's critical metal demands. LIBs are a secondary source of valuable metals such as Li (5%-7%), Ni (5%-10%), Co (5%-25%), Mn (5-11%), and non-metal graphite. Recycling is essential for the battery industry to extract valuable critical metals from secondary sources to develop new and novel high-tech LIBs for various applications such as eco-friendly technologies, renewable energy, emission-free electric vehicles, and energy-saving lightings. LIB waste is currently undergoing high-temperature pyro-metallurgical or hydrometallurgical processes to recover valuable metals, and these processes have proven to be successful and financially feasible. These methods, however, are not advisable due to the difficulties in controlling the process, secondary waste produced, high operational cost, and high risk of scaling up. Biotechnological approaches can be promising alternatives to pyro-metallurgical and hydrometallurgical technologies in metal recovery from LIB waste. Microbiological metal dissolution or bioleaching has gained popularity for metal extraction from ores, concentrates, and recycled or residual materials in recent years. This technology is eco-friendly, safe to handle, and reduces operating costs and energy demands. The pre-treatment process (material preparation), microorganisms used in the bioleaching of LIBs, factors influencing the bioleaching process, methods of enhancing the leaching efficiency, regeneration of electrode materials, and future aspects have been discussed in detail.

Keywords: Bioleaching, lithium-ion batteries, pre-treatment, microorganisms, metal recovery, cathode regeneration

Abbreviations

Al	Aluminum
CE	Consumer electronics
Co	Cobalt
Cu	Copper
ESS	Energy storage system
EV	Electric vehicle
Fe	Iron
HEV	Hybrid electric vehicle
HF	Hydrogen fluoride
LCO	Lithium cobalt oxide
Li	Lithium
LIBs	Lithium-ion batteries
Mn	Manganese
Ni	Nickel
NiMH	Nickel-metal hydride
NMC	Nickel manganese cobalt

PED	portable electronic device
PHEV	Plug-in hybrid electric vehicles
PVDF	Polyvinylidene fluoride
TEA	Techno-economic analysis
XRF	X-ray fluorescence

1. Introduction

The demand for rechargeable batteries in various sectors such as mobile phones, computers, gadgets, electric vehicles, and storage devices is continuously growing, and lithium-ion batteries (LIBs) stand for the most rechargeable batteries market and their usage expanding every day (Chen et al., 2019). The expanding usage of portable electronic devices (PEDs) increases the demand for LIBs; subsequently, raising the volume of LIBs entering the global market every day, increasing their production and waste (Kang et al., 2013). LIB’s attractive characteristics such as high energy density, high voltage, extended lifespan, lower self-discharge, broad working temperature range, compact size and weight, and excellent electrochemical features make them choose among other types of batteries (Fergus, 2010). LIBs are more compatible than other energy storage devices that make them superior to other energy storage devices for most consumer electronics (CEs) (Kim et al., 2012; Goodenough and Park, 2013; Huang et al., 2018b; Khor et al., 2018). There is a substantial increase in the production of LIBs due to the advancements in microchip technology, constant upgrades of CEs, also reduced updating period increases the rapid production of spent LIBs in recent years (Gu et al., 2017). A substantial quantity of metal resources, such as lithium, cobalt, nickel, copper, aluminum, etc. is required for the production of LIBs used in portable electronic devices, electric vehicles (EV), plug-in hybrid electric vehicles (PHEV), and hybrid electric vehicle (HEV) in recent years. Thus, increasing demand for LIBs is projected to reach $95 billion by 2025, increasing 16.5% annually (Georgi-Maschler et al., 2012; Swain, 2017; Zheng et al., 2018). The electric vehicle (EV) market's high demand required a considerable number of LIB packs, which is estimated to be 1 million in 2030 (Chen et al., 2019).

The toxicity of LIB is caused by the use of toxic materials such as metals, electrolytes, binders, and plastics; all are hazardous to the environment. Some of the resources used for LIB production are also scarce in recent years (Chen et al., 2017). Toxic metals such as nickel, cobalt, manganese, and chemicals such as electrolytes and binders found in LIBs pose severe threats to ecosystems and human health, and they contaminate soil and groundwater. The electrolytes in LIBs such as $LiPF_6$, $LiBF_4$, or $LiClO_4$ can readily react with water and release harmful gases such as HF and PF_5 into the atmosphere (Zeng et al., 2015). The binders such as PVDF require toxic NMP (N-methyl-pyrrolidone) solvent for electrode preparation, and they can also react with graphite and lithium to form LiF and CF on the surface of the electrode at elevated temperatures (Versaci et al., 2017). During the lifecycle of LIBs, Li seeks to deposit on the anode owing to overcharge and reacts with water producing hydrogen gas (H_2) and lithium hydroxide (LiOH), thus cause explosions or fire accidents (Zeng et al., 2014). The predicted output of the disposed of LIBs is expected to increase from 10,700 tons in 2012 to 464,000 tons in 2025, with a growth rate of 59% (Siqi Zhao, 2019). Growing environmental risk on the waste disposal of LIBs and the increasing demand for critical metals for their production have significant attention paid for developing efficient, economical, and environmentally friendly processes in their recycling (Zheng et al., 2018). A few countries dominate the production of the primary metals used in LIBs; 59% of worldwide cobalt production is from the Republic Congo, and 78% of lithium production from Australia and Chile, and China controls 67% of graphite production (Figure 1) (Chen et al., 2019). Metal extraction from waste LIBs is more profitable than using pure metals because of its cost-effectiveness and high-circulability with no supply risk. It is essential to recover high-value metals such as cobalt, lithium, and nickel from spent LIBs to reduce the supply risk from the dominant countries. The environmental and safety challenges are decisive to ensure viable development in the efficient recycling of spent LIBs (Fan et al., 2020).

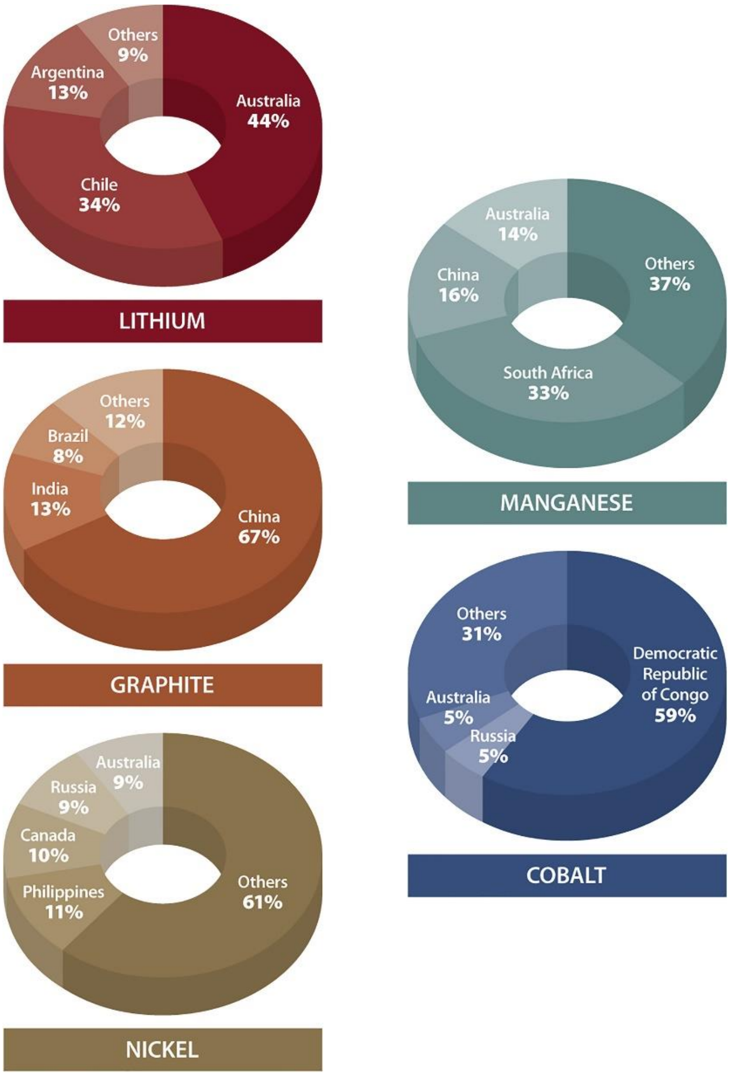


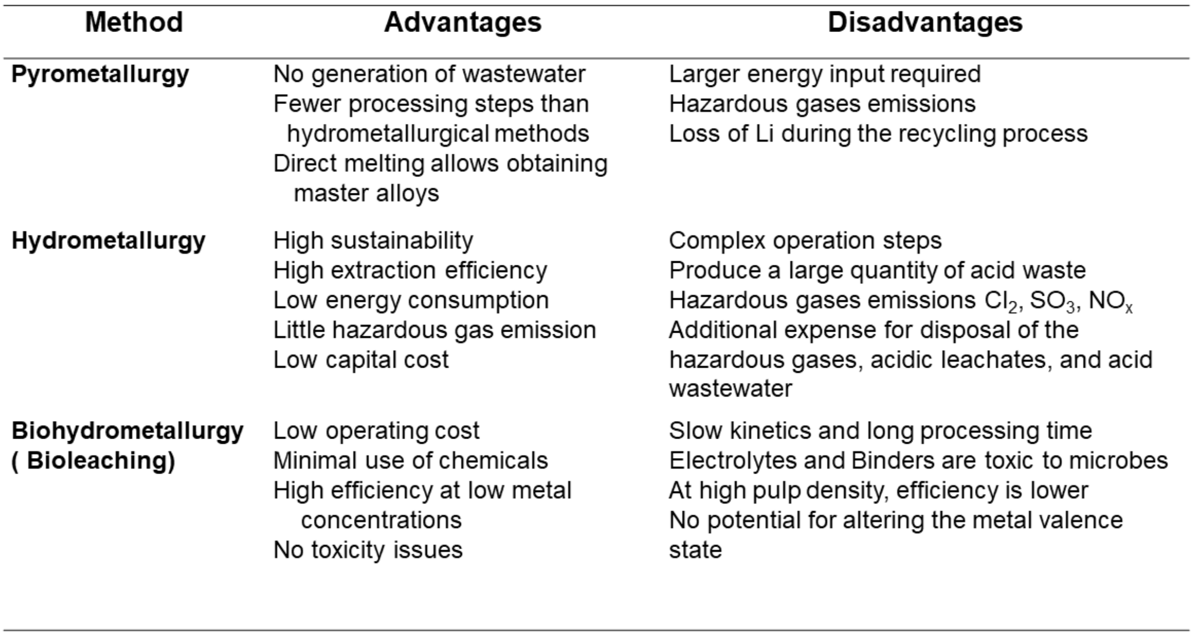
Figure 1. Global production of metals used in Lithium-Ion Battery, Reprint with permission from Joule 2019, 3(11), 2622-2646 Copyright (2019) Elsevier.

The recovery of metals from spent LIBs was currently performed by three conventional methods, such as pyro-, hydro-, and biohydro- metallurgical processes (Espinosa et al., 2004). Commercial recycling companies such as Umicore Group uses the pyrometallurgical process by treating spent LIBs like natural ores (Dunn et al., 2012). A high-temperature smelting reduction process is used in pyrometallurgy to recycle metals from spent LIBs, where the valuable metals are reduced and recovered as alloys (Bahgat et al., 2007; Fouad et al., 2007). However, pyrometallurgical technologies are inadvisable due to high energy consumption, hazardous gas emissions, lithium loss during the recycling process, and strict requirements for the treatment equipment (Sun and Qiu, 2011; Xiao et al., 2017b; Xiao et al., 2017a). Nowadays, hydrometallurgy has surpassed pyrometallurgy regarding sustainability, extraction efficiency, energy consumption, hazardous gas emissions, and capital cost. (Lv et al., 2018). Usually, the hydrometallurgical recycling process involves low-temperature acid leaching or bioleaching, purification, and separation procedures such as solvent extraction, chemical precipitation, electrochemical deposition to get the pure metals from spent LIBs (Chagnes and Pospiech, 2013). The hydrometallurgical process's recovery efficiency is higher than the pyrometallurgical process because of metals' high solubility in acid solutions. However, the hydrometallurgical process has some challenges such as complicated operation steps, produce a considerable amount of acid wastes, and some toxic gases emission such as Cl_2, SO_3, NO_x, which cause harm to human life and the environment; their disposal also required additional expenses (Zheng et al., 2018; Ashiq et al., 2019; Liu et al., 2019). The necessary condition for discharging acid waste into the environment is that it should not be too acidic or alkaline; to avoid harming aquatic life, environmental regulators define acceptable pH ranging from 5 to 9. Usually, hydrometallurgical acid waste is treated with strong alkali to neutralize the pH around 7 before discharging into the environment. Few organic acids such as acetic acid, citric acid, malic acid, oxalic acid, and tartaric acid have been explored in the bioleaching of spent LIBs (Golmohammadzadeh et al., 2018; Meshram et al., 2020). Citrus fruit juice, which contains citric acid and malic acid, and some citrus flavonoids, was also used to extract metals from NMC- based LIBs. These flavonoids can act as a reducing agent to reduce metal valency

(Pant and Dolker, 2017). Another recent study found that fruit peel waste can be used as a green reductant for metal extraction from lithium-ion batteries (Wu et al., 2020).

Bio-hydrometallurgy, a branch of hydrometallurgy that uses microorganisms to recover metals from ores, concentrates, and recycled or waste materials in the aqueous extractive metallurgy (Watling, 2015; Kaksonen et al., 2018). The bio-hydrometallurgical process is an emerging technology that attracts many researchers; and has potential advantages in waste treatment in an environmentally friendly route. Bioleaching is a well-developed multidisciplinary process that includes chemistry, biology, and metallurgy; that is one of the several applications within biohydrometallurgy to recover metals from ores and wastes. (Brierley and Brierley, 2013; Vera et al., 2013). Bioleaching is a cost-efficient technique that uses lixiviants (leaching agents) biologically produced by microorganisms used to treat waste disposal, which is significantly beneficial with few industrial requirements (Bosecker, 1997; Islam et al., 2020). The microbial-assisted bioleaching process converts the insoluble metal composition into water-soluble metals by bio-oxidation, and the microbe gains energy by rupturing the ores or wastes into their component metals (Rohwerder et al., 2003; Mishra et al., 2008). The bioleaching process mediates by a diverse group of microbes, such as chemolithotrophic prokaryotes, heterotrophic bacteria, and fungi that solubilize the metals from their respective ores or wastes (Johnson and du Plessis, 2015; Panda et al., 2015; Jafari et al., 2018; Quatrini and Johnson, 2019). The bioleaching process of LIBs aims to discrete the metal components of the spent batteries into separate fractions and use them to produce new batteries from extracted metals, reduce battery waste, and hazardous constituents in an energy-efficient and cost-effective manner (Vanitha and Balasubramanian, 2013; Johnson, 2014). The critical advantage of LIB bioleaching over the conventional recycling processes is that it generates mild acid waste and emits low levels of harmful gases, eliminating the need for additional treatment and reducing the treatment costs (Bahaloo-Horeh et al., 2019; Yu et al., 2020a). Table 1 compares the advantages and disadvantages of the various LIB recycling processes.

Table 1. Comparison of the various metal recovery processes for spent LIBs.



Method	Advantages	Disadvantages
Pyrometallurgy	No generation of wastewater Fewer processing steps than hydrometallurgical methods Direct melting allows obtaining master alloys	Larger energy input required Hazardous gases emissions Loss of Li during the recycling process
Hydrometallurgy	High sustainability High extraction efficiency Low energy consumption Little hazardous gas emission Low capital cost	Complex operation steps Produce a large quantity of acid waste Hazardous gases emissions Cl_2, SO_3, NO_x Additional expense for disposal of the hazardous gases, acidic leachates, and acid wastewater
Biohydrometallurgy (Bioleaching)	Low operating cost Minimal use of chemicals High efficiency at low metal concentrations No toxicity issues	Slow kinetics and long processing time Electrolytes and Binders are toxic to microbes At high pulp density, efficiency is lower No potential for altering the metal valence state

Many reviews have been published on metal recovery from spent LIBs using pyrometallurgical and hydrometallurgical processes (Huang et al., 2018a; Assefi et al., 2020; Fan et al., 2020; Yang et al., 2021). Some review articles reported the metal recovery from various e-wastes using biohydrometallurgical processes (Baniasadi et al., 2019; Zhao and Wang, 2019). Recently, few reviews have been published on the recovery of metals from spent LIBs using bioleaching processes (Huang and Wang, 2019; Moazzam et al., 2021; Sethurajan and Gaydardzhiev, 2021). The majority of the above reviews on bioleaching focused on the metals extraction processes. Detailed reviews on the bioleaching of spent LIBs that cover the entire process, including material preparation, factors influencing the bioleaching processes, methods to increase bioleaching efficiency, metal recovery, and regeneration of electrode materials for new battery fabrication, are lacking. This article aims to review the current status of research progress in bioleaching-based LIB recycling. Potential issues and future directions are also addressed.

2. Material preparation from spent LIBs for bioleaching

The success of recycling LIBs is based on the separation of constituents from used batteries into different parts that can be reused to make new batteries or other components. LIB structure is involved with different constituents; thus, pre-treatment processes are necessary to remove the casing, foils, and plastics to extract metals such as Co, Li, Ni, and Mn, which are valuable and essential for many industrial applications (Ekberg and Petranikova, 2015). Electrical short circuits, fire and explosion, and toxic chemicals such as binders and electrolytes are the three primary hazards possible in spent LIBs disposal (Balasubramaniam et al., 2020). The primary goal of the pre-treatment process is to emphasize the safety concerns in the effective separation of different constituents, especially on a large scale (Fan et al., 2020). Figure 2 shows the LIB recycling pre-treatment and recycling methods.

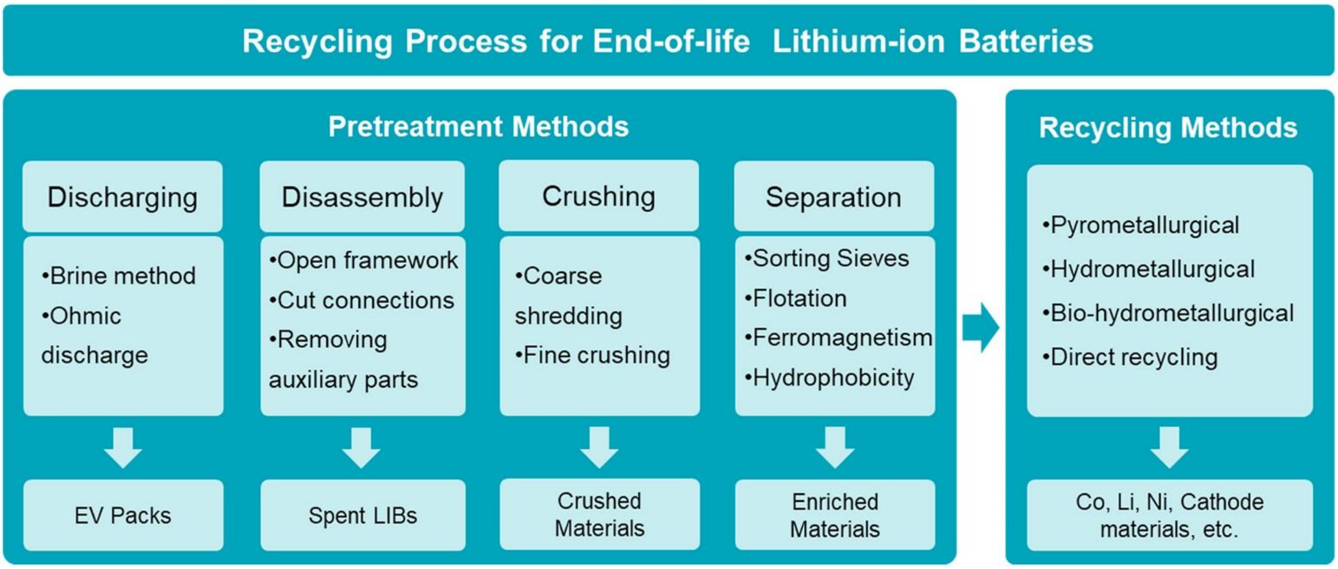


Figure 2. The pre-treatment and recycling methods of spent LIBs. Reprint with permission from Journal of power sources 2020, 478, 228753 Copyright (2020) Elsevier.

2.1 Battery Sorting and dismantling

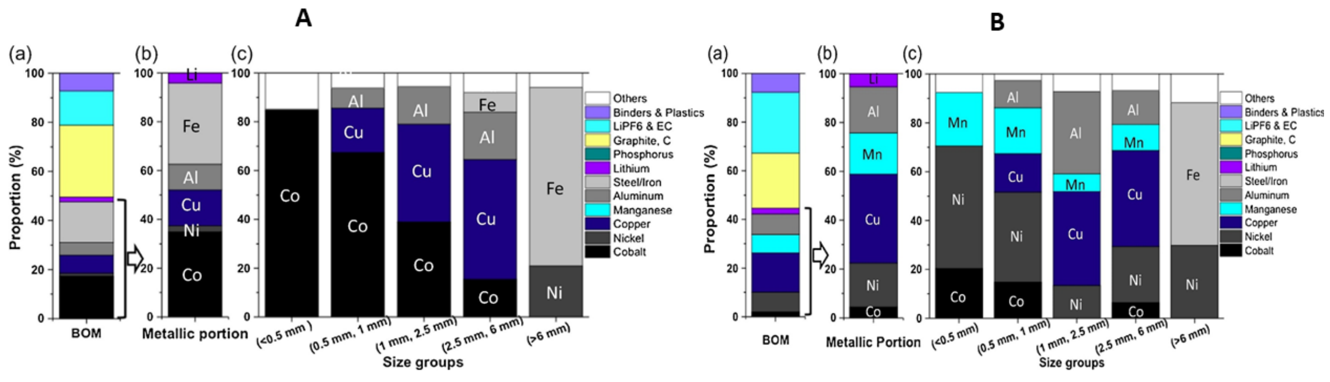
Battery sorting is an essential step in the pre-treatment of LIB recycling to separate the batteries with high accuracy. Nowadays, X-ray and X-ray fluorescence (XRF) techniques are widely used to sort the LIBs based on their composition. In industrial-scale battery sorting, automation and robots are necessary when working with a massive volume of battery packs (Zhang et al., 2012; Wang et al., 2020). LIBs have a little residual power when reaching their end of life; due to this reason, batteries explode during the recycling process. Before proceeding with the dismantling, the batteries must be completely discharged. The immersion of the batteries in a NaCl salt solution (5%-20%) is the most common method to deactivate the LIBs. The reactivity of metallic lithium in LIBs can be reduced by submerged in liquid nitrogen (Chen et al., 2015; Luidold, 2019). Manual dismantling is commonly employed to separate the plastic and metallic shells quickly and entirely from LIBs (Zheng et al., 2017; Boxall et al., 2018b). On a small scale, usually, a knife or screwdriver can remove the plastic cases. The saw is used to cut the metallic shell of the LIBs, and pliers are used for removing the shells to access the cathode and anode material of the battery. When working with large LIB packs from EVs, manual dismantling and mechanical treatments are not feasible in industrial-scale applications. In such cases, robots or high-level equipment automation can dismantle and separate the individual cells and remove the plastic and metallic shells.

Mechanical separation and organic solvent dissolution are standard physical techniques used in the LIB recycling process based on physical properties of LIBs such as density, solubility, magnetic property, etc. The current collectors and binders from LIBs should be separated from cathode materials to reduce the impurities during the physical recycling process to facilitate subsequent recycling processes (Dorella and Mansur, 2007; Goodenough and Park, 2013). Nowadays, ionic liquids are explored as an alternative to organic solvents due to their low vapour pressure, high thermal stability, and solvating properties (Huang et al., 2006; Markiewicz et al., 2013; Zeng et al., 2013b; Zeng and Li, 2014).

2.2 Crushing and sieving

A massive amount of LIBs produced after sorting and dismantling are used to reduce the volume and concentrate the valuable elements; crushing of spent LIB is an essential process. During the crushing process, all the constituents in the LIBs have been exposed, so a possibility of micro-short circuits due to contact between the cathode and anode materials (Zhang et al., 2014; Diekmann et al., 2016). The temperature may increase to 300°C during crushing because of the violent friction and high-speed impact of crushing; this generates many toxic gases such as HF when the electrolyte decomposes and volatilizes. The release of toxic gases can be prevented by crushing the LIBs under an inert condition (N_2 or CO_2) or liquid N_2.

Sieving is the last pre-treatment step to separate and concentrate the metallic fraction of spent LIBs, which provides an overview of the valuable metal distribution at various particle sizes in the crushed sample. The metals can be separated as five size fractions: <0.5 mm (ultrafine), 0.5–1 mm (fine), 1–2.5 mm(mid), 2.5–6 mm (coarse), and >6 mm(largest) using multiple sorting sieves aided with a vibration machine system. The size separation process has yielded 82% cobalt in ultrafine fraction, 68% in the fine fraction, and almost zero in the largest fraction for $LiCoO_2$ –based cathode materials, and for mixed cathode material, most of the cobalt and nickel were collected in ultra-fine and fine fractions (Figure 3A & 3B) (Wang et al., 2016; Wang et al., 2019). The particle size, distribution, and surface morphology of LIB black mass can be altered during the pre-treatment processes such as crushing, sieving, fine crushing, and separation (Al-Thyabat et al., 2013; Wang et al., 2016).



(a)The mass percentage of $LiCoO_2$ cathode batteries
(b) the metallic portion in (a); (c) the distribution of
metallic components in each size fraction of battery pack

(a) The mass percentage of mixed-metal cathode batteries
(b) the metallic portion in (a); (c) the distribution of
metallic components in each size fraction of battery pack

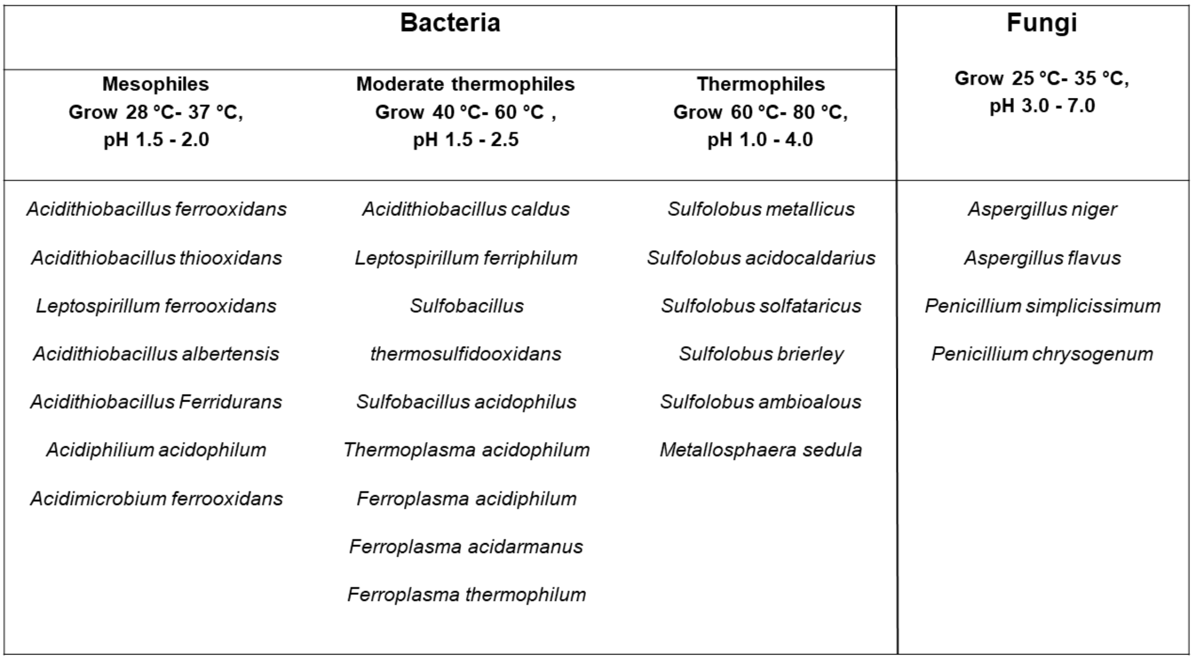
A) Sieving fractions of black mass from $LiCoO_2$ based LIBs **B) Sieving fractions of black mass from mixed LIBs**

Figure 3. Particle size separation of LIBs after crushing and sieving, Reprint with permission from Waste management 2016, 51, 204-213 Copyright (2016) Elsevier.

3. Bacteria and fungi used for bioleaching

Metal extraction from LIBs by bioleaching is an environmentally friendly process due to its ambient performing conditions, less energy requirement, less greenhouse gas emissions, less operational cost, and less contamination and processing hazards than conventional pyrometallurgical and hydrometallurgical processes (Krebs et al., 1997; Hoque and Philip, 2011; Kaksonen et al., 2018). However, the challenges encountered during the bioleaching of LIBs are directly linked to the toxic components such as metals, binders, electrolytes as well as secondary reactions of the process. A hugely diverse group of microorganisms involved in the bioleaching of metals are chemolithotrophic prokaryotes, heterotrophic bacteria, and fungi (Table 2) (Hedrich et al., 2011; Johnson, 2014; Quatrini and Johnson, 2019). Bioleaching technologies that were initially developed for the metal extraction from ores are currently applied to the recycling of LIBs. In the past 30-40 years, 20 bioleaching plants for the extraction of metals from ores have been commercialized; however, commercial LIB recycling is still developing. Four groups of microorganisms can be used to recycle LIBs based on their nutritional requirements (chemolithotrophs, heterotrophs, and facultative heterotrophs) (Bosecker, 1997; Johnson and Roberto, 1997; Mishra and Rhee, 2014; Jafari et al., 2018). According to the temperature of metabolism, these microbes organized into mesophiles (below 40 °C), moderate thermophiles (45 °C – 70 °C), and thermophiles (70 °C or above) (Duarte et al., 1993; Norris, 1997; Zhao and Wang, 2019). Microbes use inorganic materials and carbon-based material as their energy source aerobically named chemolithotrophs and heterotrophs, respectively; however, some of the heterotrophs can metabolize energy anaerobically without oxygen. The microorganisms can be used as an individual and combination (consortia) to test the bioleaching process's synergy (Işıldar et al., 2017; Yu et al., 2020b).

Table 2. Bacteria fungi are used in the biomining of metals.

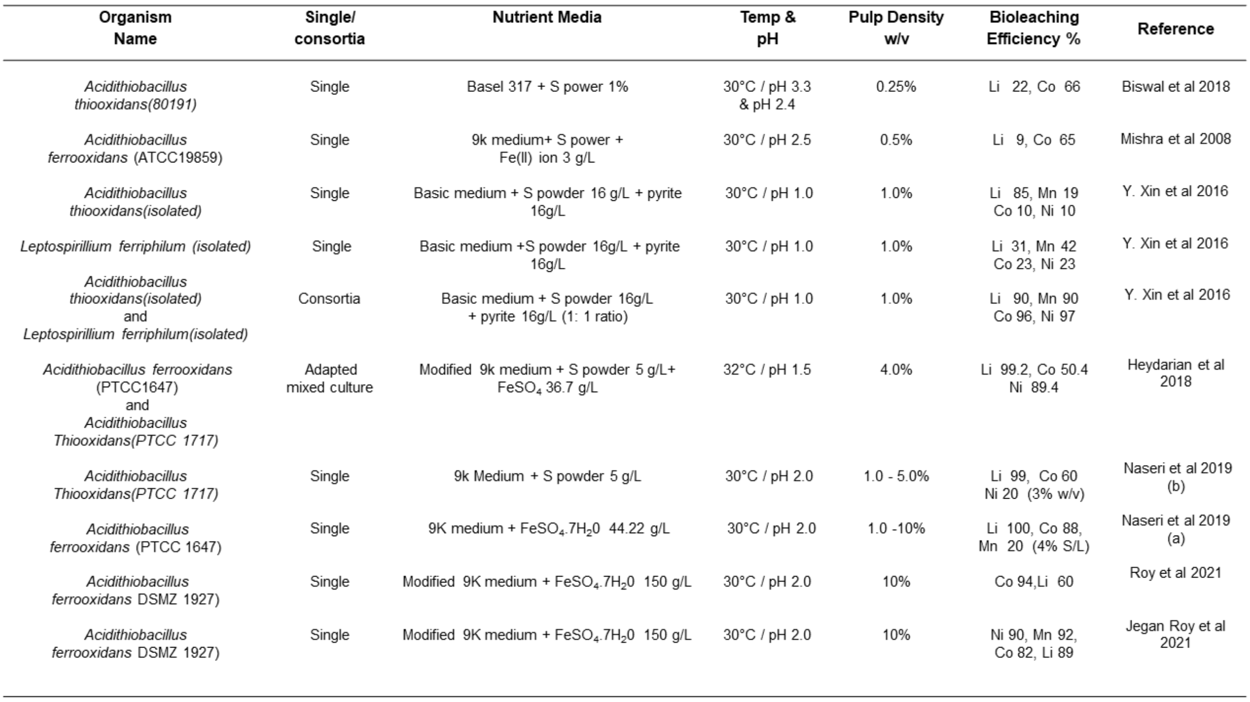


Bacteria			**Fungi**
Mesophiles Grow 28 °C- 37 °C, pH 1.5 - 2.0	**Moderate thermophiles Grow 40 °C- 60 °C , pH 1.5 - 2.5**	**Thermophiles Grow 60 °C- 80 °C, pH 1.0 - 4.0**	**Grow 25 °C- 35 °C, pH 3.0 - 7.0**
Acidithiobacillus ferrooxidans	*Acidithiobacillus caldus*	*Sulfolobus metallicus*	*Aspergillus niger*
Acidithiobacillus thiooxidans	*Leptospirillum ferriphilum*	*Sulfolobus acidocaldarius*	*Aspergillus flavus*
Leptospirillum ferrooxidans	*Sulfobacillus*	*Sulfolobus solfataricus*	*Penicillium simplicissimum*
Acidithiobacillus albertensis	*thermosulfidooxidans*	*Sulfolobus brierley*	*Penicillium chrysogenum*
Acidithiobacillus Ferridurans	*Sulfobacillus acidophilus*	*Sulfolobus ambioalous*	
Acidiphilium acidophilum	*Thermoplasma acidophilum*	*Metallosphaera sedula*	
Acidimicrobium ferrooxidans	*Ferroplasma acidiphilum*		
	Ferroplasma acidarmanus		
	Ferroplasma thermophilum		

3.1 S/Fe-Oxidizing bacteria (Mesophilic)

Mesophilic microorganisms, also named Fe/S-oxidizing bacteria, can solubilize the metallic fractions in LIBs shown in Table 2. The genus *Acidithiobacillus* are gram-negative, non-spore-forming rods, grow under aerobic conditions, and having a higher tolerance to metal toxicity have dominated the critical research in the bioleaching of LIBs (Rawlings, 1997; Jafari et al., 2018; Naseri et al., 2019a, b; Quatrini and Johnson, 2019). These bacteria employ atmospheric carbon dioxide (CO_2) as a carbon source and use ferrous iron (Fe^{2+}) or elemental sulfur (S^0) as their primary energy sources. They promote metal dissolution by producing biogenic sulfuric acid (H_2SO_4) and ferric ion (Fe^{3+}), which support metal oxidation (Xin et al., 2009; Wu et al., 2019). Bacterial bioleaching is executed in an acidic pH range of 1 to 3, where most metal ions remain in solution. The most broadly studied mesophilic microorganisms in bioleaching of LIBs are *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, and *Leptospirillum ferrooxidans*; some moderate thermophilic microbes are sulfur-oxidizing *Acidithiobacillus caldus* and iron-oxidizing *Leptospirillum ferriphilum* (Bosecker, 1997; Krebs et al., 1997; Schippers et al., 2014). Table 3 indicates the bioleaching efficiency of single and mixed bacteria. The leaching efficiency of the bioleaching process considerably decreases with an increasing pulp density due to the alkaline nature of waste LIBs and non-metallic components such as electrolytes and binders that are toxic to bacteria (Yu et al., 2020a).

Table 3. Mesophilic bacteria (S/Fe-Oxidizing) used in the bioleaching of spent LIBs.



Organism Name	Single/ consortia	Nutrient Media	Temp & pH	Pulp Density w/v	Bioleaching Efficiency %	Reference
Acidithiobacillus thiooxidans(80191)	Single	Basel 317 + S power 1%	30°C / pH 3.3 & pH 2.4	0.25%	Li 22, Co 66	Biswal et al 2018
Acidithiobacillus ferrooxidans (ATCC19859)	Single	9k medium+ S power + Fe(II) ion 3 g/L	30°C / pH 2.5	0.5%	Li 9, Co 65	Mishra et al 2008
Acidithiobacillus thiooxidans(isolated)	Single	Basic medium + S powder 16 g/L + pyrite 16g/L	30°C / pH 1.0	1.0%	Li 85, Mn 19 Co 10, Ni 10	Y. Xin et al 2016
Leptospirillium ferriphilum (isolated)	Single	Basic medium +S powder 16g/L + pyrite 16g/L	30°C / pH 1.0	1.0%	Li 31, Mn 42 Co 23, Ni 23	Y. Xin et al 2016
Acidithiobacillus thiooxidans(isolated) and *Leptospirillium ferriphilum(isolated)*	Consortia	Basic medium + S powder 16g/L + pyrite 16g/L (1: 1 ratio)	30°C / pH 1.0	1.0%	Li 90, Mn 90 Co 96, Ni 97	Y. Xin et al 2016
Acidithiobacillus ferrooxidans (PTCC1647) and *Acidithiobacillus Thiooxidans(PTCC 1717)*	Adapted mixed culture	Modified 9k medium + S powder 5 g/L+ $FeSO_4$ 36.7 g/L	32°C / pH 1.5	4.0%	Li 99.2, Co 50.4 Ni 89.4	Heydarian et al 2018
Acidithiobacillus Thiooxidans(PTCC 1717)	Single	9k Medium + S powder 5 g/L	30°C / pH 2.0	1.0 - 5.0%	Li 99, Co 60 Ni 20 (3% w/v)	Naseri et al 2019 (b)
Acidithiobacillus ferrooxidans (PTCC 1647)	Single	9K medium + $FeSO_4.7H_20$ 44.22 g/L	30°C / pH 2.0	1.0 -10%	Li 100, Co 88, Mn 20 (4% S/L)	Naseri et al 2019 (a)
Acidithiobacillus ferrooxidans DSMZ 1927)	Single	Modified 9K medium + $FeSO_4.7H_20$ 150 g/L	30°C / pH 2.0	10%	Co 94,Li 60	Roy et al 2021
Acidithiobacillus ferrooxidans DSMZ 1927)	Single	Modified 9K medium + $FeSO_4.7H_20$ 150 g/L	30°C / pH 2.0	10%	Ni 90, Mn 92, Co 82, Li 89	Jegan Roy et al 2021

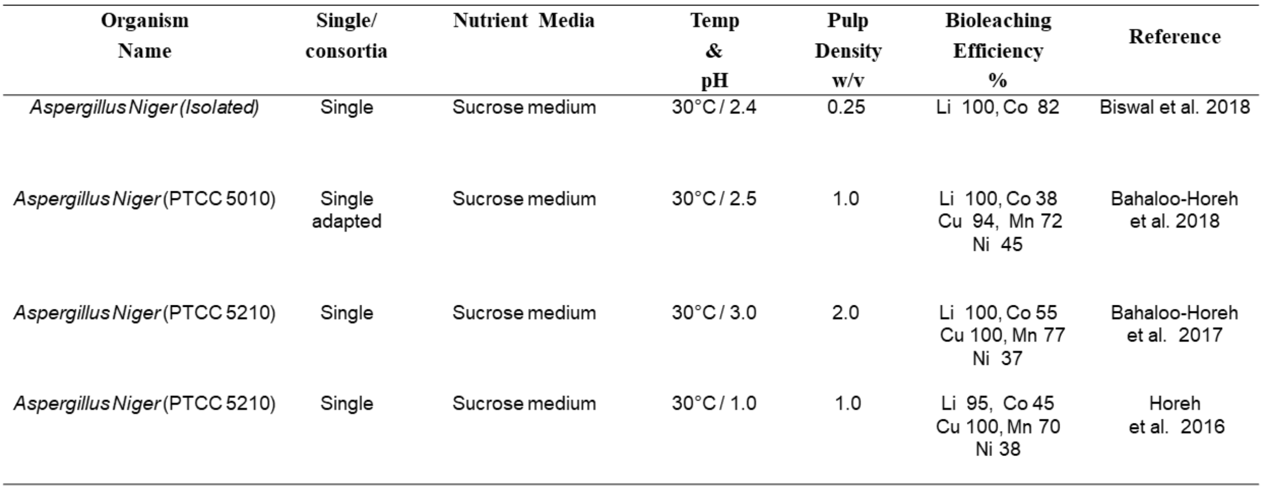
3.2 Thermophilic S/Fe-Oxidizing bacteria

Bacteria that can grow at higher temperatures are beneficial for bioleaching because elevated temperatures promote process kinetics. The most essential moderately thermophilic bacteria that have been used in bioleaching of e-waste are from the *Sulfolobus* genus such as *Sulfobacillus thermosulfidooxidans*, *Sulfobacillus acidophilus,* and *Thermoplasma acidophilum*, which can grow up to 50 °C and use as an individual or consortia (Ilyas et al., 2007; Ilyas et al., 2018). Extreme thermophiles such as *Sulfolobus acidocaldarius, Sulfolobus solfataricus, Sulfolobus brierley, and Sulfolobus ambioalous* can grow at 75-80 °C and pH in between 1 to 3 are isolated from volcanic springs (Plessis et al., 2007). These extreme thermophiles have higher bioleaching rates of metals than moderate thermophiles and mesophiles due to high-temperature growth. However, now no paper has been published to apply extreme thermophiles for the bioleaching of LIBs.

3.3 Heterotrophic fungi

Heterotrophic fungi utilize carbon substrates, such as waste sugars and agricultural waste as their primary nutrients, to produce organic acids such as citric acid, gluconic acid, lactic acid, malic acid, oxalic acid, etc. that contribute to metal leaching. Bioleaching studies of e-waste have been reported with heterotrophic fungi such as *Aspergillus niger, Penicillium simplicissimum, and Penicillium chrysogenum* (Bosecker, 1997; 2016; Liang and Gadd, 2017). *A. niger* is the essential fungi over many others due to ease of handling, harvesting, fermentation of a wide range of organic acids and high yields (Horeh et al., 2016; Kim et al., 2016). The advantages of fungal bioleaching of LIBs over bacteria are the bioleaching occurs at near-neural pH, and the LIBs are alkaline. Bioleaching of LIBs mediated by fungi carried out by acidolysis, complexolysis (generation of organic acids), redoxolysis. Moreover, the metals solubilization occurs under near alkaline conditions where organic acid metabolites are complexing with the metallic fractions, and the complexed metals have lower toxicity (Biswal et al., 2018) (Bahaloo-Horeh and Mousavi, 2017). The difference in metal removal efficiency has been reported with six *A. niger* species, when various carbon sources were used as their primary nutrient, there is a relation between carbon source and organic acid production (Zhuang et al., 2015; Pollmann et al., 2018). As the LIBs lack carbon-based nutrients, glucose, sucrose, or other waste carbon substrates should be supplemented during the bioleaching process, but it is costly. The metal recovery obtained by fungi bioleaching is usually lower than the acidophilic bacteria, and the pulp density used in fungi bioleaching is below 3% (Table 4).

Table 4. Heterotrophic fungi used in the bioleaching of spent LIBs



Organism Name	Single/ consortia	Nutrient Media	Temp & pH	Pulp Density w/v	Bioleaching Efficiency %	Reference
Aspergillus Niger (Isolated)	Single	Sucrose medium	30°C / 2.4	0.25	Li 100, Co 82	Biswal et al. 2018
Aspergillus Niger (PTCC 5010)	Single adapted	Sucrose medium	30°C / 2.5	1.0	Li 100, Co 38 Cu 94, Mn 72 Ni 45	Bahaloo-Horeh et al. 2018
Aspergillus Niger (PTCC 5210)	Single	Sucrose medium	30°C / 3.0	2.0	Li 100, Co 55 Cu 100, Mn 77 Ni 37	Bahaloo-Horeh et al. 2017
Aspergillus Niger (PTCC 5210)	Single	Sucrose medium	30°C / 1.0	1.0	Li 95, Co 45 Cu 100, Mn 70 Ni 38	Horeh et al. 2016

4. Bioleaching mechanisms

The bioleaching mechanism occurs through 3 pathways redoxolysis, acidolysis, and complexolysis, during the metal dissolution process. The kinetics of the bioleaching process relies on a) how the bacteria promote redox reaction, b) bacteria produced metabolites that complex with metals c) binding ability to metal substrates (Rohwerder et al., 2003; Vera et al., 2013; Wu et al., 2019). However, still, now debates are going on to conclude the exact mechanism of microbial metal extraction from ores and waste materials. Metals such as lithium, cobalt, nickel, copper, and manganese exist as complexes in LIBs in their nonvalent element form. Microbes require additional energy sources such as iron or elemental sulfur for the chemolithotrophic autotrophic bacteria and additional carbon sources (sugar) for heterotrophic fungi to break these element complexes (Schippers et al., 2014; Yu et al., 2020a).

4.1 Bioleaching mechanism of chemolithotrophic autotrophic bacteria

Autotrophic bacteria use atmospheric carbon dioxide as their carbon source and ferrous iron or elemental sulfur as their primary energy sources (Bosecker, 1997; Mishra et al., 2008; Xin et al., 2016). These bacteria are widely used for the bioleaching of LIBs due to their high tolerance for heavy metals toxicity (Jerez, 2009). Two mechanisms have been proposed for the metal bio-oxidation mediated by the autotrophic bacteria (a) direct oxidation of the metal substrate (b) indirect oxidation mediated by a redox couple, such as Fe^{3+} and Fe^{2+} present in the metallic substrate (Mishra et al., 2008; Mishra and Rhee, 2014).

4.1.1 Contact leaching or Direct oxidation

Bacterial cells attach directly themselves to the metal complex's surface (physical contact) indirect bacterial bioleaching and allow the metal substrates to directly accept electrons from the reduced metals as they are oxidized. Elemental sulfur and ferrous iron are the primary nutrients of chemolithotrophic bacteria and play as electron donors; however, they do not typically occur in spent LIBs to be added externally to the leaching medium. The bacteria oxidize elemental sulfur to sulfuric acid and ferrous ion to ferric ion, the production of biogenic sulphuric acid and ferric ions, which play the role of an oxidizing agent, solubilizes the metal ions from the spent LIB (Eqns 1-4).

$$S^0 + 1.5\ O_2 + H_2O \rightarrow 2H^+ + SO_4^{\ 2-} \rightarrow H_2SO_4 \quad \text{(Microbial)} \qquad (1)$$

$$H_2SO_4 + M \rightarrow MSO_4 + 2H^+ \quad \text{(M = metal)} \qquad (2)$$

$$4LiCoO_2 + 6\ H_2SO_4 \rightarrow 2\ Li_2SO_4 + 4CoSO_4 + 6\ H_2O + O_2 \qquad (3)$$

$$2Fe^{2+} + 1/2O_2 + 2H^+ \longrightarrow 2Fe^{3+} + H_2O \quad \text{(Microbial)} \qquad (4)$$

In $LiCoO_2$-based LIBs, the protonation of the oxygen atom results in the detachment of metals from LIB matrices because of the hydrolysis caused by the protonated oxygen atom.

The dissolution of cobalt and lithium is linked to the formation of their sulfate intermediates during the bioleaching process. The formation of cobalt and lithium sulfate intermediates by *A. ferrooxidan's* sulfur metabolism also promotes the dissolution of other metals in the leaching solution. The reaction indicates the metal dissolution occurs through a joined acidolysis-redoxolysis process; the kinetics are affected by the initial pH and ferrous iron concentration and the oxidation rate (Niu et al., 2014). The final extraction efficiency and leaching rate are directly correlated to Fe^{3+} ion formation (Vera et al., 2013; Niu et al., 2014).

$$4LiCoO_2 + 12H^+ \longrightarrow 4Li^+ + 4Co^{2+} + 6H_2O + O_2 \qquad (5)$$

$$Fe^{2+} + LiCoO_2 + 4H^+ \longrightarrow Fe^{3+} + Co^{2+} + Li^+ + 2H_2O \qquad (6)$$

$$Li_2O + 2H^+ \longrightarrow 2Li^+ + H_2O \qquad (7)$$

$$Fe^{2+} + Co^{3+} \longrightarrow Fe^{3+} + Co^{2+} (CoO) \qquad (8)$$

$$CoO + 2H^+ \longrightarrow Co^{2+} + H_2O \qquad (9)$$

$$2FeSO_4 + 2LiCoO_2 + 4H_2SO_4 \rightarrow Fe_2(SO_4)_3 + 2CoSO_4 + Li_2SO_4 + 4H_2O \quad (10)$$

Xin et al (2016) proposed the bioleaching mechanism for the NMC-based LIB by Fe^{2+}/Fe^{3+} redox reaction followed by the acid dissolution is shown below Eqns. (11) – (15) (Xin et al., 2016).

$$4Fe^{2+} + O_2 + 4H^+ \longrightarrow 4Fe^{3+} + 2H_2O \qquad (11)$$

$$Li_2O + 2H^+ \longrightarrow 2Li^+ + H_2O \qquad (12)$$

$$M^{4+}/M^{3+} (M{=}Mn, Co, Ni) + Fe^{2+} \longrightarrow M^{2+} (MO) + Fe^{3+} \qquad (13)$$

$$MO + 2H^+ \longrightarrow M^{2+} + H_2O \qquad (14)$$

$$6LiNi_{1/3}Mn_{1/3}Co_{1/3}O_2 + 12H_2SO_4 + 6FeSO_4 \longrightarrow 3Fe_2(SO_4)_3 + 2MnSO_4 + 2NiSO_4 + 2CoSO_4 + 3Li_2SO_4 + 12H_2O \qquad (15)$$

4.1.2. Contactless leaching or Indirect Oxidation

In indirect leaching, the bacteria only have their catalytic function and not in contact with the waste LIB’s surface because they accelerate the reoxidation of ferrous ion, which is about 10^5-10^6 times faster than the chemical oxidation at pH 2-3 (Wang et al., 2018; Huang et al., 2019; Wu et al., 2019). The bacteria oxidize elemental sulfur to sulfate, and protons lead to the formation of biogenic sulphuric acid (H_2SO_4); also, Fe^{2+} is oxidized to Fe^{3+}. In indirect bioleaching, biogenic sulphuric acid leaches out the metals from the waste LIBs through proton attack (acidolysis) and redoxolysis. Ferric ions act as an oxidizing agent to enhance the leaching reaction (Eqns 16-18)

$$S^0 + 3\,O_2 + 2H_2O \rightarrow 2\,H_2SO_4 \quad \text{(Microbial)} \quad (16)$$

$$Fe^{2+} \rightarrow Fe^{3+} + e^- \quad \text{(Microbial)} \quad (17)$$

$$2\,Fe^{3+} + Cu^0 \rightarrow Cu^{2+} + 2\,Fe^{2+} \quad \text{(chemical)} \ (18)$$

4.2 Bioleaching mechanisms of heterotrophic bacteria and fungi

In heterotrophic bacteria and Fungi mediated bioleaching, no specific descriptions were involved with metal contact during the dissolution process. These bacteria and fungi excrete protons, organic acids during their metabolism, oxidize the metals from LIB with the aid of O_2/H_2O redox couple followed by making complexation with the protonated metals (Eqns 19-26) (Bahaloo-Horeh and Mousavi, 2017; Bahaloo-Horeh et al., 2018; Biswal et al., 2018). However, fungal-mediated bioleaching is involved by organic acids excreted by the fungi via acidolysis and complexolysis. They also change the medium's oxidation potential (redoxolysis) or the combination of the above three (Bosecker, 1997; Liang and Gadd, 2017). Protonation results in the release and increased mobility of free metal cations produced by acidolysis, favour the metal solubilization at low pH (Eqn (19)-(26)). Complexolysis is the crucial mechanism of fungal leaching in which the metal cations form a complex with the anions of organic acids (Horeh et al., 2016; Bahaloo-Horeh and Mousavi, 2017; Biswal et al., 2018). *A. niger* and *P. simplicissimum* are the fungi that have received the most attention in the bioleaching of metals from waste LIBs by producing various organic acids as their metabolites in sucrose ($C_{12}H_{22}O_{11}$) medium (Deng et al., 2013; Mishra and Rhee, 2014; 2016).

$$\underset{\text{(Sucrose)}}{C_{12}H_{22}O_{11}} + H_20 \rightarrow \underset{\text{(Glucose)}}{C_6H_{12}O_6} + \underset{\text{(Fructose)}}{C_6H_{12}O_6} \quad (19)$$

$$C_6H_{12}O_6\,\text{(Glucose)} + 1.5O_2 \rightarrow C_6H_8O_7\,\text{(citric acid)} + H_2O \quad (20)$$

$$C_6H_8O_7\,\text{(citric acid)} \rightarrow (C_6H_7O_7)^- + H^+ \quad (21)$$

$$(C_6H_7O_7)^- + M^+\text{(metal)} \rightarrow \text{metal citrate complex} \quad (22)$$

$$C_6H_{12}O_6\,\text{(Glucose)} + 4.5O_2 \rightarrow 3C_2H_2O_4\,\text{(oxalic acid)} + 3H_2O \quad (23)$$

$$C_2H_2O_4\,\text{(oxalic acid)} \rightarrow (C_2HO_4)^- + H^+ \quad (24)$$

$$(C_2HO_4)^- + M^+\text{(metal)} \rightarrow \text{metal oxalate complex} \quad (25)$$

$$7\,C_2H_2O_4 + 2LiCoO_2 \rightarrow 2LiHC_2O_4 + 2Co(HC_2O_4)_2 + 4H_2O + 2CO_2 \quad (26)$$

5. Factors affecting the bioleaching process

The leaching efficiency of the bioleaching process mainly depends on the capacity of microorganisms and the composition of LIBs. The yield of the metal extraction can be maximized by optimizing the microbe's growth conditions, increasing the production of necessary metabolites for bioleaching. The factors such as growth nutrient, pH, redox potential, temperature, O_2, and CO_2 supply, metal toxicity, and pulp density affect the metal leaching efficiency (Bosecker, 1997).

5.1 Microbial nutrients

The type of specific nutrient and their dosage is necessary for microbial growth and their metabolites production, which is involved in the metal solubilization of LIBs. Chemolithoautotrophic bacteria such as *A. ferrooxidans* required ferrous salts as their primary nutrients to produce biogenic sulphuric acid and Fe^{3+} ions production, and *A. thiooxidan* required elemental sulfur (Mishra et al., 2008; Dolker and Pant, 2018; Zhang et al., 2018a; Quatrini and Johnson, 2019). The growth media is also supplemented with ammonium, phosphate, and magnesium salts for their optimum growth. These bacteria do not require any carbon source because they utilize CO_2 from atmospheric nitrogen. However, fungi required sucrose or glucose as their carbon source, and it can be substituted with food-processing wastes, potato processing waste, sugarcane bagasse, which are cost-effective (Kim et al., 2016; Bahaloo-Horeh and Mousavi, 2017).

5.2 O_2 and CO_2 supply

Sufficient oxygen supply is necessary for proper growth and high activity of the microorganism during the bioleaching process, and this can be achieved by aeration, stirring, or shaking. On a massive scale of LIBs bioleaching, it is tricky to make enough oxygen supply. CO_2 supply is required when the bioleaching perform in anaerobic conditions with specific microorganisms (Naseri et al., 2019a).

5.3 pH

According to the optimum microbial growth and leaching conditions, it is necessary to adjust the medium's correct pH value to solubilize the metals from LIBs. The optimum pH for the *Acidiophilic* bacteria is in the range of 2.0 – 2.5, and the oxidation of ferrous salt and element sulfur(S^0) is high at this range (Bosecker, 1997; Mishra et al., 2008). At pH below 2.0, *A. ferrooxidans* can adapt to the environment when acid production increases (Heydarian et al., 2018). The optimum pH value of *A. niger* mediated bioleaching is around 5.0. Due to the LIB powder’s alkaline nature, the pH increases according to the pulp density (S/L) in the bioleaching process (Bahaloo-Horeh et al., 2018).

5.4 Microbiological redox potential

Redox potential is a crucial and essential factor in bioleaching mediated by ferric sulfate, which has already been proven to be necessary (Sandström et al., 2005; Córdoba et al., 2008). The Fe^{3+}/Fe^{2+} redox couple primarily determines the oxidation/reduction potential (ORP), with high concentrations of Fe^{3+} indicating high potentials. (Li et al., 2013; Zhao et al., 2015; Billy et al., 2018; Masaki et al., 2018). By the addition of chemical reductants or limiting the oxygen supply, the redox potential can be controlled. The redox potential increases when the ferrous ions oxidize into ferric ions during the bacterial growth; however, it dropped sharply in the early days of LIB bioleaching and more severe at high pulp densities (S/L) (Naseri et al., 2019a). Still, no publication is reported the optimum ORP range to increase the leaching efficiency of metals in the LIB bioleaching.

5.5 Temperature

Temperature is also an essential factor in microbial-mediated bioleaching of LIBs. The leaching efficiency can be increased by performing the bioleaching process at the optimum temperature of the microorganism. The optimum temperature for the oxidation of ferrous to ferric ions is between 28 to 30 °C by *A. ferrooxidans* (Naseri et al., 2019a). Most of the *A. niger* mediated bioleaching has been performed at 30 °C (Horeh et al., 2016). Even though higher temperatures favour improving the kinetics of the bioleaching process, the microbes can have died at high temperatures. However, moderately thermophilic and thermophilic bacteria can be used at their optimum temperature to increase the leaching efficiency (Plessis et al., 2007).

5.6. Metal tolerance and pulp density

Microorganisms such as acidophilic bacteria have a high tolerance to metals such as nickel, copper, and zinc; however, different strains have different sensitivity to metal tolerance. Individual strains or consortia can be made to adopt a higher concentration of metals by step by step increase of metal concentration from low to high (Bosecker, 1997). The metal toxicity is high at high pulp densities, reducing the metal dissolution due to less microbial activity. Bioleaching of LIBs at high pulp densities increases the leaching medium's viscosity, thus reducing the dissolved oxygen and air distribution to the microorganism. When the viscosity is high, the penetration of oxygen into the depth of the leaching medium is less; it changes the metabolism of the microorganism that reduces metal leaching efficiency (Naseri et al., 2019a).

6. Process enhancements and metal recovery

6.1 Adoptive culture

Toxic metals present in the LIBs are the abiotic stresses to the microorganism that leads to cellular responses. The growth, metabolism, and survival of the microorganism are influenced by the surrounding metals which leached out during the leaching process. The metabolic pathways of microbes responsible for producing biogenic acids are inhibited by the metals leaching out from LIBs during the bioleaching; they also denature the cells' nucleic acid and proteins. The toxicity of LIBs to the microorganism is influenced by the heavy metal concentration and the increasing contact time. The microorganism should have a stable population with competent functionality during the bioleaching process and sustain an acidic environment with inhibitory factors. Before starting the bioleaching process, the microorganism must be adapted and resistant to the toxic LIB system and continue their growth and activity during the process. Serial adaptation of microorganisms over a prolonged period by gradually increasing the pulp densities of the LIBs can result in the high tolerance of the bacterial or fungal strain to the metal toxicity. There is a correlation between the decrease in bacterial growth and activity with an increased pulp density of LIBs. The microbial cells can adapt to the LIB powder through a sequential sub-culturing process by exposing them to higher concentrations of toxic metals step by step.

Heydarian et al. (2018) have studied the bioleaching of spent LIBs from laptops using a mixed culture of adapted acidophilic bacteria *A. ferrooxidans* and *A. thiooxidan*. The adaptation studies were carried out with a pulp density start from 2.5g/L to 40g/L of LIB powder at pH 1.5 with $FeSO_4$ dosage 36.7 g/L and sulfur powder(S^0) 5.0 g/L in the medium with an inoculum ratio of 3/2. The number of days required for this adaptation study was 128, and 40 g/L is the threshold of the bacterial tolerance of LIBs powder. The bioleaching process performed with adapted bacteria culture could be leached out 99.2% lithium, 50.4% cobalt, and 89.4% nickel from the spent LIBs. Another study performed using adapted metal tolerant *Aspergillus niger* for the bioleaching of metals from spent LIBs in mobile phones (Bahaloo-Horeh et al., 2018). The adaptation process started from 0.3% (w/v) of LIB powder and end with 1.0 % (w/v) in sucrose culture medium at 30 °C with 130 rpm. The adapted *A.niger* strain produced biogenic organic acids such as citric, oxalic, malic, and gluconic acids and 100% lithium, 94% copper, 72% manganese, 62% aluminum, 45% nickel, and 38% cobalt were leached out when 1% (w/v) of LIB powder.

6.2 Addition of metal ions in bioleaching

The extensive scale application of the bioleaching process has the main drawback of the slow kinetics of metal dissolution; that can be improved by adding specific metal ions such as Ag^+, Cu^{2+}, Hg^{2+}, Co^{2+}, etc. These metal cations can increase the leaching efficiency by speeding up electron transfer, thus improving the metal dissolution process by oxidation and form metal intermediate products that are soluble easily in water. Zeng et al. reported that silver ions (Ag^+) could be used in $LiCoO_2$ based bioleaching process mediated by *Acidithiobacillus ferrooxidans* (Zeng et al., 2013a). The crucial role of the silver ion is the formation of $AgCoO_2$ intermediate product, and Fe^{3+} ions oxidize the intermediate to cobalt (II) ions, and the consumed Ag^+ is released into the solution can be recovered and used again.

$$Ag^+ + LiCoO_2 \longrightarrow AgCoO_2 + Li^+ \qquad (27\ )$$

$$AgCoO_2 + 3Fe^{3+} \longrightarrow 3Fe^{2+} + Ag^+ + O_2 + Co^{2+} \qquad (28)$$

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{A.\ ferrooxidans} 4Fe^{3+} + 2H_2O \qquad (29)$$

The same group has also reported using Cu^{2+} ions instead of Ag^+ ion to increase the cobalt dissolution in LIBs using *A. ferrooxidans* (Zeng et al., 2012). The leaching efficiency of cobalt was increased to 99.9% in 6 days with the presence of 0.75 g/L Cu^{2+} ions; however, the leaching was only 43.1% even after 10 days in the absence of copper ions. Copper ions formed the intermediate $CuCo_2O_4$ by cationic exchange reactions, followed by Fe^{3+} ions oxidize the intermediate to release the Co^{2+} ions.

$$Cu^{2+} + 2LiCoO_2 \longrightarrow CuCo_2O_4 + 2Li^{+} \qquad (30)$$

$$CuCo_2O_4 + 6Fe^{3+} \longrightarrow 6Fe^{2+} + Cu^{2+} + 2O_2 + 2Co^{2+} \qquad (31)$$

The challenge of using these metal ions in the bioleaching process is the inhibitory/toxic effects on the microorganisms. If the Ag^{+} ions use above 0.1 mg/L, the Fe^{2+} ions can be replaced by the Ag^{+} ions in the active site of the enzyme (ferric ion oxidoreductase) in *A. ferrooxidans*, which has the oxidizing ability. Thus, it is essential to engineer the microorganism to tolerate a higher concentration of metallic ions such as Ag^{+} and Cu^{2+} (Pathak et al., 2017).

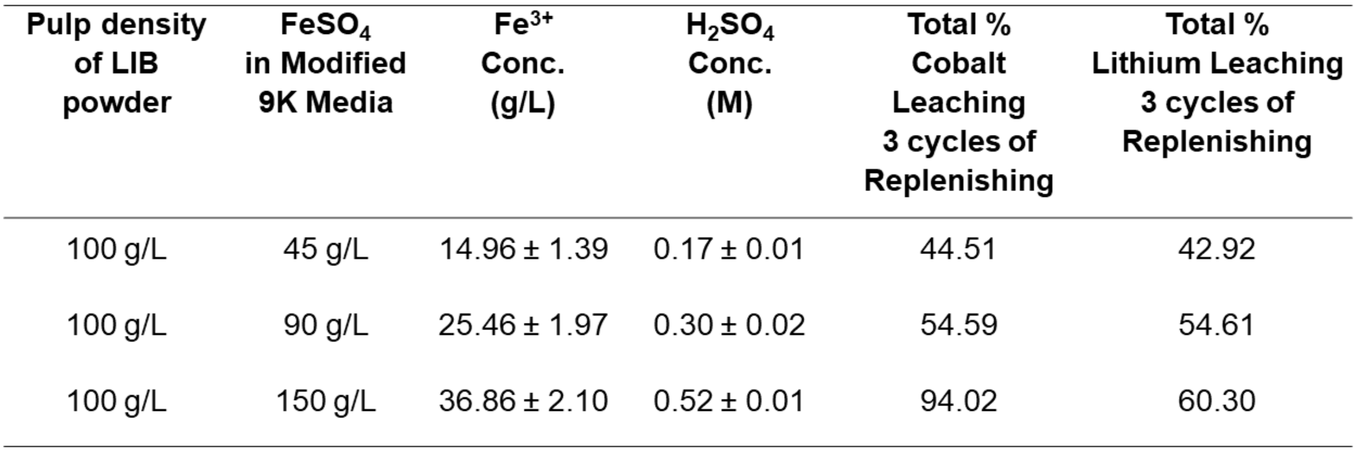
6.3 Ultrasound bioleaching or Sonobioleaching

Another approach to overcome the drawback of slow kinetics is using ultrasound in a bioleaching process called sonobioleaching; the metal dissolution using metabolites produced by the microbes increased by using ultrasonic in the form of micro-jet and shock wave (Anjum et al., 2014). The penetration of bio-lixiviant to the solid particles becomes easier when ultrasonication is used in the bioleaching process, and the leaching rate is enhanced even at a low concentration of biogenic acids (Vyas, 2018). Ultrasonication increases the stirring at macroscopic and microscopic levels, thus improving microbial metabolic activities and changing cell membranes' permeability. Ultrasonication with low-frequency leads the microbial growth improvement; however, high-frequency favours cell wall disruption (Vargas et al., 2004). Still, now no work has been reported for the use of ultrasonication in waste LIB bioleaching.

6.4 Increasing the metabolite production and replenishing bacterial culture

The bioleaching process's leaching efficiency is determined by the metabolites present in the culture media. Metabolite production can be increased by increasing the nutrient of the microbes. In our study, increasing the $FeSO_4$ concentration in the nutrient media could increase the biogenic sulphuric acid production and ferric ion concentration in the culture media. A high concentration of biogenic sulphuric acid could destroy the battery particles and release valuable metals from the inner part of the battery powder. The leaching efficiency of metals from LCO and NMC based LIBs could increase up to 90% at a high pulp density of 100 g/L by three cycles of bacterial culture replenishment, which has a high level of biogenic H_2SO_4 and ferric ion (Jegan Roy et al., 2021; Roy et al., 2021). Table 5 shows that cobalt and lithium's leaching efficiency increases with increasing the biogenic H_2SO_4 and the ferric ion concentration in the culture medium during the bioleaching process by *A. ferrooxidans.*

Table 5. Cobalt and lithium’s leaching efficiency correlated with H_2SO_4 and Fe^{3+} concentration, when a different dosage of $FeSO_4$ used in the modified 9K media for *A. ferrooxidans* growth, reproduced from *Journal of cleaner production* 2021, 280, 124242 Copyright Elsevier.



Pulp density of LIB powder	$FeSO_4$ in Modified 9K Media	Fe^{3+} Conc. (g/L)	H_2SO_4 Conc. (M)	Total % Cobalt Leaching 3 cycles of Replenishing	Total % Lithium Leaching 3 cycles of Replenishing
100 g/L	45 g/L	14.96 ± 1.39	0.17 ± 0.01	44.51	42.92
100 g/L	90 g/L	25.46 ± 1.97	0.30 ± 0.02	54.59	54.61
100 g/L	150 g/L	36.86 ± 2.10	0.52 ± 0.01	94.02	60.30

6.5 Microbial consortia for bioleaching

Microbial consortia are a diverse range of different microbial species that are more tolerant in extremes and variable environmental conditions than individual cultures. Microorganisms such as *A. thiooxidans, A. ferrooxidans, A. caldus, L. ferrooxidans, and L. ferriphilium,* have been mixed and made defined microbial consortia and used for biomining of metals (Gumulya et al., 2018). Usually, microbial consortia form a biofilm and create a microenvironment, increasing the leaching kinetics; however, their functions and activities, such as interaction and communication, are still unknown. Gumulya et al. (2018) have consolidated different bacterial consortia groups and their design purposes in biomining. A similar kind of bacterial consortia can be developed for LIB bioleaching. A mixed culture of iron-oxidizing and sulfur-oxidizing bacteria such as *A. ferrooxidans* and *A. thiooxidans L. ferriphilum, Alicyclobacillus* sp, and *Sulfobacillus* sp. have been used in the bioleaching of LIBs in the combination of two strains (Niu et al., 2014; Xin et al., 2016; Boxall et al., 2018a; Heydarian et al., 2018).

6.6 Genetic engineering of microorganism for bioleaching

The Bioleaching process is often hindered by the presence of inhibitory metals present in LIBs; however, synthetic biology tools such as genetic engineering can enhance the tolerance and robustness of bioleaching microbes to various stress factors present in harsh environments, thus increase bioleaching efficiency. The genetically engineered microbes have the potential to improve the bioleaching process by increasing tolerance to fluctuating and challenging process conditions and shortening the time required for metal extraction. Synthetic biology tools can alter metabolic pathways of novel microbes such as an acid endurance pathway, heavy metal resistance, and Rubisco-free carbon fixation pathway for the bioleaching of LIBs (Baker-Austin and Dopson, 2007; Dopson and Holmes, 2014; Gumulya et al., 2018).

Gumulya et al. proposed four potential targets of engineering pathways for the biomining microorganisms (Figure 4).

A. **Acid tolerance**. Acidophilic bacteria survive at low pH conditions by inhibiting the proton (H^+) penetration into the cell and overproducing enzymes/chemicals to bind and sequester protons to maintain pH. Microorganisms also increase their acid tolerance by increasing the synthesis of organic acids and involving in DNA and protein repair systems.

B. **Metal tolerance.** Microorganisms are detoxifying the cells by multiplying several carriers for the efflux of metal cations and toxic compounds.

C. **Osmotolerance:** Microorganisms gain the ability to withstand high levels of osmotic stress by compiling different osmoprotectants.

D. **Thermotolerance:** Thermostability of the cells can be increased by incorporating thermostable enzymes and proteins, expressing heat shock proteins, increasing the DNA repair system, and modifying the membrane composition with fatty acids or tetraether lipids

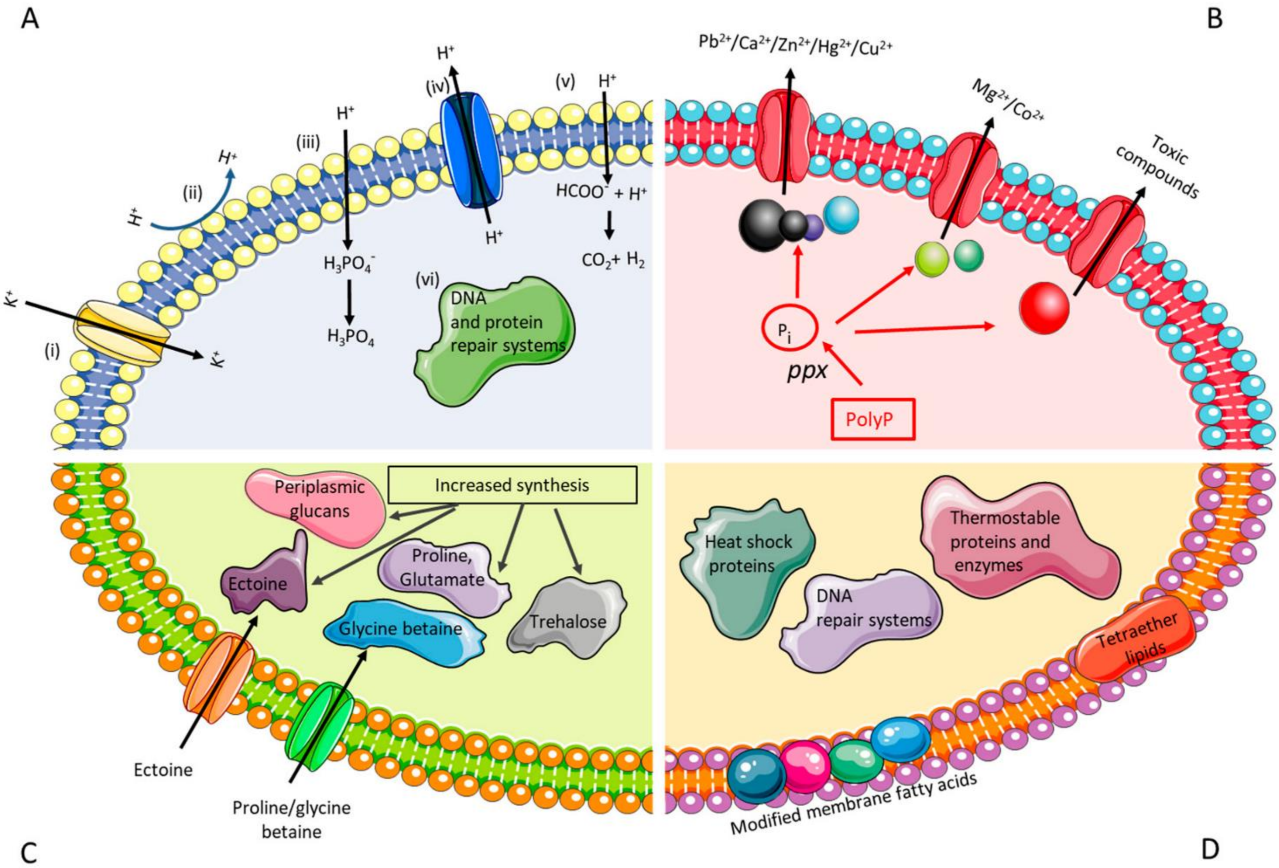


Figure 4. Potential targets A. Acid tolerance, B. **Metal tolerance, C. Osmotolerance, D. Thermotolerance for engineering microorganisms for bioleaching.**

Adopted from *Genes* **2018**, 9, 116 Copyright MDPI

6.7 Metal recovery and regeneration Process

The bioleaching liquor contains valuable metals such as Co, Li, Ni, Mn, and other metals Fe, Al, and Cu. It is necessary to remove these impurities before performing the cathode regeneration because these metals affect the testing results. Thus, these metals can be removed by serial precipitation at pH 3-5 using NaOH or NH_4OH, followed by oxalate or carbonate precipitation (Gratz et al., 2014; Verma et al., 2019). The cathode regeneration can be accomplished through a hydrothermal or calcination process that involves LiOH or Li_2CO_3 addition (for lithiation) and other metals salts in the appropriate proportions (Yang et al., 2017; Zhao et al., 2020). Graphite can be regenerated from the bioleaching residue by cleaning it with acid and then calcining it at high temperatures. (Ma et al., 2019; Yang et al., 2019; Natarajan and Aravindan, 2020).

7. Challenges and prospects

The extraction of valuable metals from ores, concentrates, and wastes by bioleaching have enticed many academic and commercial researchers, extensively studied significant progress in recent days, and emerged as an attractive option to the conventional methods. The extraction of metals from waste LIBs can be facilitated by molecular and microbial engineering solutions of microorganisms, such as microbial consortia, increasing the metabolite production, adaptation to toxic environments, and genetic engineering. Understanding the identity, function, and interactions of microbial catalysts can benefit in optimizing the bioleaching processes (Baniasadi et al., 2019). The recycling process's systematic engineering implies source control, processing control, treatment, and the development of green and straightforward processes. The designing of the recycling process is most important because the environmental constraints of LIBs recycling depend on the recycling approaches. Metal recovery from LIBs by bioleaching depends on its environmental impact, cost-effectiveness, smooth operation, and future development (Innocenzi et al., 2017). The bioleaching process of waste LIBs is still far from industrial application due to some drawbacks such as low adaptability and rigorous leaching conditions. However, waste LIBs have more metal content than natural ores; thus, with the aid of inorganic or organic acid, active cathode materials can be leached out well in an eco-friendly and energy-efficient way. Factors such as leaching conditions, nutrient cost, temperature, liquid−solid ratio, and reaction time determine the process cost and energy consumption (Zhang et al., 2018b).

The problems and challenges that need to be addressed during the recycling process are

1. The recovery of the electrolytes such as $LiPF_6$, binders such as PVDF, and the anode materials such as graphite, should also be considered; more research needs to be done to recycle them properly by the bioleaching process (Zhang et al., 2018b; Fan et al., 2020).
2. The overall cost of the bioleaching process should be reduced to get more benefits for manufacturers to recycle spent LIBs. Compared to the conventional processes, the bioleaching process does not require any critical instrumentation or sampling to deliver high metal recovery. It requires simple services such as assaying, instrument engineering, and maintenance to be implemented on a commercial scale for LIBs recycling (Moazzam et al., 2021). The capital costs of bioleaching are associated with the construction of bioreactors, the supplement of nutrients and reagents, operating costs, provision services, and services (Thompson et al., 2017; Li et al., 2018). The overhead costs of bioleaching are less than conventional methods such as pyrometallurgy (high energy involved for smelting and roasting) and hydrometallurgy (additional cost for acid waste disposal) (Chen et al., 2019; Xu et al., 2020). Most of the economic profits obtained from the LIBs recycling process come from the recovery of the valuable metals, so the bioleaching processes should focus on the recovery of high-value electrode materials (Baniasadi et al., 2019; Gu et al., 2019). The graphite present in the bioleaching residue can be recycled and reused by removing the unleached metals from the residue. The LIBs bioleaching process's profit can be raised by using low-cost nutrients of microbes, simple and few operational processes, and obtaining high-purity of metals in higher yields (Kim et al., 2016; Bahaloo-Horeh and Mousavi, 2017).
3. The impurities present in the final products can negatively affect their performance when reusing in the direct synthesis of cathode materials; so the type and amount of impurities in the final product should be reduced as much as possible during the recycling process (Chu et al., 2020; Zhao et al., 2020).

4. The environmental concern of the bioleaching process can be considered based on non-toxic reagents and recycling technologies with less CO_2 emissions and energy consumption. The end treatment should reduce pollution emissions based on the concept of sustainable development and pollution control strategy (Fan et al., 2020; Yu et al., 2020b).

8. Conclusions

The bioleaching process is a sustainable, eco-friendly, cost-effective, and energy-efficient process with low greenhouse gas emissions for the metal recovery from LIBs. The microorganisms used in bioleaching produce biogenic sulfuric acid or organic acids. The bacteria oxidize ferrous ions to ferric ions, which are then used as a reductant to convert the metal ion's valency. These bacteria can solubilize the metals from LIBs through the acidolysis and redoxolysis process. The fungi produce organic acids that can dissolve the metal ions with the aid of oxidants such as H_2O_2 via the complexolysis process. However, the bioleaching approach has some limitations towards the metal extraction from LIBs on a massive scale due to the significant challenges, such as cultivation time, slow process kinetics, low solid to liquid ratio (pulp density), and metal toxicity. The success of LIB bioleaching is dependent on the production of metabolites, the microorganism's ability to tolerate toxic metals, the cultivation of microbes with low-cost nutrients, and the enhancement of process kinetics.

The time required to cultivate microorganisms can be reduced by optimizing the culture conditions. The omics approaches can be used to identify potential molecular targets for modifying microorganisms to enhance their competencies, such as tolerance against metal toxicity, acidic environment, and genetically enhance the metabolites’ production (Baniasadi et al., 2019; Nazanin Bahaloo-Horeh, 2019). With improved process kinetics, increased pulp density, and enhanced the microorganism's tolerance, the bioleaching approach can be potentially adapted to the recycling industry. By using the bioleaching method, a leaching efficiency of 80% to 90% was achieved at a high pulp density of 100 g/L (Jegan Roy et al., 2021; Roy et al., 2021), which is a good indication for the potential application of bioleaching for industrial-scale recycling of spent LIBs in the near future. Conclusively, LIB’s recycling strategy should be based on the principles of high efficiency, high economic return, high environmental benefit, and high safety by redesigning, reusing, recycling, or refurbishing spent LIBs (Fan et al., 2020).

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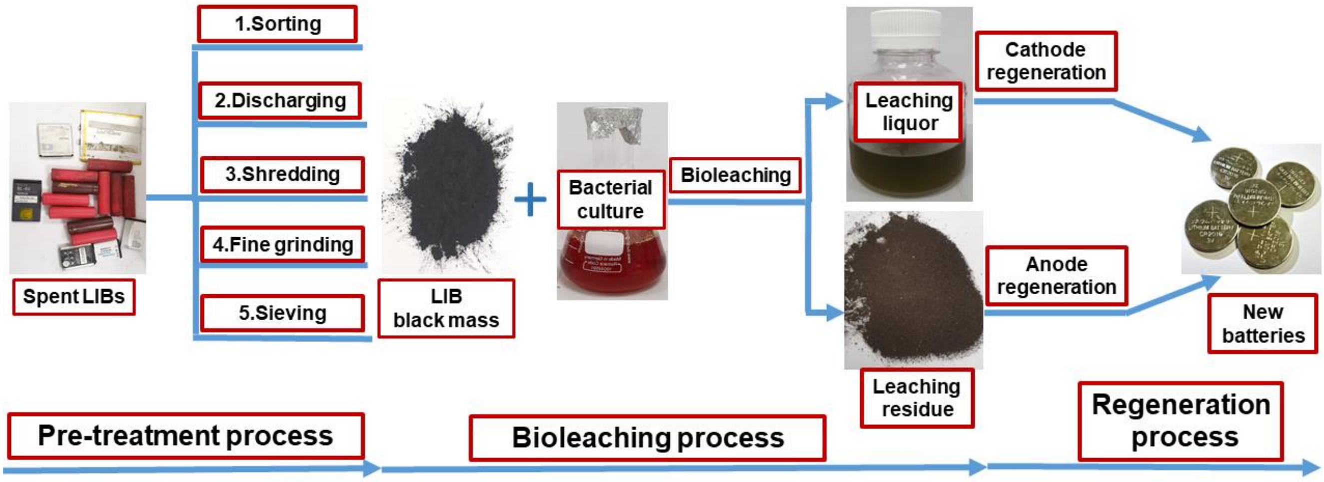
Graphical abstract



Highlights

- The bioleaching process, an emerging trend for extracting valuable metals Co, Li, Ni, and Mn from Lithium-ion batteries (LIBs) is discussed.
- Extensive information on the pre-treatment process, microorganisms, and their mechanisms in the bioleaching of LIBs.
- Factors influencing the bioleaching process, methods for improving leaching efficiency and metal recovery, challenges, and prospects are discussed in detail.