Bioleaching of valuable metals from spent lithium-ion mobile phone batteries using *Aspergillus niger*

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Abstract

In this paper, a bio-hydrometallurgical route based on fungal activity of *Aspergillus niger* was evaluated for the detoxification and recovery of Cu, Li, Mn, Al, Co and Ni metals from spent lithium-ion phone mobile batteries under various conditions (one-step, two-step and spent medium bioleaching). The maximum recovery efficiency of 100% for Cu, 95% for Li, 70% for Mn, 65% for Al, 45% for Co, and 38% for Ni was obtained at pulp density of 1% in spent medium bioleaching. The HPLC results indicated that citric acid in comparison with other detected organic acids (gluconic, oxalic and malic acid) had an important role in the effectiveness of bioleaching using *A. niger*. The results of FTIR, XRD and FE-SEM analysis of battery powder before and after bioleaching process confirmed that the fungal activities were quite effective. In addition, bioleaching achieved higher removal efficiency for heavy metals than the chemical leaching. This research demonstrated the great potential of bio-hydrometallurgical route to recover heavy metals from spent lithium-ion mobile phone batteries.

Keywords: Bioleaching; Li-ion spent batteries; Metals recovery; Organic acids; *Aspergillus niger*

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1. Introduction

Nowadays, by reason of the development of industry, communication, and new technologies, the usage of such portable electronic devices as mobile phones, laptops, notebooks, and cameras, to name a few, has substantially risen. Therefore, the demand for rechargeable batteries as an electrochemical power sources has rapidly increased. The increase in the production and consumption of rechargeable batteries has augmented the amount of spent batteries [1]. Among rechargeable batteries, lithium-ion batteries (LIBs) have extensively been employed. For example, the consumption of LIBs reached $4.49 \times 10^9$ units in 2011 [2] and in China, the quantity and weight of spent LIBs in 2020 can exceed 25 billion units and 500 thousand tons, respectively [3]. Indeed, LIBs have played a leading role in the global rechargeable battery market today [4], by virtue of their high energy density, long life cycle, low self-discharge rate, safety, high cell voltage, wide temperature domain of use, light weight, absence of memory effect, and the fact that, unlike older generation of batteries like nickel–cadmium (Ni–Cd) batteries, they do not contain hazardous heavy metals such as cadmium [5,6].

LIBs consist of an anode, a cathode, a separator, collectors, electrolyte, and a metal protective shell. The electrolyte, consisting of a lithium salt such as LiPF$_6$, LiBF$_4$ or LiCF$_3$SO$_3$, is dissolved in organic solvents like dimethyl carbonate (DMC), ethylene carbonate (EC) and diethyl carbonate (DEC). Polyethylene (PE) or polypropylene (PP) is used as a separator between the two electrodes. The anode contains graphitic carbon, and the anode collector is Cu foil. The cathode is made of lithium mixed metal oxide, and the cathode collector is Al foil. LiCoO$_2$ is used as the cathode material in 37.2% (w w$^{-1}$) of the LIBs, LiNi$_{0.33}$Mn$_{0.33}$Co$_{0.33}$O$_2$ is employed in 29.0% (w w$^{-1}$), LiMn$_2$O$_4$ in 21.4% (w w$^{-1}$), LiNiO$_2$ in 7.2% (w w$^{-1}$) and LiFePO$_4$ is made use of in 5.2% (w w$^{-1}$) of the LIBs. Furthermore, polyvinylidene fluoride (PVDF) is used in the
electrodes so as to hold the material particles together [7,8]. Cathode and anode are folded into a prismatic or cylindrical form and the assembled battery is fitted into a metal shell [4]. Nonetheless, owing to the vast amount of spent LIBs and its concomitant environmental and economic ramifications, recycling has become a significant issue. Safe disposal of hazardous wastes has led to strict global regulations. For instance, according to the EU Battery Directive 2006/66/EC, by 2011, at least, 50% by mean weight of waste batteries should be recycled into substances for their primary application or for other applications, except energy recovery [9]. Although spent LIBs are not deemed as dangerous waste, due to their toxic and flammable ingredients, their disposal in the environment entails a potential hazard to the environment and human health [10]. Unfortunately, most spent batteries are disposed of in landfills as domestic waste. In landfills, organic electrolytes and heavy metals present in spent batteries gradually leach into the soil, groundwater, or surface water. Additionally, the capacity of landfill spaces is limited. Therefore, landfilling is undesirable. On the other hand, LIBs contain valuable metals like aluminum, copper, nickel, lithium, cobalt, and manganese. LIBs can, as a matter of fact, act as a secondary and a cheaper mineral source of valuable metals and be even richer than mineral ores [11]. Previous studies have indicated that if only the cobalt and nickel of LIBs are recycled and reused instead of withdrawing from virgin mineral sources, 51% of natural resources will be saved [9]. It is anticipated that the usage of LIBs will expand more in the future, therefore, finding new methods and technologies for recycling spent batteries is as crucial as optimizing the current strategies [4]. Pyrometallurgical and hydrometallurgical processes or combination both of them are traditional metal recovery methods [4,11]. In all pyrometallurgical LIBs recycling processes, lithium cannot be recovered and it is the major disadvantage of this method[12]. It is
indispensable, however, to find an economic and eco-friendly process that can discard certain disadvantages of traditional methods, including low efficiency, high energy consumption, being expensive and involving risk, not to mention the secondary pollution caused by chemical reagents and by-products [13]. Bio-hydrometallurgy, environmentally friendly and suitable for low-grade sources, is an effective method of metal recovery that consumes less energy, and needs a mild reaction condition along with a few industrial requirements [14]. In this method, interactions between microorganisms (including both bacteria and fungi) and surfaces of ore or waste cause the metals solubilization [15]. Most previous studies conducted on bio-recovery of spent LIBs were done using bacteria [2,13,16–19]. To the best of our knowledge, no studies have been reported as to the fungal leaching of LIBs. Compared to bacteria, fungi have more ability to tolerate toxic materials, have a shorter lag phase and a faster leaching rate; what is more, they grow in alkaline and acid-consuming materials. Moreover, the metabolites excreted by fungi, like organic acids, help leach the metals [20] by way of replacing metal ions from the waste with hydrogen ions, or by forming soluble metal complexes and chelates [21]. Accordingly, heavy metal toxicity is reduced for biomass thanks to the formation of metal complexes or precipitation by excreted metabolites. In fact, such excreted metabolites act as lixiviants for bioleaching of solid wastes [20,22]. Several fungi like Penicillium simplicissimum, Penicillium chrysogenum, Aspergillus niger and so forth, are used for the recovery of heavy metals from different solid wastes such as municipal solid waste incinerator fly ash [20,23–25], spent catalyst [20,25–26], electronic scraps [29] and red mud [26,30]. The main objective of this study was to examine the ability of A. niger to leach heavy metals from spent LIBs and to find out the most suitable method among one-step, two-step and spent
medium bioleaching methods. In addition, the characteristics of spent LIBs including their composition, component phases, and acid neutralization capacity were examined. Prior to bioleaching experiments, the properties of fungal growth, including pH, biomass and organic acid concentration were determined. In order to investigate the progress of bioleaching, the XRD, FTIR, and micromorphology analysis of spent LIB particles were done and the metal recovery from spent LIBs was ultimately examined. Chemical leaching was performed to compare the bioleaching efficiency of spent LIBs.

2. Experimental

2.1. Spent LIBs

Spent mobile phone batteries were collected from mobile phone markets. All batteries were Li-ion made from different manufacturers with different sizes and types of cathode. At first, all spent batteries were discharged to avoid self-ignition and short-circuiting. Spent batteries were then dismantled and separated manually into cathodes, anodes, plastic separators and metal cases. Afterwards, the classified portions were weighed. Next, cathodes and anodes were dried at 60 °C for 24 hours to remove moisture. After drying, the classified cathodes and anodes were once again weighed so as to calculate the percentage of battery components. The average percentage of different parts of batteries is shown in Fig. 1.
Cathodes and anodes were manually crushed into small particles with a pair of scissors and then ground by a ball mill (Fritsch, Germany) until transmuted to fine powder. The powder was sieved by vibrator shifter with mesh# 200 to get homogeneous mixture. All battery powders used in this research were of a particle size less than 75 \( \mu \text{m} \). The final mixture was used for all subsequent experiments, yet prior to use, it was autoclaved at 121 °C for 15 min.

2.2. Characterization of spent LIBs

2.2.1. LIBs composition

The metal composition of LIB powder was determined by both chemical digestion and submitting to X-ray fluorescence (XRF) and subsequent analysis with XRF analyzer (PW2404, Philips, Netherland). In chemical digestion, the metal contents were determined using four acids, namely HCl, HNO₃, HClO₄ and HF. At first, 0.5 g of powder was subjected to 5 mL HNO₃ in a

\[ \text{Fig. 1: Average percentage of different parts of batteries.} \]
Teflon beaker and heated to 150 °C for 0.5 h. After cooling, 10 mL mixture of HClO$_4$ and HF in a ratio of 1:6 was added and heated to 200 °C. After cooling, 20 mL 1:1 HCl was added and heated again for 0.5 h on a hot plate. When the digest was cooled, it was filtered and made up to 200 mL with deionized water, the final dilution factor being 400. Subsequently, the solution was analyzed through the use of an inductively coupled plasma optical emission spectrometer (ICP-OES) (Vista-pro, Varian, Australia). In addition, a control sample was prepared, whose metal concentration was subtracted from the main sample in the calculations. The results are shown in Table 1.

**Table 1: Metal composition of LIBs**

<table>
<thead>
<tr>
<th>Component (% (w w$^{-1}$))</th>
<th>XRF analyze</th>
<th>Chemical digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td>22.00</td>
<td>21.31</td>
</tr>
<tr>
<td>Co</td>
<td>17.11</td>
<td>16.54</td>
</tr>
<tr>
<td>Al</td>
<td>9.45</td>
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<td>Cu</td>
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<tr>
<td>Ni</td>
<td>2.82</td>
<td>2.56</td>
</tr>
<tr>
<td>Li</td>
<td>nd</td>
<td>2.22</td>
</tr>
<tr>
<td>Fe</td>
<td>0.19</td>
<td>0.04</td>
</tr>
<tr>
<td>S</td>
<td>0.17</td>
<td>nt</td>
</tr>
<tr>
<td>Si</td>
<td>0.11</td>
<td>nt</td>
</tr>
<tr>
<td>Mg</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Na</td>
<td>0.06</td>
<td>nt</td>
</tr>
<tr>
<td>Ti</td>
<td>nd</td>
<td>0.02</td>
</tr>
<tr>
<td>Nb</td>
<td>0.02</td>
<td>nt</td>
</tr>
<tr>
<td>P</td>
<td>0.01</td>
<td>nt</td>
</tr>
</tbody>
</table>

nt: not tested
nd: not detected
2.2.2. CHN analysis

The carbon, hydrogen, and nitrogen contents of both initial powder and bioleached residue were evaluated employing CHNS-O analyzer (ECS 4010, Costech, Italy). 1 mg of the sample was primarily wrapped in a tin capsule and combusted in a CHNS-O analyzer. Samples were then combusted under the oxygen stream in a 980 °C furnace. The gasses produced by the combustion (CO₂, H₂O, SO₂, and NO₂), were passed through the column and detected. Helium was employed as a carrier gas. So as to prepare the calibration curve, acetalilide (C=71.09%, N=10.36%, H=6.71%, O=11.84%) was used as a standard.

2.2.3. Acid neutralizing capacity test

The acid neutralizing capacity (ANC) test is the leaching procedure for solidified products that give information as to hydration progression and the overall buffering capacity of materials against acidification and chemical attack. ANC is defined by measuring the amount of acid needed to change the pH value of the sample to a different chosen amount. The test was performed in Erlenmeyer flasks (containing 1 g battery powder and 100 mL deionized water) to which various amounts of 1.0 M HNO₃ were added so as to have disparate pHs ranging from that of the sample to about 4. All flasks were shaken at room temperature for 5 days. After shaking and equilibration, the pH values of the solution were measured.

2.3. Microorganism and growth condition

A. niger(PTCC 5210) was provided by Iranian Research Organization for Science and Technology (IROST) in Tehran, Iran. The fungus, on which the impurity test was done, was received in lyophilized form. The strain was cultured on a 3.9% (w v⁻¹) potato dextrose agar
(PDA) slant. The culture was then incubated for 7 days at 30 °C. The spores for inoculation were obtained from washing the surface of PDA using sterile distilled water. The spores were counted under a phase-contrast microscope (Standard 25, Zeiss, Germany) using a Neubauer counting chamber with a depth of 0.1 mm and an area of 0.0025 mm². In order to obtain the desired number of spores (approximately 10⁷ spores mL⁻¹), the suspension was diluted using sterile distilled water. Next, 1 mL of spore suspension was added to 100 mL of sucrose medium composed of sucrose (100 g L⁻¹), NaNO₃ (1.5 g L⁻¹), KH₂PO₄ (0.5 g L⁻¹), MgSO₄.7H₂O (0.025 g L⁻¹), KCl (0.025 g L⁻¹), and yeast extract (1.6 g L⁻¹) in a 250 mL Erlenmeyer flask. Prior to inoculation, sucrose medium was autoclaved at 121 °C for 15 min.

2.4. A. niger pure culture

In all experiments, spore collection and inoculum preparation were performed as described in section 2.3. 1 mL of spore suspension was inoculated into 100 mL sucrose medium and incubated for 40 days in a shaker-incubator (WiseCube® WIS-20, Daihan Scientific, South Korea) at 130 rpm and 30 °C (as in the bioleaching condition). At regular time intervals, the fungal culture from each flask was filtered to determine the growth characteristics. The residue was used for calculating the dry weight biomass and the filtrate was employed for measuring pH and organic acid concentrations.

2.5. Different methods of bioleaching

All Bioleaching experiments were performed in 250 mL autoclaved Erlenmeyer flasks with 100 mL sucrose medium at a pH of 6 containing 1% (w v⁻¹) sterilized battery powder. All the flasks were shaken in an orbital shaker-incubator at 130 rpm and 30 °C. One-step two-step and spent
medium bioleaching were conducted. In the one-step bioleaching process, 1 mL of spore suspension was inoculated into sucrose medium (containing battery powder) and incubated for 30 days. In the two-step approach, the fungi were, firstly, pre-cultured in sucrose medium; no battery powder was added until the fungi entered the logarithmic phase. The logarithmic phase commences with a sudden reduction in the pH, meaning the beginning of organic acid production. It took 3 days to observe a sudden pH reduction in \textit{A. niger} growth medium. The autoclaved battery powder was then added to the culture medium and bioleached in a shaker-incubator for 27 days. In the spent medium process, the fungi were primarily cultured in sucrose medium without battery powder until the fungi entered the stationary phase, which took 14 days for the \textit{A. niger}. After 14 days of cultivation, in order to obtain a cell-free spent medium, the suspension of mycelia and liquid medium of each flask was filtered through Whatman 42 filter paper. At last, the battery powder was added to the filtrate which included fungal metabolites and then bioleached in a shaker-incubator for 16 days.

So as to determine the biomass, pH, residual powder, biosorption, organic acids and metal ion concentrations, the culture from each flask was filtered through Whatman 42 filter paper at the end of the desired time of the bioleaching process. A control experiment was performed in a parallel manner, using fresh sucrose medium without inoculation. During all experiments, water evaporation was compensated for through adding sterilized distilled water via the weight difference method. All experiments were run in triplicate, and the mean values were reported.

2.6. \textit{Biomass determination}

The biomass dry weight was determined through the approach reported in Aung and Ting [27]. Briefly, in the pure culture, the mycelium obtained from the filter paper was put in a pre-weighed
porcelain dish and was dried in an 80 °C oven (WiseVen® WON-50, Daihan Scientific, South Korea) for 24 h. In the bioleaching experiments, the mycelium and residual powders obtained from the filter paper were dried as described for biomass determination in the pure culture. Subsequently, the dried residue was burned in a furnace (WiseTherm® FP, Daihan Scientific, South Korea) at 500 °C for 4 h. At last, the final residue was put in a desiccator to cool. The biomass dry weight was specified by calculating the differences between the weight of the residues before and after burning.

2.7. Chemical leaching experiments

Chemical leaching of the spent battery powder was carried out to evaluate the leaching effectiveness of organic acids through the use of a mixture of commercial citric acid, oxalic acid, malic acid and gluconic acid at a concentration similar to the bioleaching experiment. The leaching test was performed in 250 mL Erlenmeyer flask containing 100 mL of acid and 1% (w v⁻¹) battery powder. As in the bioleaching, the flasks were shaken at 130 rpm and 30 °C. At the end of the experiment, samples were filtered and analyzed for heavy metal concentrations employing ICP-OES.

2.8. Analytical methods

A digital multi meter (CP-500L, ISTEK, South Korea) was used to measure the pH of the battery powder, medium and filtrate. The component phases of powder were detected using X-ray diffraction (XRD) (X’Pert MPD, Philips, Netherland) with Co Kα radiation. The tube voltage was 40 kV and the current was 30 mA. The powder was scanned from 5° to 90° making use of a
scanning speed of 0.8 s step\(^{-1}\) with a step size of 0.04. ICP-OES was employed in order to analyze metal recovery. Multi-element standard (Merck) was used for calibration standards. Fourier transform infrared spectroscopy (FT-IR) (Frontier, Perkin-Elmer, USA) was used to study the chemical structure, functional groups and the bonds in the battery powder before and after bioleaching. FT-IR studies were carried out in the range of 500–4000 cm\(^{-1}\) at room temperature. Samples were prepared through mixing the solid sample with KBr powder. The morphology of both initial powder and the bioleached residue was observed using field emission scanning electron microscope (FE-SEM) (S-4160, HITACHI, Japan), operating at 30 kV. The sample was attached to sticky carbon tubes and then coated with a 30 nm layer of gold. The concentration of such primary metabolites as gluconic, oxalic, citric and malic acid was specified via high performance liquid chromatography (HPLC) (Sykam, Germany) equipped with a Nucleodur C18ec column (5 µm, 250 mm × 4.6 mm, Macherey-Nagel, Germany) and a UV-VIS diode array detector (DAD) at 210 nm. The injection size was 20 µL and all samples and standards were injected in triplicate. The operation was performed at 30 °C with an operating pressure of 5.7 MPa. 5mM sulfuric acid was made use of as a mobile phase with a flow rate of 0.5 mL min\(^{-1}\). Prior to injection, in order to prevent the fine particles from blocking the chromatography column, the samples were filtered using a 0.22 micron syringe filter.

3. Results and discussion

3.1. LIBs characterization

The composition (% (w w\(^{-1}\))) of LIBs was determined via XRF analysis (Table 1). The main elements were Mn (22.0%), Co (17.1%), Al (9.4%), Cu (6.6%) and Ni (2.8%). Trace amounts of Mg, Fe, Nb, S, Si, Na and P were also detected. The amounts of P were related to LiPF\(_6\) as an
Li was not detected by XRF, hence the acid digestion test so as to identify the amount of Li. The results of acid digestion are shown in Table 1. Through CHN analysis, the carbon, hydrogen, and nitrogen contents (\% (w w\(^{-1}\))) of battery powder were determined. The results showed 24.8\% carbon, 0.5\% hydrogen and 0.1\% nitrogen. The initial pH was found to be close to 9, by reason of the presence of alkaline components in the battery powder. For instance, Li is an alkali metal reacting vigorously with water, producing an aqueous solution of strongly alkaline hydroxides. (So as to measure the pH, 1 g battery powder was added to 50 mL deionized water and shaken at 140 rpm and 30 °C [23]).

The results of ANC test are shown in Fig. 2 through a titration curve, indicating the final pH as a function of the amount of mmol H\(^+\) g\(^{-1}\) battery powder. As seen in Fig. 2, the battery powder has an acid neutralizing capacity of 15.6 mmol H\(^+\) g\(^{-1}\) battery powder (ANC4). In fact, when the pH reached 4, 15.6 mmol H\(^+\) by 1 g of battery powder was consumed.

![Fig. 2: Acid neutralizing capacity (ANC) of battery powder.](image-url)
3.2. Growth of A. niger

The HPLC results showed that the organic acids secreted by A. niger were mainly malic, gluconic, oxalic and citric acid. Following inoculation, owing to such metabolic processes as glucose oxidation, A. niger produced metabolites such as organic acids and protons that cause changes in the pH values [22,31]. After 3 days of incubation, the biomass dry weight increased to 28.80 g L\(^{-1}\) and the pH decreased from 6 to 3.4, mainly because of the enzymatic action of invertase, hydrolyzing sucrose to glucose and fructose [15], meaning the beginning of organic acid production. On the third day, during the growth period, the pH was at its maximum and the most oxalic acid concentration (3445 mg L\(^{-1}\)) was secreted. Recent studies have shown that in alkaline cultures, by virtue of the induction of the enzyme oxaloacetate hydrolase by de novo synthesis, the fungi excrete mainly oxalic acid [32]. In line with the reduction in pH, the biomass concentration increased. On the 10\(^{th}\) day, the biomass reached its highest value at 33.70 g L\(^{-1}\). After the 10\(^{th}\) day, on the other hand, the biomass concentration decreased gradually, which is because of the inhibition of secreted primary metabolite (especially citric acid) for fungi growth. On the 14\(^{th}\) day, the pH was at its lowest level (2.8) while secreted gluconic acid (2126 mg L\(^{-1}\)) and citric acid (8078 mg L\(^{-1}\)) were at their highest. According to studies, citric acid is secreted more in lower pHs[33]. After the 14\(^{th}\) day, the pH gradually increased, probably due to the secretion of intracellular metabolites with an alkaline buffering nature. In the last days of incubation occurred a sharp increase in pH and a drastic decrease in biomass concentration that means the biomass lysis.
3.3. Bioleaching studies

According to the results of growth characteristic, for two-step bioleaching experiments, the 3\textsuperscript{rd} day was the proper day to add the battery powder to the medium owing to the fungi entering the logarithmic phase; the 14\textsuperscript{th} day was chosen for filtering the pure culture medium and using the filtrate for spent medium bioleaching experiments. The pH changes during alternative bioleaching approaches are shown in Fig. 3.

![Fig. 3: The pH changes during alternative bioleaching approaches.](image)

In one-step bioleaching, a sudden decrease occurred in the pH on the 5\textsuperscript{th} day, meaning, in the presence of battery powder, the growth of \textit{A. niger} lagged for 5 days. After the 5\textsuperscript{th} day, because of organic acids secretion, the pH was reduced continuously. At the end of the one-step bioleaching
process, the pH was found to be near to 5.5. In two-step bioleaching, after 3 days of incubation, by adding the battery powder to the medium, the pH of the medium rose from 3.4 to 6.8, which is due to the alkaline nature of the battery powder. In the following days, the pH did not undergo any perceivable changes. At the end of the two-step method, the pH was found close to 6.9. In spent medium bioleaching, after adding the battery powder to the medium, the pH surged from 2.8 to 5.3, indicating the fact that the secreted organic acids were consumed by battery powder components. Also, the gradual increase in the pH was caused by the continuous release of alkaline anions from battery powder. 

Although, at the end, the biomass concentrations in one-step and two-step bioleaching were 10.16 and 15.23 (g L⁻¹), respectively, showing that, compared to spores, the mycelium of A. niger had a higher tolerance to the toxic material of battery powder. Moreover, by reason of the presence of excreted metabolites such as organic acids, amino acids, and polypeptides, to name a few, in the pre-culture medium of two-step bioleaching, the toxicity of the heavy metals could be reduced to the mycelium after adding the battery powder [34].

3.4. Organic acids production

Fungal leaching is based on acidolysis, complexolysis and redoxolysis. In acidolysis, we have the protonation of oxygen atoms covering the surface of metallic compounds. The protons and oxygen are associated with water, thus the metal is detached from the surface [34]. Eq. (1) is the example of acidolysis reaction, producing nickel ions [35]:

\[ \text{NiO} + 2\text{H}^+ \rightarrow \text{Ni}^{2+} + \text{H}_2\text{O} \]  

In complexolysis, metal complexes and chelates are formed, leading to the solubilization of the metal ions (due to the complex capacity of a molecule). For example, a complex of oxalic acid
with aluminum, iron and magnesium, a complex of citric acid with magnesium and calcium and a complex of tartaric acid with iron, calcium, silicium, magnesium, and aluminum. Furthermore, metal ions solubilized into solution by acidolysis, are stabilized in complexolysis[34]. Eq. (2) is the example of complexolysis reaction that produces nickel citrate [35]:

\[
\text{Ni}^{2+} + C_6H_8O_7^- \rightarrow \text{Ni}(C_6H_5O_7)^- + 3\text{H}^+
\]  

Eq. (2)

In redoxolysis, the oxidation-reduction processes help the fungal leaching. Eq. (3) illustrates a redoxolysis reaction that produces manganese ion [35]:

\[
\text{MnO}_2 + 2\text{e}^- + 4\text{H}^+ \rightarrow \text{Mn}^{2+} + 2\text{H}_2\text{O}
\]  

Eq. (3)

Eqs.(4) to (15) list the related reactions between metal ions and different organic acids (that Mn\(^{n+}\) corresponds to the metal ions with certain valence) [26]:

Gluconic acid:

\[
\text{C}_{6}\text{H}_{12}\text{O}_{7} \rightarrow \text{C}_{6}\text{H}_{11}\text{O}_{7}^- + \text{H}^+ \quad (\text{PK}_{\text{a}} = 3.86)
\]  

Eq. (4)

\[
n[C_6H_{11}O_7^-] + \text{M}^{n+} \rightarrow \text{M}[C_6H_{11}O_7]_n \quad (\text{Gluconic metallic complex})
\]  

Eq. (5)

Oxalic acid:

\[
\text{C}_2\text{H}_2\text{O}_4 \rightarrow \text{C}_2\text{HO}_4^- + \text{H}^+ \quad (\text{PK}_{\text{a1}} = 1.25)
\]  

Eq. (6)

\[
\text{C}_2\text{HO}_4^- \rightarrow \text{C}_2\text{O}_4^{2-} + \text{H}^+ \quad (\text{PK}_{\text{a2}} = 4.14)
\]  

Eq. (7)

\[
n[C_2\text{HO}_4^-] + \text{M}^{n+} \rightarrow \text{M}[C_2\text{HO}_4]_n \quad (\text{Oxalic metallic complex})
\]  

Eq. (8)

\[
n[C_2\text{O}_4^{2-}] + 2\text{M}^{n+} \rightarrow \text{M}_2[C_2\text{O}_4]_n \quad (\text{Oxalic metallic complex})
\]  

Eq. (9)

Citric acid:

\[
\text{C}_6\text{H}_8\text{O}_7 \rightarrow \text{C}_6\text{H}_7\text{O}_7^- + \text{H}^+ \quad (\text{PK}_{\text{a1}} = 3.09)
\]  

Eq. (10)

\[
\text{C}_6\text{H}_7\text{O}_7^- \rightarrow \text{C}_6\text{H}_6\text{O}_7^{2-} + \text{H}^+ \quad (\text{PK}_{\text{a2}} = 4.75)
\]  

Eq. (11)
C_6H_6O_7^- \rightarrow C_6H_5O_7^- + H^+ \quad (P_{ka3} = 6.40) \quad (12)

n[C_6H_7O_7^-] + M^{n+} \rightarrow M[C_6H_7O_7]_n \quad (Citric metallic complex) \quad (13)

n[C_6H_6O_7^-] + 2M^{n+} \rightarrow M_2[C_6H_6O_7]_n \quad (Citric metallic complex) \quad (14)

n[C_6H_5O_7^-] + 3M^{n+} \rightarrow M_3[C_6H_5O_7]_n \quad (Citric metallic complex) \quad (15)

Fungi secrete metabolites like amino acids, and organic acids that are effective in metal leaching. As described above, among these metabolites, organic acids as leaching agents play important roles in fungal leaching of metal ions [14]. Type and concentration of secreted organic acids are significantly affected by the pH and metals in culture medium [25]. Accordingly, it is crucial to analyzed organic acids if one is to understand the bioleaching mechanism. Table 2 shows organic acid concentrations at the end of the three methods of bioleaching.

**Table 2**: Organic acids concentration at the end of the three methods of bioleaching

<table>
<thead>
<tr>
<th>Organic acid (mg L^-1)</th>
<th>1% (w v^-1) pulp density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One-step</td>
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<tr>
<td>Citric</td>
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<td>Malic</td>
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<td>Gluconic</td>
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</tbody>
</table>
As said before, alkaline cultures (above neutral pH) are preferable when secreting oxalic acid, due to the induction of enzyme oxaloacetate hydrolase by de novo synthesis [32]. Therefore, as is clear in the results, by virtue of the alkaline buffering nature of battery powder, the amount of secreted oxalic acid was higher in one-step and two-step bioleaching, compared to the pure culture. Also reported is the higher secretion of oxalic acid due to the presence of heavy metals like Ni [28].

*A. niger* was able to produce citric acid at a higher concentration in the absence of battery powder (spent medium bioleaching) while the oxalic acid was produced at a higher concentration in the presence of battery powder (one-step and two-step bioleaching). The presence of metal ions such as manganese and copper might be the reason behind citric acid not being produced in the presence of battery powder. Among these metal ions, manganese (a cofactor for the enzyme isocitrate dehydrogenase) is reported to have a strong inhibition to accumulating citric acid [20]. In the Krebs cycle, this enzyme catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate. Accordingly, in a manganese-deficient medium (in the absence of battery powder), α-ketoglutarate is not produced and the citric acid is accumulated in the culture medium. Nonetheless, in a manganese-efficient medium (in the presence of battery powder), citric acid accumulation is significantly reduced because of the conversion of citrate to organic acids like succinate, fumarate, malate, and so forth in the Krebs cycle [36]. On the other hand, more citric acid is secreted in lower pHs [33], rendering the amount of secreted citric acid in one-step and two-step process lower than that in a pure culture.
3.5. Extraction of heavy metals

3.5.1. Alternative bioleaching approaches

The purpose of bioleaching process is to extract metals from solid waste into solution as efficiently as possible. Both one-step and two-step bioleaching affected the metabolism of *A. niger*, consequently, the bioleaching recovery of heavy metals. Metal recovery in alternative bioleaching approaches is shown in Fig. 4. The result indicated that the total recovery in spent medium bioleaching was higher than the others. In spent medium bioleaching, 100% Cu, 95% Li, 70% Mn, 65% Al, 45% Co and 38% Ni were leached. In one-step bioleaching, however, 100% Li, 58% Al, 11% Cu and 8% Mn were leached (the amount of leached Ni and Co was negligible). In two-step bioleaching, 100% Li, 61% Al, 10% Mn, 6% Cu and 1% Co were leached (the amount of leached Ni was negligible).

![Fig. 4: Metals recovery in alternative bioleaching approaches.](image-url)
According to the results (Table 2), in comparison with other organic acids, oxalic acid was secreted much higher in the presence of battery powder. On the other hand, most oxalate complexes like nickel oxalate and cobalt oxalate were insoluble. In addition, among the metals of battery powder, lithium oxalate and aluminum oxalate were much more soluble [37]. Moreover, oxalic acid is reported to have a major role in the bioleaching of Al [28]. All of Li and most of the aluminum, therefore, turned into soluble form. However, other metal oxalates were precipitated and remained in un-leached battery powder residue after filtration, rendering justifiable the lower recovery of metals such as Co and Ni.

Higher recovery in the spent medium bioleaching and the results of organic acid production (Table 2) indicate that citric acid had a more significant role in the bioleaching of battery powder than other specified organic acids. In addition, as reported, Aspergillus produced 145 different secondary metabolites [38] that might have had an effect on the leaching of metals and thus on the recovery yields.

In addition to the higher metal recovery of battery powder, spent medium bioleaching proved to involve a shorter processing time and a more facile handling. Moreover, in spent medium approach, optimization for higher organic acid production and higher metal recovery can be done separately, not to mention that metal toxicity to the fungi did not occur [27]. Therefore, it can be concluded that among the bioleaching methods of battery powder using A. niger, the spent medium bioleaching is the most propitious for the leaching of heavy metals.

3.5.2. Control

In control experiments, fresh sucrose medium was used as leachant. Unlike Li and Cu, metal recovery of Al, Mn, Ni and Co was negligible in control medium (Fig. 4). Fresh medium leached
10% Cu and 18% Li, the latter occurring probably by virtue of the high reactivity of lithium with water.

3.5.3. Chemical leaching

Chemical leaching experiments were carried out using commercial organic acids (citric, oxalic, malic and gluconic acid) as leaching agents to define the role of secreted organic acids by fungi. The concentration of commercial organic acids was the same as that of the secreted organic acids present in the 14-day-old *A. niger* culture. Fig. 5 shows the results concerning metal recovery by chemical leaching and spent medium bioleaching of 1% (w v⁻¹) battery powder. According to the results, except for Li and Mn, the recovery of all metals was higher using the bioleaching method than the chemical one. Additionally, based on the results, other metabolites like amino acids and undetermined organic acids might be involved in the bioleaching process.

In general, bioleaching by biogenic organic acids secreted by *A. niger* was more effective than chemical leaching, due to the higher recovery of metals and the lower cost of the leaching agents[21].
3.6. Surface morphology of battery powder

To investigate the progress of bioleaching, the surface morphology was studied before and after bioleaching using FE-SEM photomicrograph[17]. Fig. 6 (a, b) illustrates the FE-SEM images of the battery powder in two distinct magnifications prior to and following bioleaching. Fig. 6 (a) shows the FE-SEM photomicrograph of the original powder which has a smooth surface. However, the morphology of the bioleaching residue particles is much different from the raw particles. Fig. 6(b) shows the bioleached residue of battery powder (after bio-treatment) having a rough surface with some holes on it (increasing porosity)[2,16]. These micro-morphological changes revealed that fungal metabolite such as organic acids and amino acids slowly eroded the battery powder particles through chemical corrosive action and cause the metal mobilization to the solution phase [26].

![Fig. 5: Comparison between spent medium bioleaching and chemical leaching for metals recovery at 1% (w v⁻¹) pulp density.](image)
3.7. XRD analysis of battery powder

Comparing the phase of battery powder before and after bioleaching through XRD analysis, the progress of bioleaching process was studied. Prior to bioleaching, the crystalline phase of LiCoO₂ and LiNi₀.₅Mn₁.₁Ti₀.₄O₄ and after bioleaching, LiCoO₂ and LiNiO₂ were clearly detected.
As shown in Fig. 7 (b) after bioleaching, no Cu combination was observed, confirming the results of ICP which indicated that 100% of Cu was leached. Although the ICP results showed 65% leaching of Al, no sign of Al was observed in the XRD pattern, which may be attributable to the formation of the amorphous state of Al. As in Mn, the diffraction of the amorphous material was not visible due to the short range of atomic order.

**Fig. 7:** X-ray diffraction of (a) the original powder and (b) bioleached residue.
3.8. FTIR analysis

FTIR spectra of the battery powder before and after bioleaching are presented in Fig. 8 (a, b). In the spectra of powder before bioleaching (Fig. 8 (a)), the peak around 550 cm\(^{-1}\) is associated with the asymmetric stretching of M-O (where M corresponds to the Ni, Co, and Mn). The peak in the range of 607-800 cm\(^{-1}\) could be ascribed to Li-O and Al-O bonds and the peak at 3400 cm\(^{-1}\) is related to the bending vibration of water molecules (O-H)[39–42]. In addition, the peaks observed at 1630 cm\(^{-1}\) and 1700 cm\(^{-1}\) are associated with the asymmetric and symmetric stretching of the C=O bond. The peaks at 870 cm\(^{-1}\) and 1080 cm\(^{-1}\) could be attributed to \(-\text{C-H}\) and \(-\text{C-O}\) bending modes, respectively[22,43].

In the spectra of powder after bioleaching (Fig. 8 (b)), the peak in the range of 607-800 cm\(^{-1}\) is related to the metal oxalates. The peak at the 550 cm\(^{-1}\), for instance, specifies the vibration bond of Ni-O in nickel oxalate (totally M-O where M corresponds to the Ni, Co, and Mn). Additionally, the peak around 800 cm\(^{-1}\) is a characteristic of lithium oxalate[44,45]. The broad peak beginning at 1600 cm\(^{-1}\) and extending up to 2900 cm\(^{-1}\) is concerned with the formation of metal complexes with acid where more exchange causes a wider peak. The observed peak at around 2900 cm\(^{-1}\) could be ascribed to C-H stretching bond. The peak at about 1600 cm\(^{-1}\) is associated with a C=O stretching group that shifts from 1700 cm\(^{-1}\), emphasizing that the metal oxides were connected with \(-\text{COOH}\). The peaks at 1400-1480 cm\(^{-1}\) (the specified peak at 1398.61 cm\(^{-1}\)) could be attributed to the symmetric vibrational frequencies of the carboxylate group. Moreover, the small peak observed between 2300 cm\(^{-1}\) and 2500 cm\(^{-1}\) was caused by the presence of CO\(_2\) molecules in the air. The strong OH bond at 3711 cm\(^{-1}\), is related to the absorption of surface hydroxyl groups, not the stretch vibration of water molecules[37,43].
4. Conclusion

In this research, we examined the fungal bioleaching of spent mobile phone batteries in laboratory-scale using A. niger through one-step, two-step and spent medium bioleaching. In this
regard, investigated were the growth characteristics of *A. niger* including pH variation, biomass and organic acid concentration; based on the results, the 3\textsuperscript{rd} and 14\textsuperscript{th} day of growth were respectively chosen for the two-step and spent medium bioleaching experiments. The result indicated that the total recovery in spent medium bioleaching was higher than that in other methods. 100% Cu, 95% Li, 70% Mn, 65% Al, 45% Co and 38% Ni were leached in spent medium experiments while in one-step bioleaching 100% Li, 58% Al, 11% Cu and 8% Mn were leached with Ni and Co having negligible leaching. In two-step bioleaching, 100% Li, 61% Al, 10% Mn, 6% Cu and 1% Co were leached (the amount of leached Ni was negligible). Furthermore, according to the results, citric acid was the major lixiviant among the other determined organic acids produced by the fungus. The XRD, FTIR and micro-morphological analysis of battery powder indicated that the appearance and structure of battery powder particles underwent a change during the bioleaching process. In addition, bioleaching, in comparison with chemical leaching, entails a higher efficiency as far as the recovery of heavy metals is concerned. With respect to the high metal recovery results, the fungal leaching as an eco-friendly and cost-effective method is an effective way to recover heavy metals in spent batteries.

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