Biohydrometallurgy for Cobalt Recovery from Spent Li-ion Batteries using *Acidophilic* Bacteria Isolated from Acid Mine Drainage

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ABSTRACT

Nowadays, recycling Li-ion batteries is an important thing to do to minimize the risk of exposure to this hazardous solid waste. On the other hand, extracting these materials can optimize the economic potential of valuable minerals in the active cathode. Bioleaching was developed as an environmentally friendly method to extract cobalt from the cathode by utilizing the metabolic activity of A. ferrooxidans cells. Microorganisms were isolated from Acid Mine Drainage in a 9K liquid medium at pH=2.5 and used as an inoculum. The Bioleaching process was carried out with various cell concentrations of 5, 10, and 20 %v/v. A positive response was shown by monitoring the microorganism activity through changes in physical appearance, decreasing the pH value, and increasing the Fe^{3+} concentration. The structural analysis of cathode by XRD and SEM has shown the effect of Co dissolution before and after bioleaching. Optimum recovery of cobalt was achieved up to 57.81% after 14 days of incubation.

1. Introduction

Cobalt is one of the rarest metals and is currently in great demand by the market because of its vast use in various advanced material applications. Cobalt limitations in nature make this metal price so expensive. In material science, cobalt plays an important role as a coating material (coating) and the mixture of metal alloy (alloy) due to having a unique property of increasing alloy resilience from hydrogen's durability, optimizing engine capabilities, and increasing material violence [1]. Cobalt was used in the United States for superalloys, aircraft gas turbine engines, and various chemicals [2]. Over the past 1 decade, cobalt's global consumption as a raw material for energy sources reached more than USD 5.0 million for various cell phone battery applications, gadgets, and electric vehicles/hybrids [3]. Searching for alternative cobalt sources is an interesting need for further research.

Using used lithium-particle batteries (LiBs) as an energy source hugely delivers a lot of strong waste, a severe issue presently being confronted [4]. These battery wastes contain various weighty metals and toxic electrolytes that can represent a specific danger to biological systems and health. Regulation of the Minister of the Environment of the Republic of Indonesia No. 8 of 2021 has classified LiBs as hazardous and toxic materials, so they need serious handling before they are defiled into the environment. The public authority's support for the reuse of utilized batteries is considered proper because this material has financial potential since it contains important metals, some of which are much higher than the metal substance in the natural mineral [5, 6]. The dynamic cathode material from LiBs based on $LiCoO_2$ has a high measure of cobalt, so the extraction of cobalt particles from this waste has the potential as an option for different new, further developed applications, for example, the creation of covering specialists and battery chargers [1].

Cobalt metal extraction from LiBs has been accounted for by a few investigations utilizing hydrometallurgical strategies. Some incorporate leaching using acids (inorganic and natural acids) and aqueous and electrochemical deposition techniques [7, 8]. Albeit this interaction has been broadly utilized, it requires high energy, poisonous synthetics, outrageous physicochemical circumstances, and eventually delivers hurtful side-effects into the climate. It supports the improvement of harmless to the ecosystem with fewer energy necessities and insignificant unsafe waste. The bio-hydrometallurgical strategy (bioleaching) is tentatively evolved in stages as a substitute for traditional metal extraction in light of its lower cost, natural agreeableness, and high effectiveness. Metal leaching with this method utilizes microorganisms fit for oxidizing iron and sulfur as an energy source. The arrangement of metabolic items, for example, inorganic and natural acids by microbial movement, is utilized to oxidize the objective metal from the mineral [8, 9].

Further examination of expected microorganisms for bioleaching frameworks should be completed. *Acidithiobacillus ferrooxidans* microbes from unadulterated cultures have been accounted for by filter specialists for utilizing lithium-particle batteries with lithium recuperation up to 79% [9, 10]. This bacterium was viewed as ready to live in outrageous local circumstances. The ex-coal mining pit region in Bentuas, East Kalimantan, makes this ex-mining region have a high natural substance, high sharpness (pH 3-5), poor in supplements, and by and large less prolific. The pool of water framed in this opening because water presented to sulfur oxides will shape an answer with high and perilous acridity and is alluded to as Mining Acid Water (AAT). This environment is ideal for developing *acidophilic* microbes [11–13]. The presence of *acidophilic* microbes in AAT should be explained by separation and development on specific media in light of their actual capacity as harmless to the natural filtering specialist. This exploration is relied upon to have the option to use the secludes contained in AAT with the goal that it can deal with this loss into a significant item and lessen the sum and impacts it causes to help ecological protection endeavors.

2. Research Methodology

2.1. Battery cathode preparation

In this study, physically, $LiCoO_2$-based cathode waste materials from cell phones and PCs were isolated from the plastic, zinc, and copper layers and afterward gathered. The battery capacity was purged for security reasons ahead of time by submerging the battery in 10% NaCl for 24 hours until the battery limit was <2 V. Then, at that point, the cathode was cut into 1 x 1 cm size. The cathode was calcined utilizing an electric heater at 600 ºC for 5 hours to eliminate the electrolyte and fastener [14]. After that, the cathode was ground until smooth homogeneous particles were gotten and sieved through a 150 lattice sifter. Preceding the bioleaching test, the powder was cleaned utilizing an autoclave (121 ºC, 20 minutes, 15 psi). The $LiCoO_2$ stage was apparent and portrayed by XRD (Brucker type D8 Advance) for subjective investigation and XRF (Horiba type Mesa-50) for quantitative examination of metal content.

2.2. Mining Acid Water Sampling

Mining Acid Water samples were taken from a coal mine pond in Bantuas, East Kalimantan. Sampling was done by checking the acidity test with a convenient pH meter. Water with a pH of <4 was an example since it demonstrated that it contained ferric iron (a decent climate for the presence of bacteria). Water samples were put away in a fridge at 4 °C.

2.3. Selective Media

Silverman/Lundgren (9K) type fluid media containing basal salt (arrangement A) joined with iron-sulfur (arrangement B) was utilized for the development of *acidophilic* bacterial strains, and its shown in Table 1.

Table 1. Component in liquid A and liquid B

Liquid A[a]*		**Liquid B[b]***	
$(NH_4)_2SO_4$	3.0 gr	$FeSO_4.7H_2O$	44.22 gr
K_2HPO_4	0.5 gr	Sulfur	4 g/L
$MgSO_4.7H_2O$	0.5 gr	Steril Aquades	300 mL
KCl	0.1 gr		
$Ca(NO_3)_2$	0.01 gr		
Aquades	700 ml		

[a.] * The solution was adjusted at pH = 5.5 with 2M H_2SO_4 (Merck) and sterilized at 120 ºC. 20 min, 15 psi.

[b.] * The mixture was filtered sterile using a 0.22 m membrane with a pH = 1.4

The two solutions are mixed and sterilized separately to avoid iron deposition and then stored. For long-term storage, media can be stored in a dark room. Meanwhile, further observations regarding the isolation and characterization of bacteria were carried out on 9K solid media by adding 1% w/v gelatin to solution A and then sterilizing using an Autoclave (121 ºC, 20 minutes, 15 psi). After that, the media was mixed with sterile solution B at 55 ºC so that the acid did not cause significant gelatin hydrolysis [14]. The media is then poured into a petri dish under sterile conditions until it solidifies.

2.4. Cultivation of Acidophilic Bacteria

Bacteria were inoculated in a 9K fluid medium at 10% (v/v) by adding 10 ml of acid mine water sample to 90 ml of media in an erlenmeyer cup, then at that point at 30 ºC. In the meantime, to support cell development, incubation treatment was done with mixing at a speed of 150 rpm to support the air circulation process. The incubation period was done for 14 days or until the microbes reached the logarithmic stage.

Identifying *A. ferrooxidant* microorganisms were carried out by cultivating a bacterial suspension when it reached the exponential growth phase into a solid medium. Then, it was cultured at 30 ºC for 7 days. A good indicator of the metabolic activity of *A. ferroxidans* can be determined by measuring the Fe^{3+} content of the culture. The content of Fe^{3+} ions during bacterial growth was estimated using the wet destructive method [14]. In the ferric ion test, 1 ml of inoculum was diluted 10 times, then 2 ml of 4M HCl (Merck) and 5 ml of 2M KSCN complexing solution (Merck) were added and allowed to stand for 15 minutes. Detection of Fe^{3+} ions was observed using a UV Vis Spectrophotometer (Shimadzu; UV-1800) at a wavelength of 481 nm to measure the red-purple color absorption in the detection of Fe^{3+} ions.

2.5. Bioleaching

In the initial stage, samples containing bacterial strains were acclimatized by adding an active cathode (pH 5.8) with a mixed concentration of 2.5 g/1 into the bacterial suspension. Cultivation was carried out on 9K liquid media. After reaching a decrease in pH of about 1.2, the cells were harvested as they had reached the final logarithmic stage and resuspended in a new 9K medium for use as inoculum.

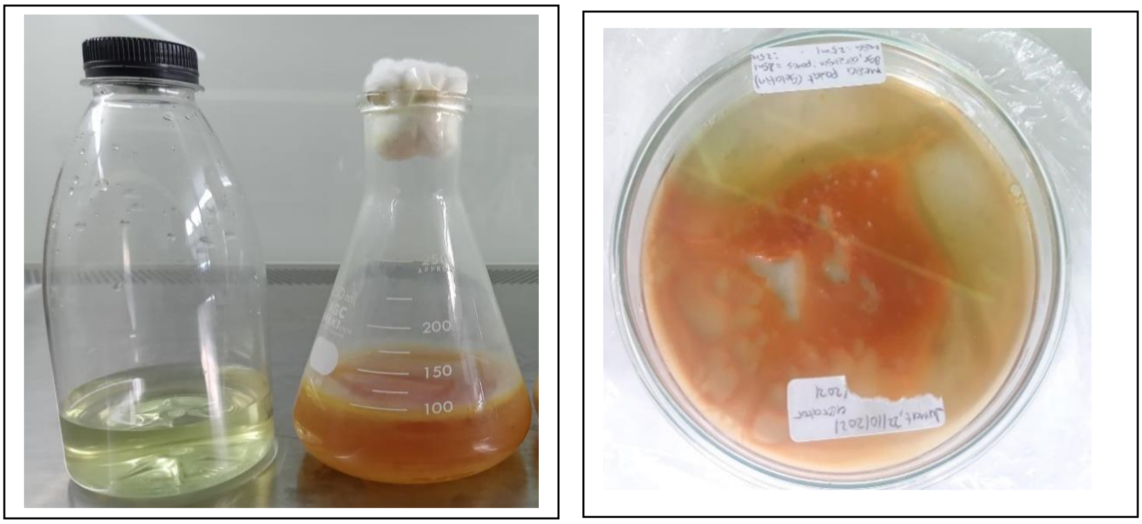
In this study, the influence of the inoculum concentration was traced starting from the concentration of 5% v/v to 20% v/v to speed up the leaching rate. Then 1 gr of active cathode powder was added, followed by the addition of inoculum in 9K liquid medium at pH = 2.5. Sterile control was carried out on media without inoculum at pH = 2.5. Sample analysis was carried out on days 0, 3, 7, 10, and 15. At the selected time, 10 mL of the sample was centrifuged and sterile-filtered to separate the leachant from the residue. Cobalt concentrations were analyzed by ICP-OES (Perkin Elmer Optima, 8000). Then the degree of metal uptake is calculated by:

$$\%recovery = \frac{Co\ dissolved}{Co\ in\ battery} x100\% \quad (1)$$

3. Results and Discussion

3.1. Identification of Bacterial Culture

The Fe^{3+} focus in AAT was estimated at 112 mg/L utilizing a UV Vis Spectrophotometer (Shimadzu; UV-1800). A high iron substance is an excellent sign of the action of acidophilic microscopic organisms. The development of *Acidophilic* microbes that have been cultured on 9K media has a positive reaction which is thought to consist of several types of bacterial strains, including *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidan,* and *Acidithiobacillus thiooxidans* where these microorganisms are the most common strains found in AAT [14, 15]. A positive reaction demonstrated that the fluid medium changed shading from light green to ruddy yellow for certain suspended solids. This is because of bacteria's movement of oxidizing ferrous metal to ferrous metal [16, 17].



(a) (b)

Fig. 1. Observation of detection of acidophilic bacterial growth on (a) 9K search media; (b) solid media

Bacteria cultured in 9K liquid media were then cultivated in solid media by the pour method until bacterial colonies were formed on the agar surface. The observed colonies were in the form of brown rust sediments. However, the growth of these bacteria became slower (new colonies formed on 7 days of observation) on solid media because the addition of gelatin as organic material could inhibit the growth of these *acidophilic* bacteria. This bacterium *A. ferrooxidans* is an obligate autotroph that cannot utilize organic compounds for composing its body cells (for growth and reproduction) and is obligate aerobic, so it requires free oxygen as an electron acceptor for metabolism [13, 18, 19].

Microorganism activity was further monitored through changes in pH and Fe^{3+} concentration in 9K liquid media during the incubation process. The pH of the solution increased during 7 days of incubation. Based on equations 2-3, an oxidation-reduction reaction occurs, which cannot be separated from microorganisms' role in increasing the metal ion degradation rate. During the initial three days of incubation, proton donors were higher than their generation. Therefore, the pH decreases when the release of H^+ ions is higher than their utilization in the reaction [20].

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 4H_2O \quad (2)$$

$$3Fe^{3+} + 6H_2O \rightarrow Fe(OH)^{2+} + Fe(OH)_2^+ + Fe(OH)_3^+ + H^+ \quad (3)$$

The oxidation of Fe^{2+} to Fe^{3+} occurs due to the bacteria *A. ferrooxidans* utilizing energy from Fe^{2+} ions from ferrous sulfate in a 9K medium to become Fe^{3+} ions and turn into iron hydroxide. Equations 4-5 show that jarosite formation occurs further when a complex reaction occurs with K^+ and NH^{4+} ions in the medium [14].

$$Fe^{3+} + (NH_4)^+ + 2(SO_4)^{2-} + 6H_2O \rightarrow (NH_4)Fe_3(SO_4)_2(OH)_6 + 6H^+ \quad (4)$$

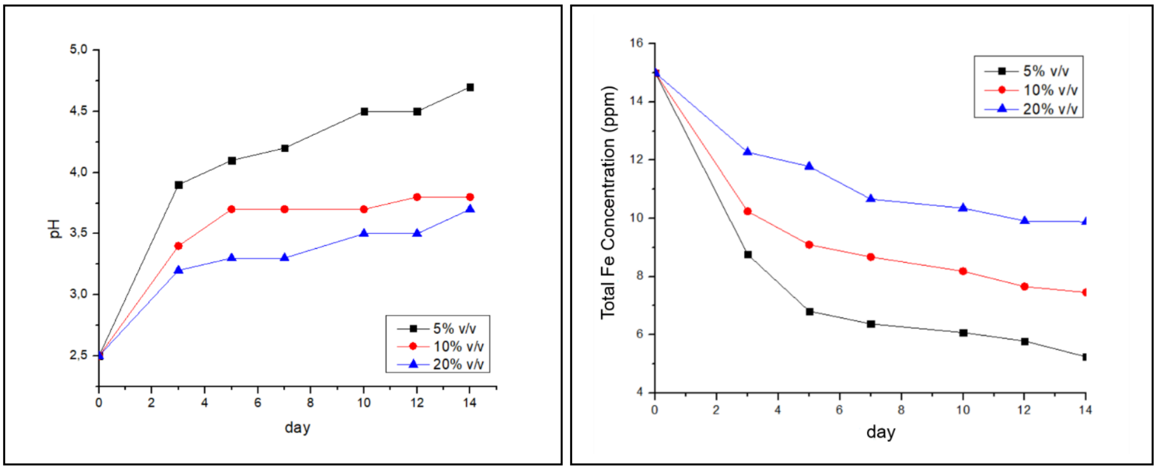
$$3Fe^{3+} + K^+ + 2(SO_4)^{2-} + 6H_2O \rightarrow KFe_3(SO_4)_2(OH)_6 + 6H^+ \quad (5)$$

3.2. Results of Cobalt Extraction by *Acidophilic* Bacteria

The cathode of LiBs is composed of metal with a $LiCoO_2$ structure with cobalt metal as the main constituent, as shown in Table 2. These results are the same as those obtained by previous studies, with a composition of 43-67% [21]. Bioleaching was carried out by immersing the battery cathode in 9K liquid media, which was added to inoculum with 3 types of concentrations, namely 5, 10, and 20% v/v, with a pulp density. of 10 g/L at initial pH = 2.5 for 14 days. Figure 2 shows that in the first 3 days of the bioleaching process, the Fe^{3+} concentration decreased drastically because the bacteria still needed to adapt to metal attack from the cathode powder. However, the Fe^{3+} concentration tends to be constant for the next few days, indicating that the bacteria are still alive [22]. LiBs contains a number of metal oxide components so that the pH of the solution increases to 3-4. However, the increase in pH in the liquid medium was still in optimum conditions for the growth of bacterial strains. These microorganisms are still able to catalyze the reduction of iron ions.

Table 2. A quantitative test of metal content in LiBs

Metal	Ni	Co	Mn	Mo	Cu	Al
% w/w	2.989	53.8241	1.67	0.2993	0.1998	0.1176



(a) (b)

Fig. 2. Profile of the activity of *A. ferrooxidans* in terms of (a) pH; (b) Fe^{3+} concentration during the bioleaching process of LiBs

Dissolution of metals is associated with sulfate formation. Bacterial cell metabolism reduces sulfur to H_2SO_4 then protons H^+ react with oxygen atoms $LiCoO_2$ followed by hydrolysis reactions by protonated oxygen atoms. The iron ion Fe^{3+} attacks the surface of the cobalt metal and then infiltrates the material's pores and dissolves the metal into the form of Co^{3+} ions through the redox reaction scheme in equations 6-11 [14].

$$2\,Fe^{2+} + \tfrac{1}{2}\,O_2 + 2\,H^+ \xrightarrow[\textit{A. ferooxidans}]{} 2\,Fe^{3+} + 4\,H_2O \tag{6}$$

$$4\,LiCoO_2 + 12\,H^+ \rightarrow 4\,Li^+ + 4\,Co^{2+} + 6\,H_2O \tag{7}$$

$$Li_2O + 2\,H^+ \rightarrow 2\,Li^+ + H_2O \tag{8}$$

The increase in inoculum concentration during this experiment positively responded to the cobalt extraction rate. The degree of metal recovery increased during bioleaching within 14 days. Up to 10 days, the Co concentration did not increase significantly due to the maximum reactivity of the bacteria, which were in the late logarithmic phase (towards the death phase) [5, 20, 22, 23].

$$Fe^{2+} + Co^{3+} \rightarrow Fe^{2+} + Co^{2+} \tag{9}$$

$$Fe^{2+} + LiCoO_2 + 4H^+ \rightarrow Fe^{3+} + Co^{2+} + Li^+ + 2H_2O \tag{10}$$

$$2FeSO_4 + 2LiCoO_2 + 4H_2SO_4 \rightarrow Fe_2(SO_4)_3 + 2CoSO_4 + Li_2SO_4 + 4H_2O \tag{11}$$

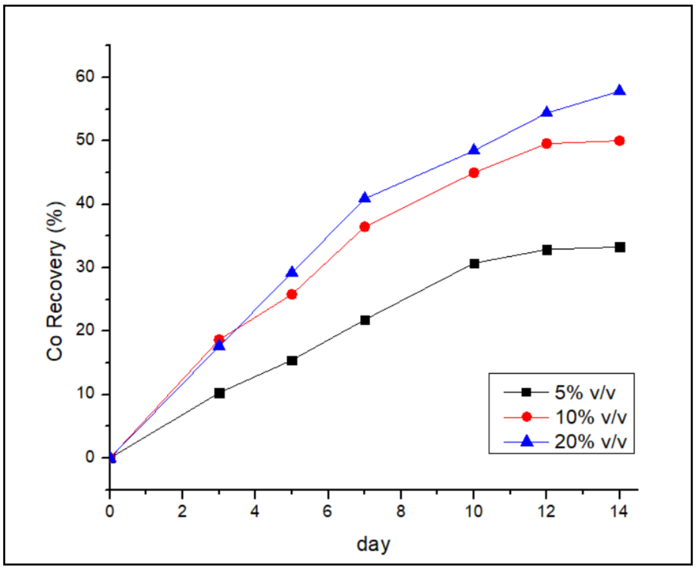
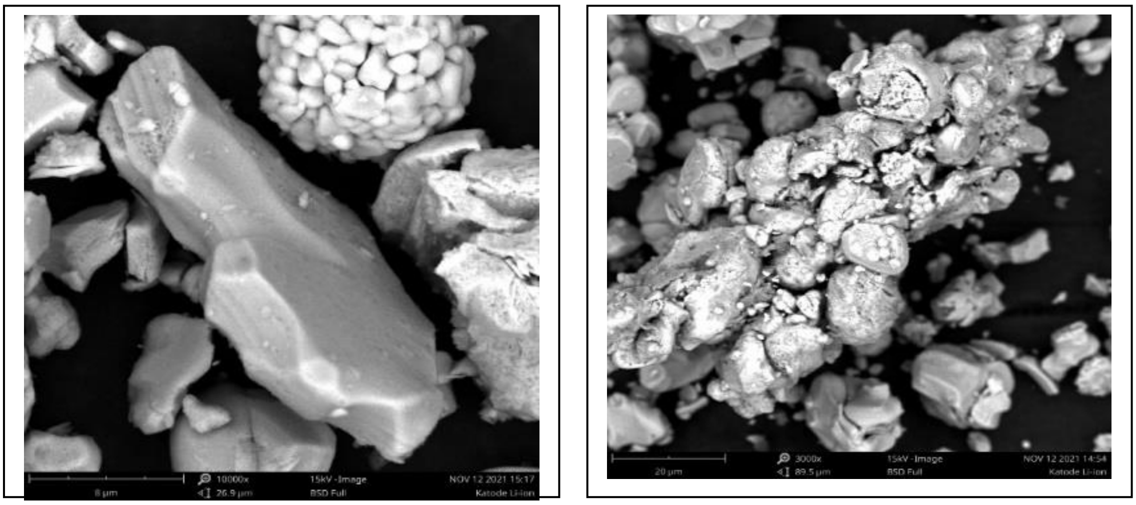


Fig. 3. Cobalt recovery during bioleaching experiment

The present work results indicated that it is possible to dissolve cobalt metals from LIBs by using acidophilic bacteria. The collected samples then need to be subjected to a further precipitation step to obtain a higher purity solid sample to return the battery raw material.

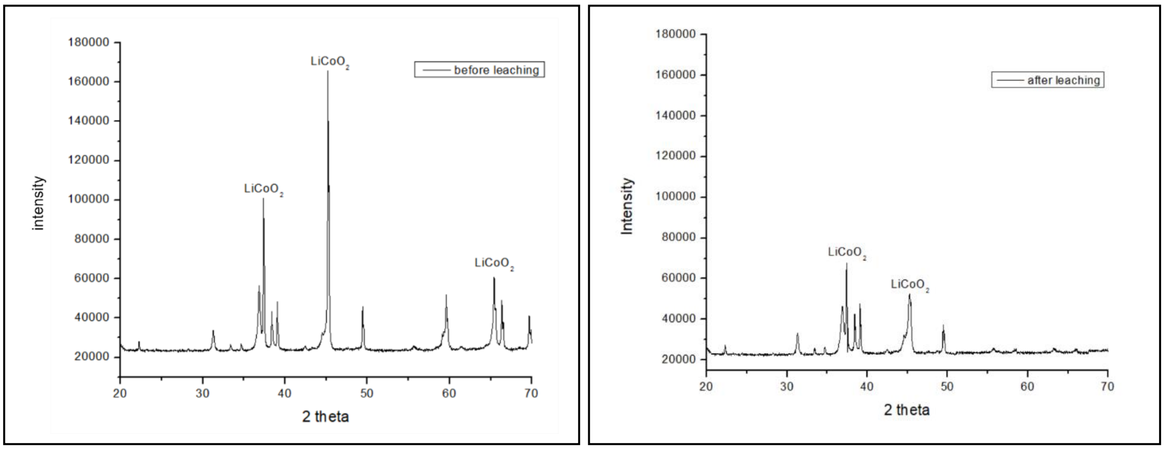
3.3. Structure Analysis

Metal structure with crystalline phase was observed at the active cathode before bioleaching. During bioleaching, acid creation and iron decrease. The semi-cracked particle structure indicates that the bacterial activity managed to infiltrate the particle pores. In addition, in the qualitative test, the $LiCoO_2$ peak decreased drastically on XRD detection after the bioleaching experiment, showing the same phenomenon based on SEM data. This shows a similar phenomenon that metal dissolution occurs during the bioleaching process [24, 25]. The morphology of the LiBs cathode powder particles when bioleaching is displayed in Figure 4.



(a) (b)

Fig. 4.$LiCoO_2$ Surface Morphology: (a) before bioleaching, (b) after bioleaching



(a) (b)

Fig. 5.XRD analysis of original LiBs powder of particle size <100 mm. (a) before bioleaching; (b) after bioleaching

4. Conclusion

This research studies a sustainable alternative method for cobalt extraction from the active cathode of LiBs based on $LiCoO_2$ by a bioleaching process using *autotrophic* microorganisms. AAT showed a positive response as a source of inoculum raw material (A. ferrooxidans bacteria), thus allowing it to be further optimized as a leaching agent. The results showed that the culture of *A. ferrooxidans* could produce sulfuric acid to dissolve metals and oxidize Fe^{2+} to Fe^{3+} to reduce the solubility of Co^{3+} from the cathode. The addition of the inoculum concentration is directly proportional to the increase in the recovery of cobalt ions. The highest cobalt recovery was obtained at a concentration of 20% v/v of 57.81% in 14 days of incubation. Structural analysis with SEM and XRD also confirmed that most of the metal was extracted within 14 days of bioleaching. Furthermore, it is necessary to develop further studies regarding the time of the bioleaching process. Selective precipitation of target metals to streamline the process.

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