



Research article

Improvement of Li and Mn bioleaching from spent lithium-ion batteries, using step-wise addition of biogenic sulfuric acid by *Acidithiobacillus thiooxidans*

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ABSTRACT

Conventional spent lithium-ion battery (LIB) recycling procedures, which employ powerful acids and reducing agents, pose environmental risks. This work describes a unique and environmentally acceptable bioleaching method for Li and Mn recovery utilizing *Acidithiobacillus thiooxidans*, a sulfur-oxidizing bacteria that may produce sulfuric acid biologically. The novel feature of this strategy is the step-by-step addition of biogenic sulfuric acid, which differs significantly from conventional methods that use chemical reagents. We expected that gradually introducing biogenic sulfuric acid produced by *A. thiooxidans* would improve metal leaching at high pulp density. To investigate this, LIBs were disassembled and bioleached with or without a step-wise addition of the biogenic sulfuric acid approach. The impact on leaching efficiency, time, and ultimate product quality was assessed.

Direct bioleaching yielded modest Li (43 %) and Mn (15 %) recoveries. However, bioleaching greatly increased metal recovery with the step-wise addition of biogenic acid. Li and Mn leaching efficiency were 93 % and 53 %, respectively, at a high pulp density of 60 g/L, while leaching time was reduced from 16 to 8 days.

Following bioleaching, $Mn(OH)_2$ and Li_2CO_3 were successfully precipitated from the leachate at more than 90 % purity. This study shows that gradually adding biogenic sulfuric acid can efficiently recover Li and Mn from waste LIBs. This approach has several environmental and economic advantages over conventional methods. The step-wise addition optimizes the leaching environment, increasing metal recovery rates while reducing the development of hazardous byproducts. This approach is environmentally friendly because it decreases greenhouse gas emissions and chemical waste. Economically, this technology offers potential cost savings through less chemical usage, shorter processing times, and lower energy needs, making it a more sustainable and cost-effective option for LIB recycling. This study shows that the step-wise addition of biogenic sulfuric acid may efficiently recover Li and Mn from wasted LIBs. This method provides a sustainable alternative to traditional procedures by limiting environmental impact while reducing process time and energy consumption.

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1. Introduction

The high energy and power density of lithium-ion batteries (LIBs) have made them an increasingly popular choice for portable electronic devices and electric vehicles (EVs) [1]. LIBs have recently become the primary power source for electric vehicles and various electronic devices such as mobile phones and laptops. Due to this significant growth, the LIB market is expected to reach a value of \$694 billion by 2025 [2]. However, there are concerns about the increasing demand for raw materials, particularly in areas with limited resources. Improper disposal of LIBs can also have environmental and safety implications [3]. Lithium (Li) and manganese (Mn) are the most critical elements in LIB cathodes. Much attention has been paid to the recovery of Li from spent LIBs, which is a valuable and scarce resource.

Recycling spent LIBs can reduce the amount of minerals needed, especially Li and Mn, which are scarce. In addition, it adheres to the principles of circular economy and sustainable development [4]. A wide range of precious metals have been recovered from spent LIBs using sulfuric acid. In various studies, sulfuric acid solutions at specific concentrations and reaction conditions have successfully extracted Li and Mn. According to Yang et al. (2020), 100 % Li and 94 % Mn were obtained using a 2M chemical sulfuric acid solution and 0.97 M H_2O_2 at 80 °C and 30 min [5]. The study of Guimaraes et al. (2022) examined metal dissolution from spent LIBs using various concentrations of sulfuric acid. Using 1M sulfuric acid and 10 % pulp density at 50 °C, they could recover 90 % Mn [6,7]. Despite this, most of these processes have contaminated natural water sources with sulfuric acid leachate, so benign lixiviants such as biogenic sulfuric acid has been proposed as a solution to this problem. Furthermore, using high temperatures and strong reducing agents such as H_2O_2 is not economical and environmentally friendly [8].

In recent decades, researchers have focused on sulfur and iron-oxidizing bacteria for the bioleaching process [9]. Metal solubilization occurs when these acidophilic, chemolithoautotrophic, and mesophilic bacteria oxidize ferrous iron (Fe^{2+}) or elemental sulfur (S^0), which allows them to generate energy through the oxidation of metals [10]. A bioleaching method is a novel technique for recovering valuable metals from cathode materials such as LMO and $LiCoO_2$ (LCO), using microorganisms' ability to produce acids to dissolve and extract the metal [11].

Our preliminary study evaluated the effectiveness of direct bioleaching for recovering Mn and Li from spent LIBs, where dissolution time and the concentration of sulfuric acid were shown to be essential factors in the direct bioleaching process [12]. To address the critical issue of leaching time and higher recovery of valuable metals at higher pulp density, this study investigated an indirect bioleaching strategy employing biogenic sulfuric acid produced by *Acidithiobacillus thiooxidans* (*A. thiooxidans*). To the best of our knowledge, it is the first study about the step-wise addition of biogenic sulfuric acid produced by *A. thiooxidans* to dissolve Li and Mn from spent LIBs. This method can solve the lack of enough presence of biogenic acid during the leaching process, which shortens the leaching time and improves the recovery rate in high pulp density. Therefore, the proposed process is a novel method for recovering acid-consuming metals in spent batteries. Compared with direct bioleaching and chemical leaching with commercial sulfuric acid, our results demonstrate that this approach is more cost-effective, quick, and straightforward in recovering valuable metals from LIBs.

Moreover, we investigated the possibility of recovering Mn and Li from the leachate, contributing to the environmental and

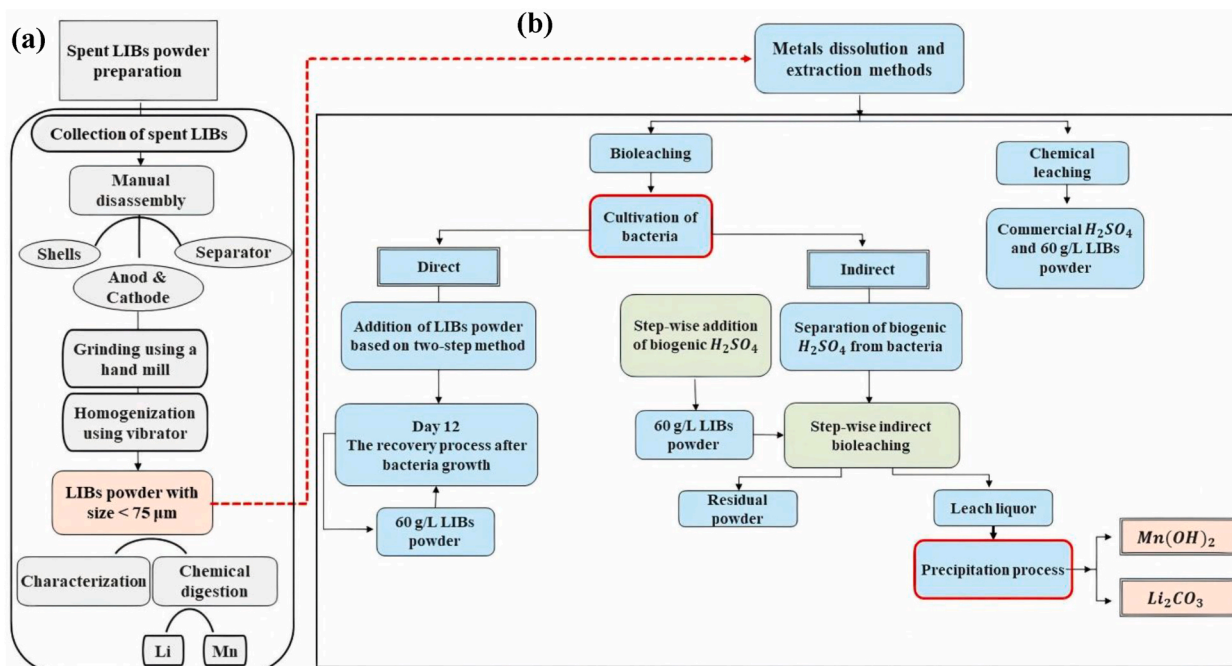


Fig. 1. The flowchart of the dissolution and recovery process of Li and Mn from spent LIBs: (a) pretreatment process, (b) recovery process.

economic benefits of recycling Li and Mn. Despite the lack of green and economical technologies for LIB recycling, a step-wise indirect bioleaching process utilizing biogenic sulfuric acid emerges as the most effective and environmentally friendly method among existing techniques. In general, this study proposes the waste-to-wealth strategy for recovering valuable metals from spent batteries and converting them into valuable salts, which applies to various industries.

2. Material and methods

2.1. Preparation and source of spent LIBs

This study used LIBs obtained from the Tehran, Iran Computer Center to separate the plastic components, shells, anodes, and cathodes from the LIBs; a manual disassembly process was performed. The mixture of cathode and anode materials was then obtained by hand milling. In order to obtain a powder with a particle size of less than 75 μm , the resulting material was sieved using mesh #200 coupled to a vibratory agitator.

Fig. 1 (part a) summarizes the steps in preparing LIBs powder. Detailed information regarding the digestion process is provided in our previous study [12]. Following chemical digestion, the composition of LIBs powder (mg/kg) shows the following metals (mg/kg): Mn (451530), Li (42810), Si (1566), Al (1232), Mg (752), Fe (522), P (208), Sn (157), and Cu (94). The final process involves three options: (a) direct bioleaching, which involves a two-step process; (b) indirect bioleaching, which utilizes biological sulfuric acid; and (c) chemical leaching by chemical sulfuric acid. Fig. 1 (part b) summarizes the recycling methods for LIBs.

2.2. The production of metabolite

2.2.1. Maximization of sulfate production

A strain of *A. thiooxidans* was obtained from the Iranian Organization for Science and Technology (IROST). A 9 K nutrient medium was prepared with the following components: $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5); $(\text{NH}_4)_2\text{SO}_4$ (3.0); K_2HPO_4 (0.5); KCl (0.1); $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (44.22 g/L); and different dosages of S (5–20 g/L). To adjust the culture medium pH to 2, sulfuric acid 98 % was used. The inoculum at 5 % (v/v) and an initial bacterial population of about 10^7 cells/ml were added to 9 K medium in each flask. The experiment was conducted in a 250 mL Erlenmeyer flask containing 100 mL growth medium at 30 °C and rotated at 140 rpm until the end of the stationary phase. Also, different *A. thiooxidans* inoculum amount (2–10 %) was investigated to obtain the maximum production of sulfuric acid by the strain. Upon reaching the optimum condition, the cells of *A. thiooxidans* were separated from the biogenic sulfuric acid by centrifuging at 10000 g for 20 min at 4 °C.

2.3. Design of bioleaching protocol

2.3.1. Contact system

A two-step direct (TSD) bioleaching method involves the culture of *A. thiooxidans* in the media, followed by recovery. Following the growth phase (when the cell density had reached a high concentration in the medium), 60 g/L of LIBs powder was added. After the LIBs were added to the flasks, they were shaken for 16 days using an orbital shaker. After the LIBs were added to the flasks, they were shaken for 16 days using an orbital shaker. At all experiments, temperatures, and agitation rates were fixed at 30 °C and 140 rpm. pH, sulfate ions, and metal ions concentrations were measured in each bioleaching flask.

2.3.2. Non-contact system

This study used biogenic lixiviants prepared from cultures of *A. thiooxidans* at the logarithmic phase of its growth curve. In order to collect the lixiviants, the growth medium containing the bacteria and the biosulfate solution was centrifuged at 10000 rpm for 20 min at 4 °C.

In order to carry out the bioleaching process, 60 g/L of LIBs powder was added to 100 mL of a solution of biogenic sulfuric acid with a concentration of 39000 mg/L. A shaker was used to maintain the mixture at 140 rpm and 30 °C. The step-wise indirect (SWI) bioleaching process began after four days. The leaching medium containing LIB powder was centrifuged at 10000 rpm for 15 min at 4 °C to separate the metabolites from the residue. Following adding a fresh medium with the concentration of 26000 mg/L of biogenic sulfuric acid to the LIBs powder, a similar concentration that bacteria produced during TSD bioleaching was maintained. Afterward, the mixture was kept at 30 °C for nine days. Similar to the TSD bioleaching method, the concentration of biosulfate ions in the leaching solution was maintained at 20000–24000 mg/L throughout the SWI bioleaching process. The fresh biogenic sulfuric acid solution was added to the leaching solution until the bioleaching process was completed. In order to replenish the biosulfate, fresh leaching solution was added at specific intervals (on days 4, 6, 8, and 10) for a total of 16 days of contact. The solid residues were then washed with deionized water following the bioleaching process. Subsequently, the washed residues were dried and analyzed using Field Emission-Scanning Electron Microscopy (FE-SEM) and Fourier transform infrared spectroscopy (FTIR). Through these analytical techniques, insight was gained into the surface morphology of the solid residues that resulted from the bioleaching process and their chemical properties.

2.4. Control leaching experiment

A sulfuric acid chemical leaching experiment was conducted to compare bioleaching and chemical leaching. In an experiment,

culture media and 60 g/L of LIBs powder were combined without adding any bacterial inoculum. A sulfuric acid solution (39.025 g, Merck Chemical Co.) was added to initiate the chemical leaching process. Sulfuric acid concentration and leaching conditions were maintained at the same level as in TSD bioleaching. A rotating orbital shaker incubated a flask containing the chemical leaching solution at 140 rpm and 30 °C for 16 days. This experiment was conducted to provide a benchmark for comparison with the bioleaching approach to assess both approaches' effectiveness and efficiency in removing valuable metals from LIBs.

2.5. Precipitation experiment

In the following steps of the SWI bioleaching process, the leachate obtained from the bioleaching solution underwent separate treatment procedures. Firstly, Mn hydroxide ($Mn(OH)_2$) was prepared using traditional methods for removing Mn from leachate [13]. A precipitation procedure was used to remove the Mn from the leachate. A subsequent step consisted of precipitating the Li remaining in the raffinate using sodium carbonate (Na_2CO_3). The raffinate was stirred at a speed of 500 rpm for 3 h with a saturated Na_2CO_3 solution at a temperature of 90 °C. As a result, Li ions reacted with carbonate ions, precipitating Li_2CO_3 [14]. The $Mn(OH)_2$ precursor and the Li_2CO_3 precipitate were filtered and dried for 12 h. These dried precipitates were then characterized using a powder X-ray diffraction meter (XRD) equipped with Cu-K α radiation.

3. Result and discussion

3.1. Maximization of sulfate production

As part of the contact bioleaching experiments, *A. thiooxidans* generated intermediate sulfur species using sulfur as an energy source. The strain converts sulfur in the culture medium through an oxidation reaction with sulfuric acid. An enzyme complex forms thiosulfate in an oxidation reaction between sulfur and sulfite. Sulfate is the end product of sulfur oxidation. (Eqs. (1)–(5)).

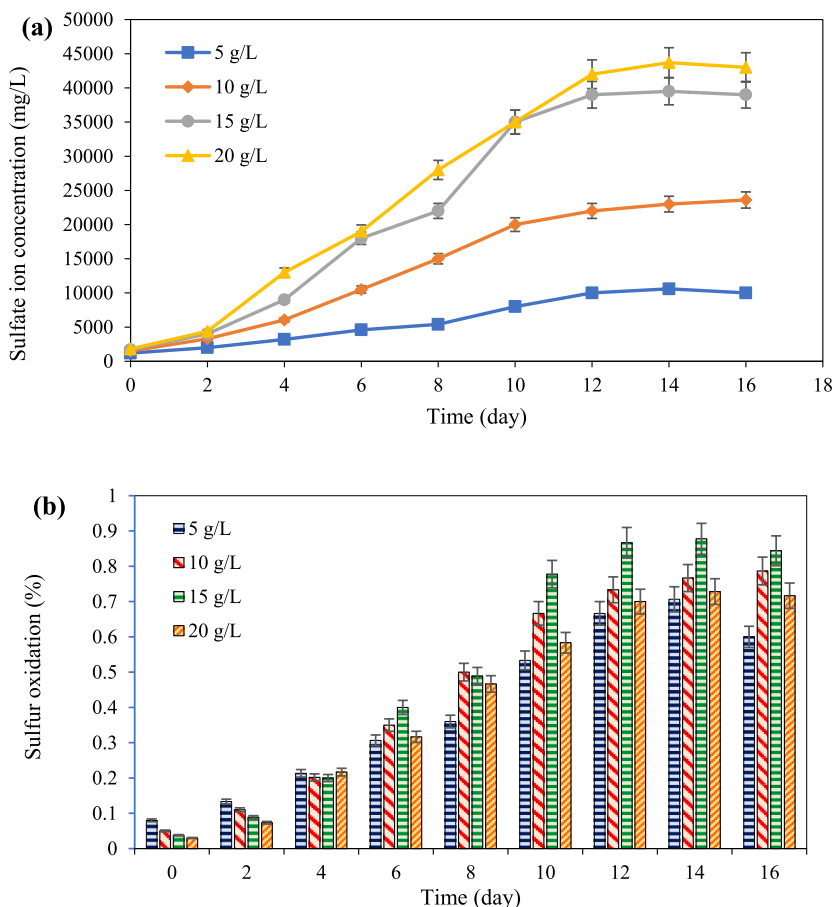


Fig. 2. (a) Biogenic sulfate production by *A. thiooxidans*; (b) sulfur oxidation at different concentration of sulfur; (c) The effect of inoculation size on sulfate production(d) changes of pH and cell concentration over time at S (10 g/L).

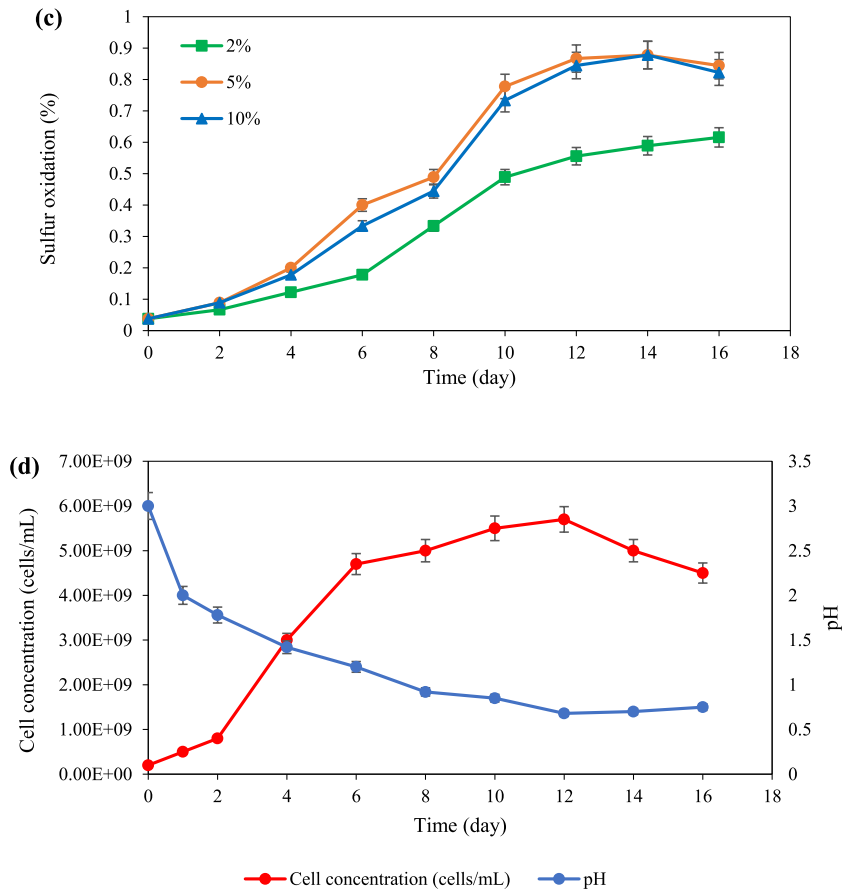


Fig. 2. (continued).



Fig. 2((a)-(b)) indicates bacterial production of sulfate ions and the rate of sulfur oxidation to sulfate ions at different sulfur concentrations (5–20 g/L) with 5 % of inoculation size. In Fig. 2(b), increasing sulfur concentration to 20 g/L results in a decrease in sulfur oxidation. Approximately 80 % of the sulfur in 15 g/L is oxidized and converted into sulfate (39000 mg/L). A sulfur concentration of 20 g/L results in 70 % sulfur oxidation to sulfate. Fig. 2(b) illustrates that sulfur oxidation has remained constant at 10, 15, and 20 g/L concentrations over 10–16 days. This may be the result of the following factors: 1) There are not enough bacterial cells to completely oxidize sulfur to sulfate; 2) it takes longer for sulfur to be completely oxidized. During oxidation, the bacterium's direct contact with sulfur is paramount. An amount of sulfur with a concentration of 15 g/L has been chosen to maximize bio-production of sulfate.

Fig. 2(c) illustrates the effect of inoculation size (2%–10 %) on sulfate production at an optimal sulfur concentration (15 g/L). This figure shows sulfur oxidation increased with increasing inoculation size from 2 % to 5 %. The sulfur oxidation does not change significantly when the inoculation size is increased from 5 % to 10 %. According to Fu et al. (2021), the opposite result is found. In their study, the authors reported that bacteria growth and bioleaching efficiency increased with an increase in inoculation size from 10 to 30 % [15]. The findings in our study indicate that the amount of inoculum at the optimum percentage significantly impacts sulfur oxidation by bacteria since there are enough bacteria to convert sulfur to sulfate ions. Also, a larger volume of inoculum would not be economically viable; therefore, a 5 % inoculation size was chosen to produce maximum sulfate.

The change of pH and the number of bacterial cells of *A. thiooxidans* at a sulfur concentration of 15 g/L and inoculation size of 5 % in a pure culture was measured over time (Fig. 2(d)). Increasing the number of bacteria in the medium results in a sharp drop in pH values, which is due to bacteria activity and the production of protons (Eqs. (1)–(5)). In the bacterial culture medium, the highest

concentration of sulfate ions was 39000 mg/L with a pH of 0.68 on the 12th day of growth. The biogenic sulfuric acid solution obtained on this day will serve as a bio-extraction agent for replacing chemical sulfuric acid and for recovery of Mn and Li from spent LIBs at the following stage.

3.2. Evaluation of different bioleaching approaches on bacteria's growth characteristics

An illustration of pH changes during 16 days of different bioleaching methods for LIBs can be found in Fig. 3(a). Under all conditions, pH values rapidly increase, indicating acid consumption during metal dissolution from LIBs [16]. Under all conditions, pH values rapidly increase, indicating acid consumption during metal dissolution from LIBs [16]. Due to the high alkalinity of LIBs and the

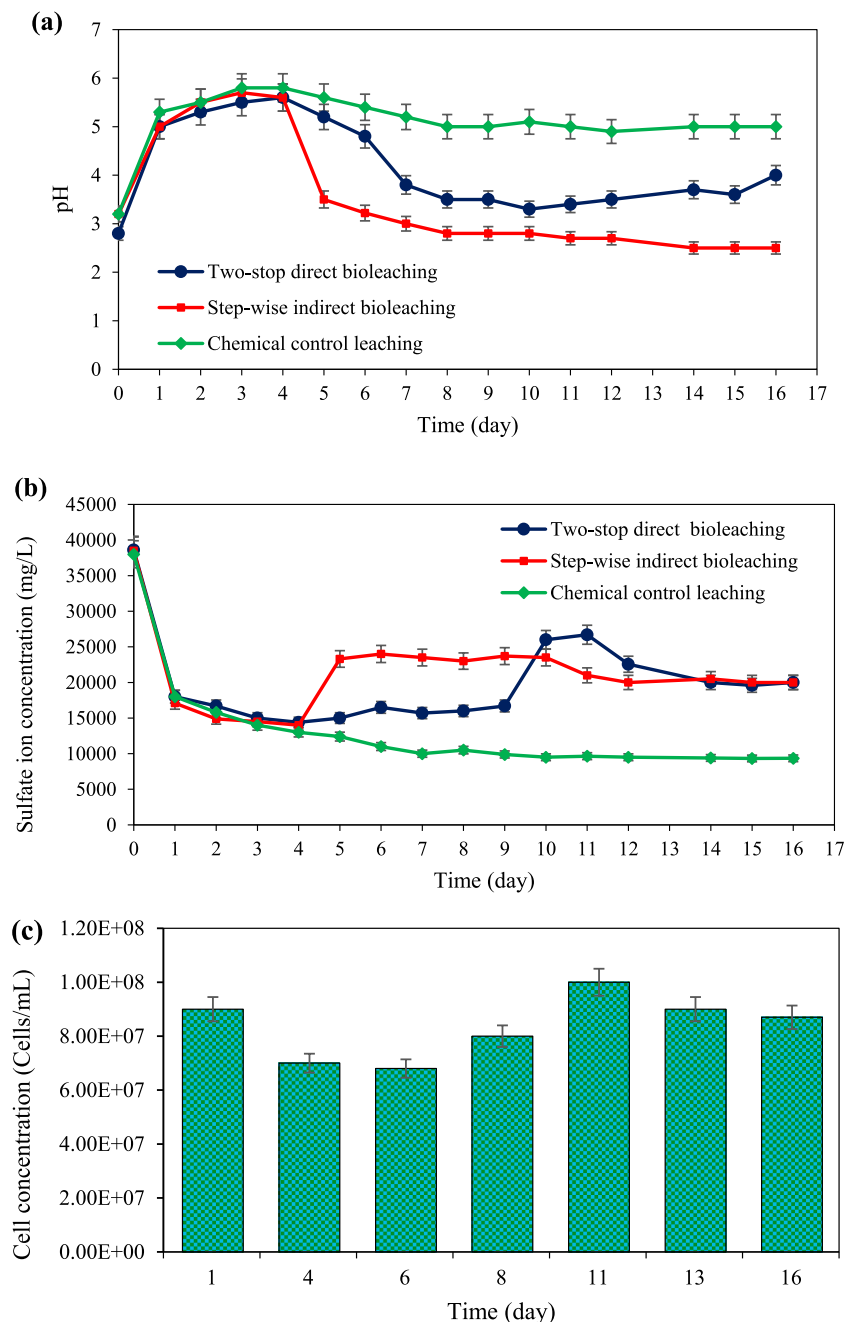


Fig. 3. Variation in (a) pH; (b) sulfate concentration during different leaching method; (c) variation of *A. thiooxidans* cell concentration during TSD bioleaching.

high content of Li as an acid-consuming material (Eq. (6)), the pH increased from about 3 to about 5. As a result of the production of sulfate ions, the pH drops from 4 to 10 days. A decrease in the number of bacteria and reduced proton production increased pH after ten days of TSD bioleaching.



It was observed that pH values as high as four were recorded after 16 days of TSD bioleaching with the growth of *A. thiooxidans*. pH values rapidly decreased in the SWI bioleaching test following the addition of the second solution of biogenic sulfate on the fourth day; due to the step-wise addition of fresh biogenic sulfate, the pH of the leach solution of SWI bioleaching by biogenic sulfate gradually dropped to 3.2–2.5.

When sulfuric acid was applied to control leaching chemically, following 16 days of LIBs addition to 9 K medium, a pH of 4.4–4.6 was consistently reached. It may result from a lack of bacterial activity under these conditions [17]. As a result of bacterial activity in TSD bioleaching systems, sulfur is bio-oxidized into sulfate. In TSD bioleaching, biogenic sulfate concentration significantly decreased (Fig. 3(b)) following the addition of LIBs powder. An increase in pH and a sharp decrease in sulfate ions resulted from bacteria adapting to LIBs powder in a new environment rich in acid-consuming compounds during TSD bioleaching. In turn, this delayed the activity of bacteria's biooxidation. Fig. 3(c) shows an increase in the bacteria population after 11 days of bioleaching with TSD, predicting a positive correlation between the concentration of biogenic sulfate and bacteria cells. In other words, more bacteria cells result in a higher concentration of biogenic sulfate (Fig. 3(b)).

The dissolved sulfate ions were consumed between 0 and 4 days after LIBs powder was added to the leaching solution. As a result, the sulfate concentration decreased from 38500 to 17100 mg/L and remained within 14000 mg/L for the next four days. Following

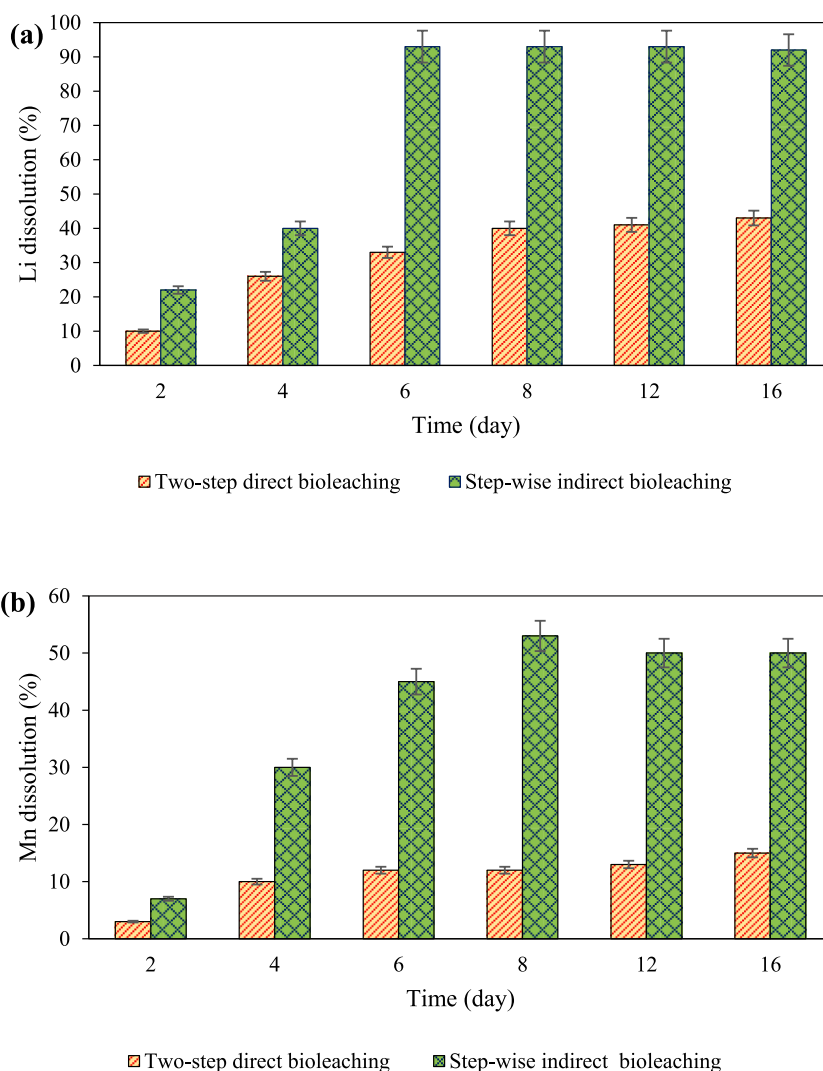


Fig. 4. Metals leaching efficiency from LIBs under TSD and SWI conditions (a) Li; (b) Mn.

four days, the pH dropped to 3.5 with the fresh leaching solution (26000 mg/L) and steadily declined until the process was completed, thereby improving metal recovery. Using step-by-step additions of fresh biogenic sulfuric acid to the leaching medium, the concentration of sulfate ions remained stable, ranging from 20000 to 24000 mg/L for 5–16 days. Based on the different concentrations of sulfate ions between direct and chemical control leaching, it can be concluded that *A. thiooxidans* are responsible for oxidizing elemental sulfur when exposed to bacterial contact.

3.3. The impact of various bioleaching approaches on metals dissolution

Fig. 4 (a) and (b) clearly show that the bioleaching methods used have a big effect on how well Li and Mn dissolve in LIBs powder. Indirect bioleaching, especially SWI bioleaching with biogenic sulfuric acid, was better at dissolving metals than TSD. At a pulp density

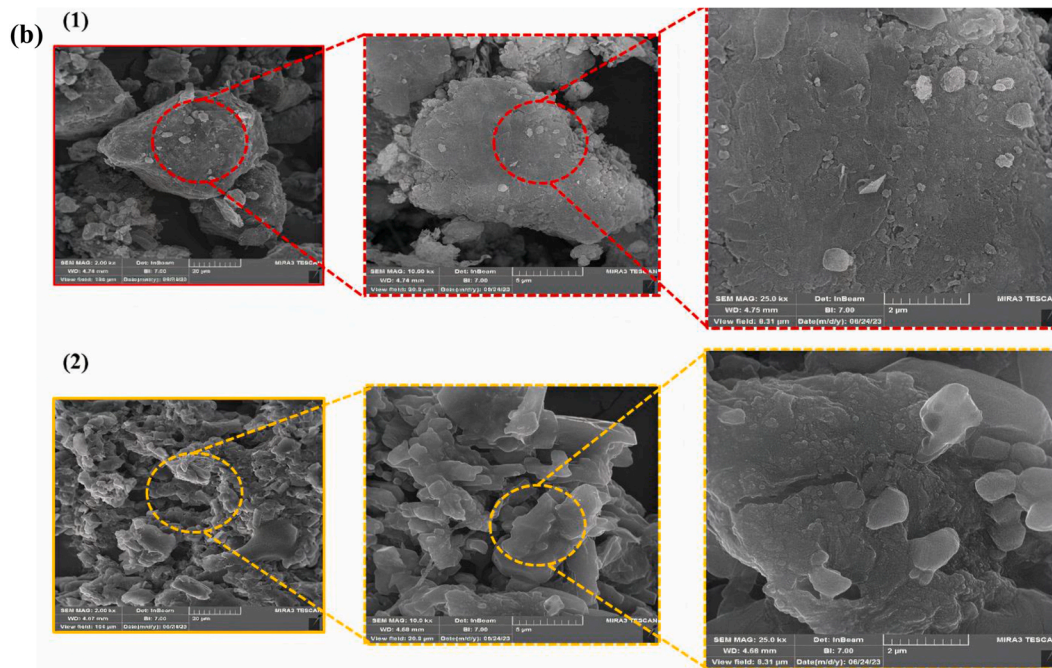
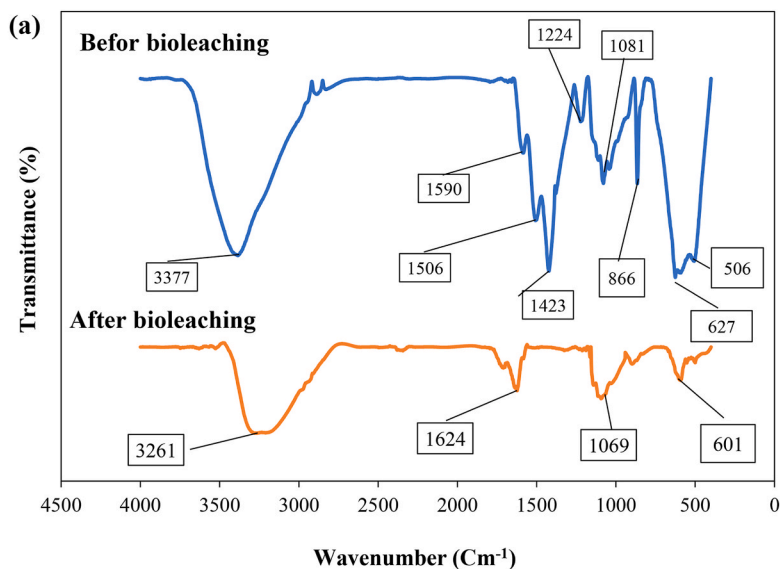


Fig. 5. (a) FTIR spectra of bioleached residue; (b-1) FE-SEM image of LIBs powder before bioleaching process; (b-2) FE-SEM image of LIBs powder after bioleaching process at a pulp density of 60 g/L.

of 60 g/L, 93 % of Li was solubilized after 6 days and 53 % of Mn after eight days. Over 12 days, the maximum effectiveness of biological leaching was 43 % of Li and 15 % of Mn under TSD bioleaching conditions. The detailed discussion on metal leaching are as follows:

- **The mechanism for metal leaching and valence state changes:**

The bioleaching process consists of a series of complex processes mediated by both biological and chemical agents. Here, we look at the main steps that Li and Mn take to dissolve from LiMn_2O_4 (the cathode material in LIBs) and the changes that happen to their oxidation states as a result [18].

Biogenic sulfuric acid protons (H^+) attack the LiMn_2O_4 lattice in the first phase. This acidic atmosphere favors the following reaction (Eq. (7)). The equation shows that Li^+ ions rapidly leach into the solution, while Mn^{4+} in LiMn_2O_4 reduces to Mn^{2+} , leaving MnO_2 as an insoluble result [12].



Manganese Oxidation and Reduction: The MnO_2 created during the first stage can participate in subsequent processes. According to Eq. (8), biogenic sulfur in the culture medium promotes the conversion of MnO_2 back to Mn^{2+} .



A. thiooxidans facilitates this process. The bacterium produces energy by oxidizing elemental sulfur to sulfate (SO_4^{2-}). During the process, MnO_2 functions as an electron donor, reducing to Mn^{2+} ions that are accessible for leaching [19].

Furthermore, ferrous ions (Fe^{2+}) in the culture medium can contribute to MnO_2 reduction, as shown in Eq. (9).



Fe^{2+} acts as a chemical reductant, aiding in Mn solubility by reducing MnO_2 to Mn^{2+} . Ferric iron reductase enzymes produced by *A. thiooxidans* regenerate the Fe^{2+} ions, resulting in a cyclical mechanism that improves Mn recovery [20].

- **Factors influencing metal leaching efficiency:**

Several factors influence metal leaching efficiency, with biogenic sulfuric acid concentration playing an important role. High amounts of biogenic sulfuric acid are required to effectively extract lithium from LiMn_2O_4 [21]. Protons have an important role in starting the attack on the cathode material lattice, releasing Li^+ ions [22].

The action of *A. thiooxidans* is also required for effective bioleaching. Conditions that are ideal for this bacterial activity are needed, because things like low oxygen levels, high pulp density (viscosity), and metal toxicity could make it harder for the bacteria to work, which would stop the manganese from dissolving by lowering MnO_2 [23].

Biogenic sulfuric acid's SWI is another factor that improves leaching efficiency. This method helps keep the pH level in a good range for *A. thiooxidans* activity, while also keeping metals from being too toxic. As a result, manganese bioleaching is more effective than TSD bioleaching.

Furthermore, in direct bioleaching, bacterial adhesion to the surface of LIB powder might form a physical barrier that impedes the leaching process. This adhesion limits the available reaction surface area between the acidic solution and the cathode material, resulting in reduced metal recovery [24].

According to this explanation, it could be said that, *A. thiooxidans* and biogenic sulfuric acid successfully mediate the bioleaching process, which dissolves Li and Mn from used LIBs. This process consists of a succession of protonation and reduction processes that result in the leaching of Li^+ and Mn^{2+} ions. The SWI bioleaching method makes this process better by keeping conditions that are good for *A. thiooxidans* activity and lowering factors that make it difficult for metals to dissolve. This better understanding of the metal leaching mechanism opens the door for further improvement of bioleaching procedures, resulting in more efficient metal recovery from wasted LIBs.

3.4. Characterization of LIBs before and after the bioleaching process

The FT-IR analysis (Fig. 5(a)) revealed significant changes in the chemical composition of the LIBs powder following SWI bioleaching.

3.4.1. Chemical composition changes

1. **Hydroxyl Groups and Water Adsorption:** The peak at 3377 cm^{-1} , attributed to hydroxyl groups and water adsorption on pre-existing metal hydroxides in the LIBs, remained present after bioleaching. This persistent peak indicates that some hydroxyl groups and water molecules are still adsorbed on the surface of metal hydroxides even after bioleaching [25].
2. **Formation of Inorganic Acids:** A broader peak emerged at 3261 cm^{-1} , suggesting an increase in water and carboxyl groups due to the bioleaching process. This potentially indicates the formation of inorganic acids by *A. thiooxidans*, which can contribute to metal dissolution by lowering the pH and enhancing the leaching environment.

3. **Graphite Structure and COH Bonds:** The peaks around 1503 cm^{-1} and 1590 cm^{-1} maintained their presence, corresponding to the graphite structure and COH bonds. This indicates that some graphite structures and COH bonds remained intact after bio-leaching [12].
4. **Persistence of C-C Bonds:** The peak at 1423 cm^{-1} confirmed the persistence of C-C bonds in the post-bioleaching sample. This suggests that not all carbon bonds were broken down during the bioleaching process [26].
5. **Jarosite Formation:** The peak at 1624 cm^{-1} , assigned to hydroxyl and hydrogen groups in jarosite (Eq. (10)), suggests the potential formation of this mineral phase during bioleaching. Jarosite is a sulfate mineral that can co-precipitate with metal cations, potentially influencing metal recovery efficiency.

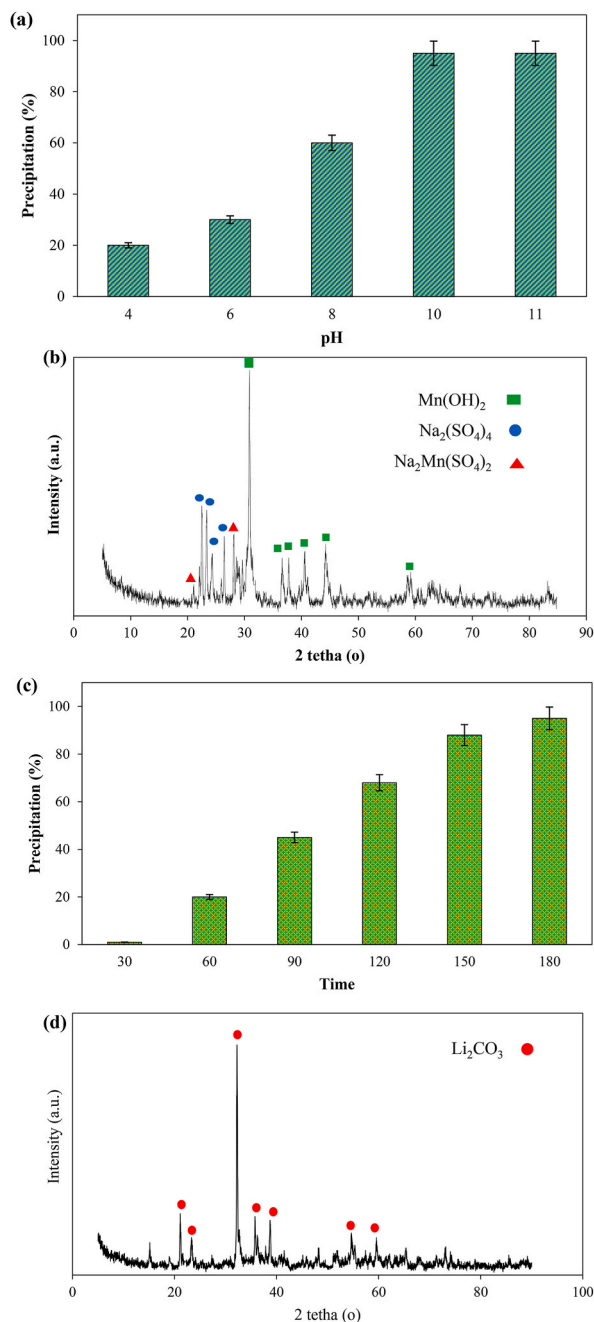


Fig. 6. (a) Effect of pH on percentage precipitation of Mn at room temperature using 1 M of NaOH; (b) XRD patterns of the solid precipitates of Mn(OH)₂; (c) effect of time on percentage precipitation of Li at 90 °C using saturate Na₂CO₃; (d) XRD patterns of the solid precipitates of Li₂CO₃; (e) Flow diagram of solid precipitate.

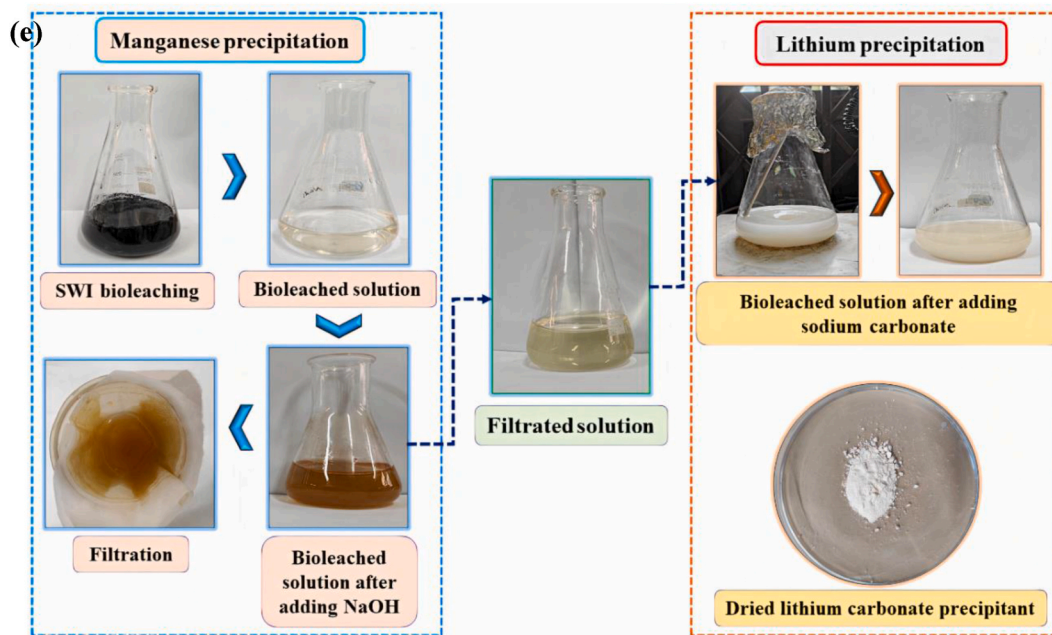
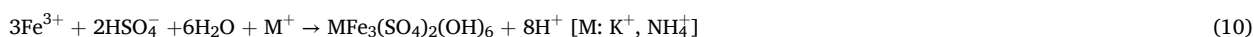


Fig. 6. (continued).



- Graphite Layer Degradation:** The most significant changes were observed in the range of 1000–1224 cm^{-1} . These peaks, associated with C-O stretching in graphite, exhibited a considerable decrease after bioleaching. This suggests that the graphite layer is breaking down effectively, which could leave the cathode materials (LiMn_2O_4) open to bioleaching [25].
- Incomplete Mn and Li Leaching:** The presence of a small peak at 601 cm^{-1} after bioleaching indicates incomplete Mn and Li leaching, which aligns with the observed recovery efficiencies (Li: 93 %, Mn: 53 %) [27].
- Removal of Organic Compounds:** The disappearance of the C-H peak at 866 cm^{-1} suggests the removal of organic compounds present in the pristine LIB powder [28].

These detailed observations from the FT-IR spectra confirm the effectiveness of the SWI bioleaching process in altering the chemical composition of LIBs powder, facilitating the breakdown of graphite layers, and promoting the formation of new mineral phases like jarosite, which together enhance the leaching of valuable metals.

The FE-SEM images (Figs. 5(b-1) and (b-2)) further corroborated the effectiveness of the bioleaching process by providing visual evidence of the morphological changes in the LIBs particles.

3.4.2. Morphological changes

- Initial Particle Structure:** Originally, the particles possessed an irregular shape and size, characteristic of spent LIB materials.
- Post-Bioleaching Morphology:** After bioleaching, the LIBs displayed a more uniform, cubic morphology with smaller particle sizes. This morphological transformation can be attributed to the step-wise addition of biogenic sulfuric acid, which gradually decreased the pH and created acidic conditions conducive to metal oxide dissolution.
- Fragmentation and Surface Porosity:** The combined effects of the biogenic sulfuric acid and the acidic medium promoted the dissolution of metal oxides and the breakdown of particle structures. This resulted in a more porous and rougher surface, making it easier for the biogenic sulfuric acid to penetrate the LIB particles and facilitate the leaching of valuable metals like Li and Mn [29, 30].
- These FE-SEM observations demonstrate that the bioleaching process not only altered the chemical composition but also significantly changed the physical characteristics of the LIBs powder. The uniform cubic morphology and increased surface area enhance the accessibility of biogenic sulfuric acid to the internal structures of the LIB particles, promoting more efficient leaching of metals.

3.4.3. Overall effectiveness of SWI bioleaching process

These comprehensive analyses emphasize the significant chemical and morphological transformations caused by the SWI bioleaching process, promoting its efficacy in recovering valuable metals from spent LIBs. By providing a comprehensive understanding of

these changes, the study improves the claim for the effectiveness and potential of this bioleaching method in recycling LIBs. The combined FT-IR and FE-SEM analyses proved the effectiveness of the SWI bioleaching process. The observed changes in the chemical composition and physical characteristics of the LIBs powder, including effective graphite layer degradation, increased surface area, and potential co-precipitation phenomena, all contributed to the enhanced recovery of Li and Mn from spent LIBs.

3.5. Precipitation experiment

SWI bioleaching leaching liquor had a pH of 2.8 before precipitation. The pH was then increased to 10 by adding 1M NaOH solution (Fig. 6(a)). NaOH concentrations of 1 M led to almost 85 % precipitation of Mn in the leaching solution, and it became fixed as pH increased (pH of 2.8 is the initial pH of the leaching solution from SWI bioleaching). The color of the solution changed from white to brown, indicating that Mn^{2+} ions interacted with OH^- ions to form $Mn(OH)_2$ [31]. As a result of the addition of NaOH to leachate, Eqs. (11) and (12) illustrate the possible reactions that could take place. It is worth mentioning that SWI bioleaching produced high concentrations of sulfate ions (23000 mg/L); therefore, Mn^{2+} ions can react with sulfate and NaOH [32]. As shown in Eq. (12), $MnSO_4$ reacts with NaOH to produce $Mn(OH)_2$ [33]. $Na_2Mn(SO_4)_2$ has also been confirmed by XRD results (Fig. 6(b)). It is also possible for the remaining $MnSO_4$ to react with Na_2SO_4 and form $Na_2Mn(SO_4)_2$ precipitants [34].



Following centrifugation, the hydroxide precipitate was thoroughly washed with distilled water, then dried at 80 °C for 5 h. In a second step, 1 M NaOH solution was added to the remaining solution to increase its pH to about 12 and then heated to 90 °C for a certain amount of time to increase the mobility of Li^+ ions [35]. Higher temperatures favor lithium carbonate synthesis because lithium carbonate’s solubility decreases with temperature, and the endothermic reaction occurs [36]. In the next step, 50 μ L of saturated Na_2CO_3 solutions were slowly added every 30 min until the desired amount of lithium carbonate precipitate. According to Fig. 6(c), the precipitation of lithium carbonate started after the first hour, and it reached its maximum percentage (93 %) after 3 h. As soon as the precipitate had formed, it was filtered and dried at 80 °C for 5 h. Lithium carbonate will be recovered from the reaction equation in Eq. (13).



Fig. 6(b) and (d) show the results of XRD analysis of the solid precipitates. A 2M sulfuric acid solution was added to the recovered lithium carbonate to examine its purity. The flowsheet of precipitation is shown in Fig. 6(e).

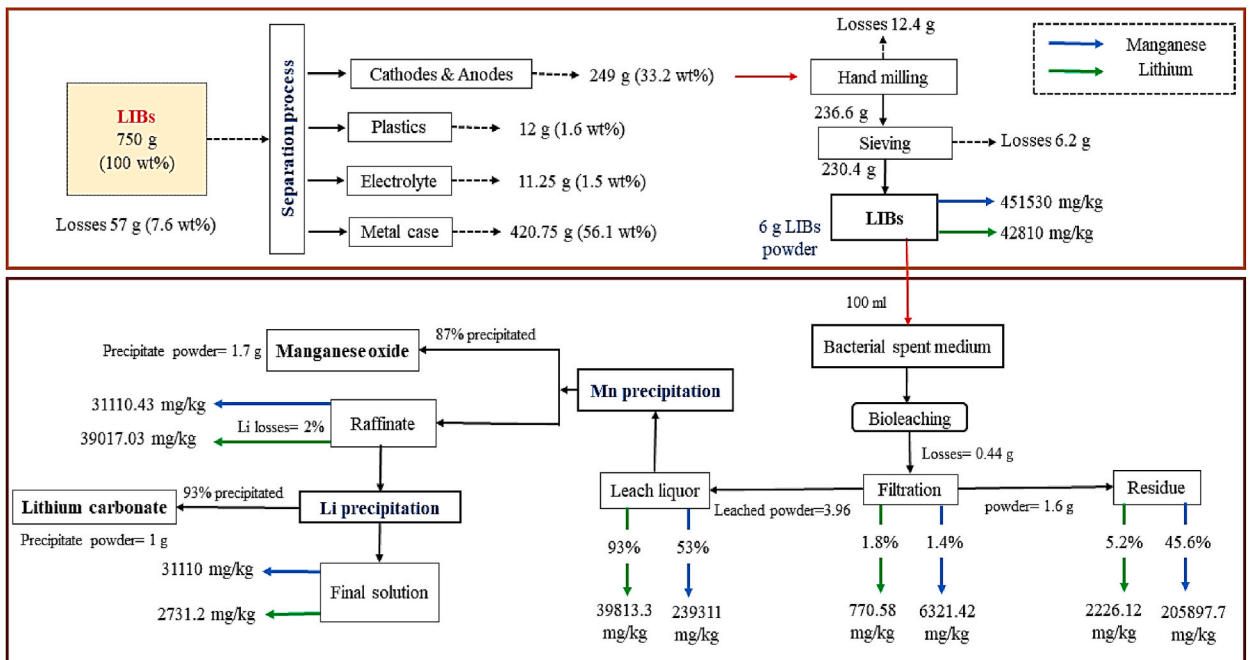


Fig. 7. The mass balance flowchart of the SWI bioleaching process of spent LIBs.

3.6. Analyzing mass balances

The flowchart shown in Fig. 7 shows the mass balance during the SWI bioleaching process of 60 g/L of spent LIBs. As a result of mass balance analysis, Mn and Li were successfully leached from LIBs powder. Since there was not enough reducing agent in the medium culture to achieve a complete recovery of Mn, the recovery of Li was higher than Mn. Through step-wise addition of biogenic sulfuric acid under 239311 mg/kg Mn of 451530 mg/kg and 39813.30 mg/kg Li of 42810 mg/kg were leached. The results also showed the loss of 6321.42 mg/kg (1.4 %) of Mn and 770.58 mg/kg (1.8 %) of Li. Therefore, 205897.7 mg/kg (45.6 %) of Mn and 2226.12 mg/kg (5.2 %) of Li remained in solid residue.

After the Mn precipitation process, the raffinate contained 31110.43 mg/kg of Mn, indicating incomplete precipitation. In addition, 39017.03 mg/kg of Li was lost during the Mn precipitation process. However, XRD and ICP analysis showed no precipitate formation of Li. According to the mass balance flowchart, the Li precipitation process was successful, and only 231.20 mg/kg of Li ions were left in the solution.

Like bioleaching, a chemical leaching experiment was carried out using commercial sulfuric acid. Chemical leaching with commercial sulfuric acid was conducted to determine the role of biogenic sulfuric acid in the bioleaching process. According to the results obtained from chemical leaching, Li recovery reached 78 % after 6 days, and Mn recovery reached 25 % after 8 days. The dissolution of metals with the SWI bioleaching method is more than chemical leaching, and this indicates that the conditions of the leaching solution, such as pH and the concentration of the extracting agent in this method, are suitable compared to the chemical method [16]. Biological sulfuric acid performed better than chemical sulfuric acid at the same concentration of sulfate, or in other words, the concentration of 39000 mg/L of chemical sulfuric acid was not enough to dissolve metals. It is worth mentioning that during the bioleaching with biological sulfuric acid, pH, the percentage of inoculation, and even different types of metabolites that have been released from bacteria, such as extracellular polymeric substances (EPS), can be one of the reasons that dissolve metals during bioleaching method compared to the chemical method. Polysaccharides and proteins are the main components of EPS; in addition to these, lipids, nucleic acids, uranic acid, and some inorganic compounds are also found in the structure of EPS [22].

3.7. Economic analysis

For a more precise evaluation of the economic potential of recycling LIBs, it is imperative to delve into the data presented in Table 1. This table provides a comprehensive overview of the economic worth of two critical elements, Mn and Li, extracted from spent LIBs. The values for these elements have been calculated, considering the most efficient metal recovery methods, including bioleaching and chemical leaching. Recycling LIBs effectively requires adopting a step-wise approach, based on an economic analysis of the reclaimed metals. This innovative technique offers an environmentally friendly method for resource recovery. It presents a compelling economic case for the sustainable management of LIBs, further reinforcing the importance of adopting such practices in battery recycling.

It is also important to specify the costs, benefits, and scalability of the biogenic sulfuric acid production process. Therefore, we discuss these factors in more detail in this section.

3.7.1. Costs

- Feedstock: The cost of the feedstock influences the production expenses of *A. thiooxidans*. Options for the chosen substrate include sulfur-containing waste streams from various industries or elemental sulfur. Using waste streams can drastically lower expenses when compared to virgin sulfur [37].
- Optimizing growth conditions for *A. thiooxidans* in bioreactors involves managing temperature, pH, and oxygen availability. The cost of operating and maintaining the bioreactor must be taken into consideration [38].
- Downstream processing involves separating and purifying biogenic sulfuric acid from bacterial culture broth, which raises manufacturing costs. Filtration and ion exchange are two techniques that can be used, and their costs should be addressed [39,40].

Table 1
The value of metals extracted from LIBs by bioleaching and chemical leaching methods.

Metals	Mn	Li
Content (mg/kg)	451530	42810
Price (\$/t)	1600	75000
Bioleaching yield (%)	53	93
Chemical leaching yields (%)	25	78
The value of bioleaching (\$/t LIBs)	421.18	3292.16
The value of chemical leaching (\$/t LIBs)	198.67	2761.16
The total value of bioleaching (\$/t LIBs)	3713.34	
The total value of chemical leaching (\$/t LIBs)	2959.83	

3.7.2. Benefits

- Environmentally friendly: biogenic sulfuric acid production reduces the environmental impact of standard sulfuric acid production processes, which burn sulfur and emit hazardous emissions like SO₂. This yields a favorable environmental externality for the recycling process [41].
- Producing biogenic sulfuric acid reduces reliance on virgin resources by providing a sustainable alternative to sulfur extraction and processing [42].
- Existing waste treatment facilities can link with the biogenic sulfuric acid manufacturing process to create a symbiotic system where waste streams become resources. Numerous factors, including the following, determine the scalability of biogenic sulfuric acid synthesis [41,43]:
- Designing larger bioreactors for optimal bacterial growth and sulfuric acid generation is crucial when scaling up the bioleaching process [44,45].
- Large-scale biogenic sulfuric acid production requires a dependable and scalable supply of feedstock. Exploring alternate or regionally available feedstocks can help with scalability [46].
- Optimizing the bioleaching process can boost efficiency and lower production costs. This could entail improving bacterial strains, growing conditions, and downstream processing methods [47].

3.7.3. Long-term economic viability and market impact

- Market demand and prices: Market demand and prices for these elements significantly influence the economic viability of extracting Mn and Li from LIBs using bioleaching. The increasing need for LIBs, encouraged by the rise of electric vehicles and renewable energy storage systems, anticipates a promising future market for recovered Li and Mn [1].
- Cost Competitiveness: While bioleaching may initially be more expensive due to bioreactor setup and maintenance, the environmental benefits and possibility for cheaper feedstock costs can make it a viable alternative to chemical leaching in the long run. Furthermore, advances in biotechnological techniques and economies of scale may help cut prices [2].
- Income Generation: Bioleaching produces larger yields of Li and Mn than chemical leaching, resulting in greater profits per ton of LIBs handled. This money can help to offset operational costs and contribute to the long-term viability of the recycling process [3].
- Regulatory and environmental incentives: Governments and environmental regulations are progressively promoting sustainable and environmentally friendly recycling solutions. Companies that use bioleaching may benefit from regulatory incentives, subsidies, and a positive public image, which increases their economic viability [4].
- Market Impact: The use of bioleaching for LIB recycling has the potential to disrupt existing recycling markets by providing a greener and possibly more cost-effective solution. This could stimulate greater industry use and investment in bioleaching technology, resulting in increased innovation and cost savings [5].

3.8. Environmental impact assessment of the present study compared to previous studies

Environmental impact assessment is crucial for understanding the potential effects of bioleaching and traditional metal recovery methods, as well as ensuring that these technologies are implemented responsibly. In this section, we go deeper into critical aspects for environmental impact assessments, concentrating on the following areas:

3.8.1. Reduced chemical footprint

- Conventional methods for metal extraction from spent LIBs use harsh chemicals such as HCl, H₂SO₄, HNO₃, and H₂O₂. These substances offer serious dangers. Accidental exposure to these acids can result in severe burns, breathing difficulties, and even death. Improper handling and spills can contaminate soil and water bodies, destroy ecosystems, and pose health risks [48].
- The proposed bioleaching method involves using *A. thiooxidans*, a naturally occurring acidophilic bacterium that produces biogenic sulfuric acid by oxidizing elemental sulfur. This removes the need for harsh chemicals, thereby lowering the risk of environmental damage and worker exposure [49,50].

3.8.2. Lower energy consumption

Pyrometallurgical procedures typically use high-temperature processes (over 700 °C–100 °C) for metal extraction [48]. This increased energy demand leads to higher greenhouse gas emissions from fossil fuel power plants that generate electricity [51].

- At ambient temperatures, bioleaching uses less energy than high-temperature pyrometallurgical processes. This results in lower greenhouse gas emissions and a smaller environmental imprint [52].

3.8.3. Reduced waste production and byproduct concerns

- Traditional procedures produce hazardous wastes that require specific management and disposal. Hydrometallurgical procedures involving H₂SO₄ and H₂O₂, for example, can generate acidic waste streams, bringing new environmental challenges [53].

- Proposed method: Bioleaching creates low waste. The principal byproducts are the desired metal compounds (e.g., $\text{Mn}(\text{OH})_2$ and Li_2CO_3) and the bacteria's residual biomass. These byproducts are far less dangerous than the trash generated by conventional procedures.

The present study also compares the valuable metal recovery procedure with previous approaches. As can be seen in Table 2, this study obtained lower leaching yields than previous studies. A recent study by Lin et al. (2019) demonstrates a green method of selectively extracting precious metals from spent LIBs using conventional sulfation roasting (thermal treatment) at 1048 K, 1073 K, and 1098 K for fixed periods of 15 min, 30 min, 45 min, 60 min, and 90 min [54]. In another study by Popescu et al. (2022), a hydrometallurgical process containing 2 M H_2SO_4 and 4 % vol H_2O_2 at 70 °C was investigated [55].

In this section, we aim to discuss the limitations of our current study and suggest potential improvements for future research.

3.9. Limitations of the current study

1. Metals Recovered: Our research focuses primarily on the recovery of Li and Mn from spent LIBs. We found no other valuable metals in this form of LIB, despite these being key components.
2. Efficiency over different pulp densities: We evaluated the bioleaching process at a high pulp density of 60 g/L and observed increased recovery rates with the gradual addition of biogenic sulfuric acid. Further research is required to optimize the process across a variety of pulp densities.
3. Process Duration and Optimization: The step-wise addition method lowered the leaching time from 16 to 8 days. Optimizing the method to reduce leaching time while maintaining recovery efficiency remains a challenge. Furthermore, operational parameters such as temperature, pH, and microbial activity need to be optimized to improve overall process efficiency.

3.9.1. Potential areas for future research

1. Future research should focus on recovering other precious metals from LIBs. Creating a complete bioleaching process capable of efficiently recovering a wider spectrum of metals will considerably improve the economic viability and sustainability of LIB recycling.
2. Optimization Across Pulp Densities: Systematic studies are needed to assess how different pulp densities affect bioleaching efficiency. Identifying the ideal pulp density that balances metal recovery rates, leaching time, and microbial activity is critical for scaling up the process for industrial use.
3. Process Intensification and Kinetics: Understanding bioleaching kinetics can significantly boost metal solubilization rates. Techniques such as better bioreactor designs, increased microbial consortia, and genetic alterations of *A. thiooxidans* could be used to accelerate the process.

Table 2

Comparison of bioleaching efficiencies from LIBs waste in this study with previous studies.

Leaching method	Type of LIBs waste	Leaching agent(s)	Temperature (°C)	Time	Pulp density (g/L)	Recovery (%)	Reference
Roasting	LCO, NCM	–	450	15 min	–	Co:97 % Mn:93 %	[56]
Hydrothermal leaching	LCO, LMO, LNO	Citric acid	120	20 min	6	Mn, Li, Co: above 90 %	[57]
Chemical leaching	LCO	Fe^{2+}	75	2 h	10	Li: 92 % Co: 98 %	[58]
Chemical leaching	LCO	H_2SO_4 Organic acid	86	2.5 h	18	Li: 90 % Co: 93 %	[59]
Chemical leaching	LMO	H_2SO_4 H_2O_2	30	1 h	10	2.52 g/L Mn 0.545 g/L of Li	[60]
Chemical leaching	LCO	Citric acid	90	50 min	20	Li: 97 %	[61]
Bioleaching (spent medium)	–	Biogenic H_2SO_4 and ferric ions	32	16 days	40	Li: 99 % Co: 50 % Ni: 89 %	[62]
Bioleaching (one-step)	LCMO	Biogenic H_2SO_4 + FeS_2	25	21 days	40	Li: 93 % Co: 91 % Mn: 87 %	[63]
Bioleaching	LCO	Ferric ions + Ascorbic acid	30	2 days	20	Li: 94 % Co: 95 %	[64]
Bioleaching (two-step)	LMO	Biogenic sulfuric acid	30	16 days	30	Li: 99 % Co: 60 % Mn: 20 %	[12]
Step-wise indirect bioleaching	LMO	Biogenic H_2SO_4	30	8 days	60	Li: 93 % Mn: 53 %	This study

- 4 Integrating bioleaching with pre-processing stages (e.g., mechanical separation, heat treatment) might improve recovery efficiency and product purity.
5. Environmental and Economic Assessment: Life cycle assessments (LCA) and techno-economic analyses (TEA) will evaluate the proposed bioleaching process's environmental impact and economic viability.

4. Conclusion

In this study, we described a new, eco-friendly way to get precious metals like Li and Mn from spent LIBs by using the step-wise addition method with *A. thiooxidans*. This biogenic sulfuric acid technology provides a sustainable alternative to conventional methods that use harsh chemicals. Our key findings are as follows:

Enhanced bioleaching efficiency: The SWI technique outperformed direct bioleaching in terms of metal recovery. The Li leaching yield increased by 50 % to 93 %, while the Mn leaching yield increased by 38 % to 53 %.

Reduced leaching time: The SWI technique effectively reduced bioleaching time by half, from 16 days to 8 days. This translates into faster metal recovery and higher process efficiency. Under ideal conditions, *A. thiooxidans* produced 39000 mg/L of sulfuric acid (15 g/L sulfur and 5 % inoculum size). This demonstrates the bacterium's ability to generate the required bioleaching agent.

Concluding remarks: The SWI bioleaching technique is a promising way to recover important metals from used LIBs. This approach's efficacy is demonstrated by the observed increase in metal leaching yields and a significant reduction in leaching time. Furthermore, using biogenic sulfuric acid reduces the need for harsh chemicals, resulting in a more environmentally friendly recycling process.

This study sets the path for further research into refining the SWI process parameters to achieve even higher metal recovery efficiency. Furthermore, future research could concentrate on scaling up the method for industrial use. Biogenic sulfuric acid generation and SWI bioleaching can make recycling wasted LIBs more sustainable and efficient.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Tannaz Naseri: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Seyyed Mohammad Mousavi:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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