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Small-scale and scale-up bioleaching of Li, Co, Ni and Mn from spent lithium-ion batteries

Sandeep Panda,[a*] Seydou Dembele,[b] Srabani Mishra,[b] Ata Akcil,[b,c*] İsmail Agcasulu,[b] Edris Hazrati,[b] Aysenur Tuncuk,[b] Pierre Malavasi[d] and Stoyan Gaydardzhiev[d]

Abstract

BACKGROUND: A bioleaching process could offer the advantage of higher metal recovery in a sustainable manner even from lithium-ion battery (LIB) samples with very low metal concentrations. In recent years, there has been a significant increase in the use of secondary resources such as LIBs for various purposes including transportation, large-scale energy storage and use in portable devices.

RESULTS: The adaptation of a mixed culture of acidophilic microorganism (lab stock culture) to a representative LIB sample allowed the setting of 0.5% of the pulp density under lab scale conditions. The maximum metal dissolution by bioleaching in a 1-L bioreactor for the as-received and thermally treated samples was found to be Li (67% & 49%), cobalt (81% & 86%), nickel (99% & 87%) and manganese (86% & 75%). Likewise, on the 10-L scale, the dissolutions observed were: Li (80% & 67%), Co (75%), Ni (91% & 88%) and Mn (63% & 75%) for the as-received and heat-treated samples, respectively.

CONCLUSION: Parameters such as particle size, leaching time, pH and iron ions (Fe^{2+}) affect the efficiency of acidophilic bioleaching of Li, Co, Ni and Mn from spent LiBs.

Keywords: acidophilic mixed culture; bioleaching; LiBs; thermal treatment

INTRODUCTION

Recently, the increasing use of electronic equipment has led to the generation of a huge amount of electronic waste (e-waste).[1] Globally, an estimated 20–50 Mt of e-waste is generated each year, representing 1–3% of municipal waste. However, a very small quantity is treated domestically. Recycling is the most pragmatic approach for value recovery from e-waste and environmental protection.[2] Batteries are considered as one of the most toxic e-wastes because they comprise various harmful components such as lithium (Li), cobalt (Co), nickel (Ni), chromium (Cr), manganese (Mn), zinc (Zn) and organic constituents.[3] Depending on the metal content, batteries can be categorized as Ni-metal hydride (NiMH), Zn—Mn, Ni—Cd, Pb-acid and Li-ion batteries.[1,3,4] The latter is one of the most interesting for recycling, as the spent lithium-ion batteries (LiBs) contain recoverable compounds such as $LiCoO_2$, $LiNiO_2$, $LiMn_2O_4$, $\mathrm{LiNixCoyMn1} - x - y\mathrm{O_2}$ or $LiFePO_4$.[5] The limited availability of Co, especially in Europe and the fact that it is very important for the EU economy, has made Co one of the vital critical raw materials for the EU. The recent forecast indicates that new emerging technologies will require nearly 300 000 t Co yr^{-1} by 2030.[6] Therefore, boosting the recycling rate of cobalt from spent electronics (e.g. spent LiB) will become a key industrial challenge.

The recycling processes of spent LiBs includes conventional approaches such as pyrometallurgy and hydrometallurgy. In addition, the bio-hydrometallurgical approach, which involves the use of microorganisms such as fungi, chemolithotrophic and acidophilic bacteria as leaching agents, is gaining momentum. The preliminary pre-treatment and extraction of metals from LiBs is carried out by the pyrometallurgical processes.[7] The pre-treatment processes such as pyrolysis[8] and the sulfation roasting processes[9] are used to convert the oxide elements in LiBs into easily soluble compounds for subsequent processes. Besides, reduction processes are used to recover metal from the oxide compounds in the LiBs.[7] Whether it is the pyrolysis process or the reduction process, all of these techniques consume a higher amount of energy.[10]

Several research works have focussed on hydrometallurgical approaches for the extraction of metals from LiBs in order to find

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* *Correspondence to: A Akcil, Department of Mining Engineering, Mineral Processing Division (Mineral-Metal Recovery and Recycling Research Group), Suleyman Demirel University, TR32260, Isparta, Turkey. E-mail: ataakcil@sdu.edu.tr; or S Panda, Department of Industrial Biotechnology Gujarat Biotechnology University (GBU), Gandhinagar, Gujarat-382355, India. E-mail: sandeep.panda@gbu.edu.in*

[a] *Department of Industrial Biotechnology Gujarat Biotechnology University (GBU), Gandhinagar, India*

[b] *Department of Mining Engineering, Mineral Processing Division (Mineral-Metal Recovery and Recycling Research Group), Suleyman Demirel University, Isparta, Turkey*

[c] *School of Mining and Geosciences (SMG), Nazarbayev University, Astana, Republic of Kazakhstan*

[d] *GeMMe-Minerals Engineering and Recycling, University of Liege, Liege, Belgium*

cheaper and more efficient methods than pyrometallurgy. Leach lixiviants such as mineral acids [hydrochloric (HCl), sulfuric (H_2SO_4), nitric (HNO_3) and phosphoric (H_3PO_4)] or organic acids (citric, oxalic and tartaric) and alkaline solutions (ammonium hydroxide, oxalate, bicarbonate and carbonate) have been used for metal leaching from spent LiBs.[1,5,11]

In LiB leaching, in addition to the use of conventional acids, some reducing agents have been used to improve leaching efficiency. Hydrogen peroxide (H_2O_2), when oxidised to O_2, is one of the most commonly used reducing agents.[12,13] However, this reagent has been shown to be a hazardous chemical and to cause fluctuation in leaching efficiency.[12] Therefore, a mineral-reducing agent such as pyrite has been tested as an alternative process for metal recovery from LiBs. Su *et al.*[13] prove that the maximum leaching efficiencies for Ni, Co, Mn and Li of 98–99.9% can be achieved with a iron disulfide (FeS_2) dosage ratio of 1:0.8.

In order to reduce or avoid the use of strong acids, Nshizirungu *et al.*[14] used waste chlorinated polyvinyl chloride (CPVC) in the presence of Ni^{2+} ions as an eco-friendly reagent for the recovery of Li and Co from LiBs. In the process, CPVC was used to provide HCl, and Ni^{2+} was used as a catalyst. Under the optimum conditions of temperature (240 °C), liquid–solid ratio (25:1 mL g^{-1}), Ni^{2+} concentration (0.04 mol L^{-1}) and time (40 min), Li and Co leaching efficiencies reached 99.05% and 98.08%, respectively. In another study, a chelating agent such as ethylenediamine tetraacetic acid (EDTA) was used for metal recovery from LiBs. Leaching efficiencies of Co (93.6%), Li (96.4%) and Cu (97.1%) were reached under optimum conditions of 0.8 mol L^{-1} EDTA concentration, 90°C temperature, 50 g L^{-1} pulp density, pH 10, and 4 h leaching time.[15]

Organic acids have emerged as alternative reagents to strong acids. Efficient optimization of these reagents could result in high metal leaching efficiency. Liang *et al.*[16] studied the applicability of a mixture of acetic acid and glucose for metal leaching from LiB samples using a mathematical model [Box–Behnken (BBD) design] four-parameter optimization. Under optimum conditions of temperature (185 °C), acetic acid concentration (1.53 mol L^{-1}), glucose dosage (1.67 g g^{-1}), solid/liquid ratio (5.19 g L^{-1}) and leaching time (67 min), leaching efficiencies of Li (99.93%), Co (97.57%), Mn (99.37%) and Ni (98.79%) were reached.

In general, all of these hydrometallurgical processes give excellent results for metal recovery from LiBs. Nevertheless, they are still techniques that require high temperatures (≤240 °C) and produce by-product chemicals. As an alternative to the previous methods, bio-hydrometallurgy has been considered as an eco-friendly technique.[17] Heterotrophic microorganisms such as *Aspergillus niger*[18] has been used for metal extraction from LiBs. Many studies have been carried out on the use of acidophilic bacteria for metal leaching from LiBs.[18] Biswal *et al.*[19] used *Acidithiobacillus thiooxidans* for metal recovery from spent lithium-ion batteries. It was concluded that this pure culture was not effective in bioleaching Li (66%) and Co (22%) after 40 days of incubation. Ghassa *et al.*[20] used a mixed culture of acidophilic thermophilic microorganisms including *Acidithiobacillus caldus, Leptospirillum ferriphilum, Sulfobacillus* spp. and *Ferroplasma* spp. for the recovery of Co, Ni and Co from LiB; at 45 °C, recoveries of 99.9% Co, 99.7% Ni and 84% Li were achieved. In another recent study, the synergistic enhancement of bioleaching efficiency was carried out using a mixed acidophilic culture where ascorbic acid was added to the leaching solution to improve metal extraction from spent LIBs.[21] However, most of these studies were conducted at laboratory scale. The current study aims to investigate bioleaching of Li, Co, Ni and Mn from thermally treated and nontreated spent LiB samples at laboratory, and semi-pilot scales respectively, with comparative insights into the characterization and pre-treatment aspects on the dissolution of these metals.

MATERIALS AND METHODS

Spent lithium-ion battery preparation

The untreated and treated LiBs samples used in the current study referred as 'black mass' were received from Exitcom (Turkey) after initial disassembly and discharge. The untreated sample was used as received in the subsequent tests. In the second case, 50 g black mass sample was placed in a porcelain crucible and then placed in a muffle furnace (Elektro-Mag, Istanbul, Turkey). The sample in the crucible was kept open in the furnace for 2 h in control mode at 700 °C. This process aims to remove organic binders (polyvinylidene fluoride).[16] The required size fractions of the sample were obtained by using two different shredders: a ZM 200 mill (Retsch, Haan, Germany) to obtain 500-μm and 250-μm size fractions; while, a Rocklabs (Aukland, New Zealand) mill to obtain 150-μm and 75-μm size fractions.

The initial sample from Exitcom was properly mixed and ≈1 kg LiB sample was taken using a quartering divider. As the Retsch ZM 200 mill incorporates a grinding and size control system, a 500-μm sieve was initially placed inside the equipment and then 1 kg LiBs was ground. A 250 g (−500 μm) amount was taken and the rest was passed through a 250-μm sieve before a second size reduction step. The oversize (+250 μm) was ground in the same equipment by changing the 500-μm sieve to a 250-μm sieve. This ground product was properly mixed with the previous undersize (250-μm) and then another 250 g was taken. The smallest sizes (150-μm and 75-μm) were obtained using a Rocklabs mill. The rest of the sample from the second size reduction step was first passed over a 150-μm sieve followed by grinding of the oversize (+150-μm) with the Rocklabs mill. The ground LiB sample from this third process also was mixed with the undersize (−150-μm) and then a third 250 g was taken. The same process was repeated with the –75-μm fraction.

Microorganisms and adaptation

A laboratory stock culture of mixed acidophilic strains was used in the current study. The consortium included acidophilic iron and sulfur oxidizers namely *Acidithiobacillus ferrooxidans* (DSMZ 583), *Leptospirillum ferrooxidans* (DSMZ 2705), *Leptospirillum ferriphilum* (DSMZ 14647) and *Acidithiobacillus thiooxidans* (DSMZ 9463) from Leibniz Institute DSMZ (Braunschweig, Germany). The 9k media with the following composition – $(NH_4)_2SO_4$ (3 g L^{-1}), $MgSO_4.7H_2O$ (0.5 g L^{-1}), K_2HPO_4 (0.5 g L^{-1}), $Ca(NO_3)_2.4H_2O$ (0.1 g L^{-1}), $FeSO_4.7H_2O$ (44.2 g L^{-1}) and elemental sulfur (S; 1 g L^{-1}) – was used in preliminary adaptation of the strains to the black mass and also for the bioleaching experiments Panda *et al.*[22]

To begin the adaptation process, the microorganism was activated through sequential subculturing with 90 mL fresh media and 10 mL mixed culture. Following activation, sequential adaptation tests were conducted via repeated transfers at varying pulp densities of 0.1%, 0.25%, 0.5% and 1%. These tests served the dual purpose of acclimating the bacteria to the LiB sample and determining a suitable pulp density that it could tolerate. During the initial phase of adaptation, a mixture of media and active culture was supplemented with 0.1% LiB. Fe^{2+} concentration and pH were monitored for control, the process ending when the Fe^{2+} concentration reduced to ≈0 g L^{-1}. The study's second phase regarding microbial tolerance capacity was carried out using the adapted cells from the first step (i.e. cells adapted with the 0.1% sample). All other parameters remained constant (10% culture and 90% fresh 9k medium) with pulp density of 0.25% LiB sample. The same process was repeated with the adapted culture from the previous step to increase the microbial tolerance to the metal ions present in the sample at 0.5% and 1% LiB. Adaptation was, however, limited to 1% LiB due to the very slow rate of Fe^{2+} oxidation. For each adaptation tests, the pH of the medium was adjusted to pH 1.8 using 1 mol L^{-1} H_2SO_4 and all experiments were performed under shaking conditions at 30 °C and 150 rpm.

Bioleaching experiments

Laboratory-scale bioleaching tests

The laboratory-scale bioleaching experiments were initially carried out using shake flasks to optimize several process parameters that had only been carried out on untreated LIB samples. Four sets of small-scale experiments were carried out using the same 0.5% pulp density from the adaptation tests. The first set of experiment was designed to evaluate the bioleaching time over 14 days. In this case, the ferrous concentration was 9 g L^{-1} and the sulfur concentration was 1 g L^{-1} at pH 1.8. After selecting the appropriate time, a second set of tests was carried out on different particle sizes such as 500-μm, 250-μm, 150-μm and 75-μm to evaluate their effect on the bioleaching efficiency under the same ferrous and sulfur concentrations and pH. Considering the first and second set of experiments, the third and fourth set of tests were conducted in parallel to investigate the ferrous concentration (3 g L^{-1}, 5 g L^{-1} and 9 g L^{-1}) at pH 1.8 (pH 1.5, 1.8 and 2) at 9 g L^{-1} on the bioleaching efficiency at the same sulfur concentration.

All experiments were conducted in 250-mL Erlenmeyer flasks with a total working volume of 100 mL. For each test, 90 mL of 9k medium and 10 mL active-adapted mixed culture were added to the flask before the addition of 0.5 g LiB sample. The pH was then adjusted with 1 mol L^{-1} H_2SO_4 according to the test. All of the flasks were kept under shaking conditions in an orbital shaker incubator (Gallenkamp Lab, Cambridge, UK). The stirring speed and temperature were maintained at 150 rpm and 30 °C, respectively. The volumetric loss resulting from evaporation was compensated with an equal volume of distilled water. Figure 1 shows the methodology for the shake flask leaching tests.

Bioreactor leaching tests

As a continuation to the shake flask tests, the laboratory-scale shake flask experiments were further validated using 1–L stirred tank bioreactors and then scaled-up to 10-L scale (using double-layered jacketed glass bioreactors; Rettberg, Göttingen, Germany). Here, the bioreactor (1 L) experiments were performed with both the treated and untreated LIB samples using the optimized parameters obtained from the shake flask experiments. The aim was to confirm the results of the shake flask tests and to perform a comparative study between the treated and untreated LIB samples. The temperature (30 °C) and stirring rate (150 rpm) were kept constant in both reactors in both reactor experiments. In the 1-L reactor, water circulating was controlled through the jacketed layer of the reactor using a heated/cooled water circulator (SMI100; Numan Teknik, Konya, Turkey). A CW-10G (Lab Companion Co., Guangdong, China) mark heated/cooled water circulator system was used for the 10-L glass bioreactor. The leaching time was 6 days for the 1-L reactor (based on the optimized result from shake flask tests) and 4 days for the 10-L reactor (based on the optimized results from the 1-L bioreactor). Figure 2 shows the steps for the 1-L and 10-L bioreactor leaching tests.

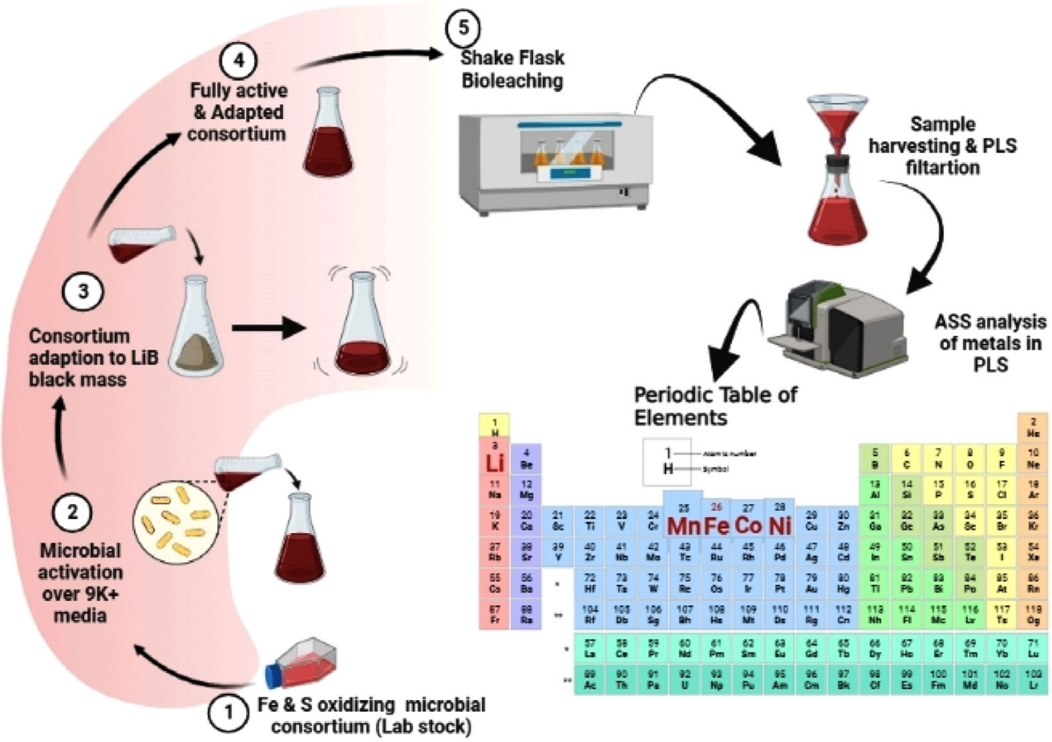


Figure 1. Methodology adapted for shake flask bioleaching tests for metal dissolution.

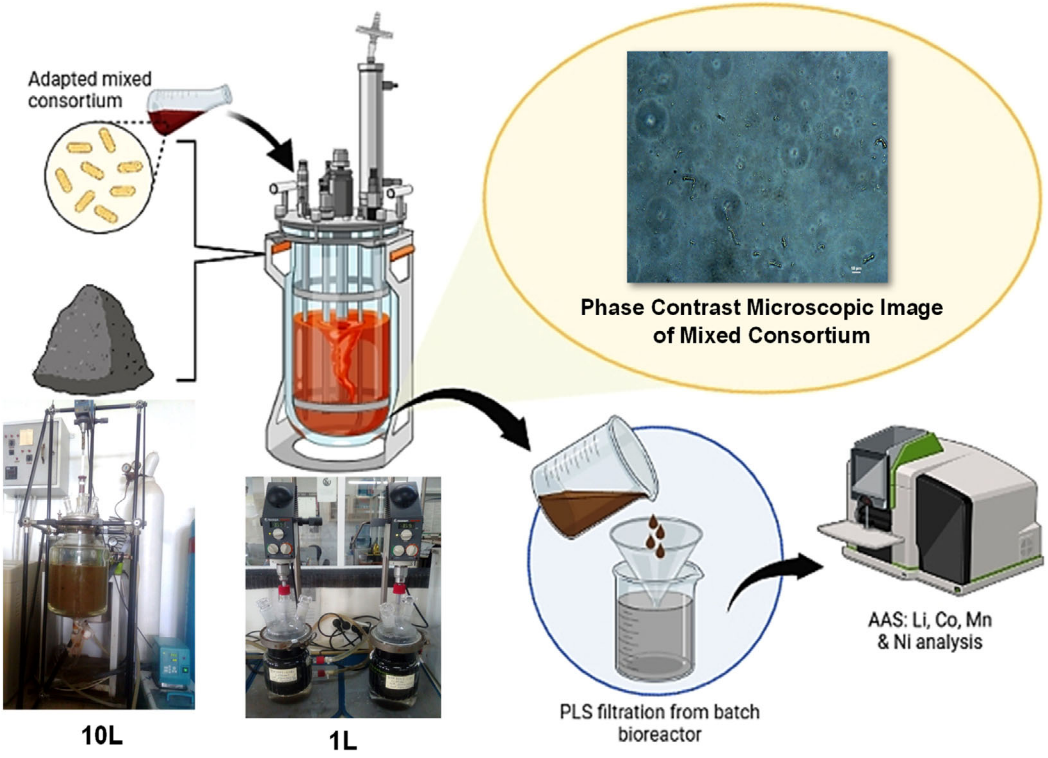


Figure 2. Stirred tank batch bioreactor system for bioleaching of lithium-ion battery black mass.

Analytical methods

For the determination of elemental metal content, a representative 1 g (triplicate) LiB sample was taken and digested in aqua regia (3HCl: $1HNO_3$). The digested sample solutions were filtered and the volume was made up to 100 mL. These solutions were then diluted at different dilution factors based on the metal concentration in the sample and submitted for AAS analysis (AA 200; Agilent Technologies, Santa Clara, CA, USA).

Mineralogical analysis of the fragmented LiBs (black mass) was done using X-ray diffraction (XRD) on a D8-ECO diffractometer (Bruker, Billerica, MA, USA), using CuK_α radiations ($\lambda = 19\,373$ $\dot{A}$). The respective sample was pulverized before being scanned between 5 and 75° 2θ at a speed of $0.02°2\theta\ s^{-1}$. The identification of all minerals from the XRD patterns was carried out using a Panalytical Xpert suite and WebPDF4 + ICDD relational database.

Black mass samples were observed for morphology and phases occurrences using a SEM-based automated mineralogy system—Zeiss sigma 300 FEG 'Mineralogic'—coupled to two Bruker EDS xFlash 630 X-ray energy dispersion (EDS) spectrometers (silicon drift detector). For this purpose, pre- and post-leach samples was cast into 30-mm-diameter mounts following an established procedure.[23] Section polishing was carried out using using polishing disks and diamond suspensions of different finesses. Scanning electron microscopy (SEM)-EDS analyses were performed using a probe current of 2.3 nA with an accelerating voltage of 20 kV at an 8.5 mm working distance. A mapping mode was performed using a with a step size of 3–5 μm and a dwell time of 55 ms. Analytical conditions such as contrast and brightness were set up manually adjusted to provide the best possible contrast between the observed phases (plastics, composites, metals). System magnification was set to ×6000 and voltage tension to 20 kV.

The changes in pH and ORP (with Ag/AgCl electrode) were measured using a digital Orion DUAL STAR™ pH/ISE meter (Thermo Fisher Scientific, Waltham, MA, USA). Each day, 3 mL bioleached solution sample was withdrawn from the respective flasks and reactors and analysed for Li, Co, Ni and Mn by atomic absorption spectroscopy. After sampling, the working volume was maintained by adding an equal volume of pH 1.8 H_2SO_4. The Fe^{2+} concentration was determined using the cerium sulfate titration method.[22,24,25]

RESULTS AND DISCUSSION

Chemical analysis of the LiB sample

Four metals (Li, Co, Ni and Mn) were considered for analysis for both treated and nontreated samples. The results are presented in Table 1. It can be seen that the metal concentration increased after thermal treatment as a consequence of the burning of some undesirable materials such as plastics.

Microscopic features of the as-received black mass

Mineralogical investigations of the black mass before and after thermal treatment was done to follow its transformation pattern resulting from the thermal treatment. The mineralogy of the untreated LiBs indicated that the black mass is presented in majority by a LCO-type cathode active material (CAM)with copper and graphite also detected [Fig. 3(a)]. The appearance of copper oxide (tennorite) after the thermal treatment indicated a prevalent oxidizing atmosphere. Nevertheless, the partial reduction of Co (III) to Co (II) could be attributed to the presence of graphite acting as reducing agent, generating CO_2 and CO. A large amount of graphite still remained in the black mass.

The SEM-EDS study gave an idea about the various CAM chemistries being present in the investigated lot of LiBs. In the upper images, CAM could be visualized as attached aggregates to an Al-Si-O-containing foil that underlies beneath. Possibly three

Table 1. Metal analysis results

Black mass	Li (%)	Co (%)	Ni (%)	Mn (%)
Untreated	2.86	19.75	2.04	6.14
Thermally treated	3.55	24.97	2.74	7.70

types of CAM chemistries could be distinguished. With an average composition of 68 wt% Mn and 30 wt% oxygen(O) (spectrum 1), the nearest known CAM type would be LMO ($LiMnO_2$ or $LiMn_2O_4$). The few percent of Co detected could arise from an adjacent second type CAM, presumably a NMC-spectrum 3. Spectrum 2 could suggest a third CAM type – thus, a LCO particle sitting on a remnant of an oxidised cathode support. It should be noted that XRD of the both nontreated and thermally treated sample revealed LCO-type CAM chemistry only (Figs 3 and 4). The fact that no LMO or NMC was detected is probably the result of a lack of data on Ni and Mn oxides in the crystallographic database or to the very high occupancy factor of the Co site.

Adaptation results

During the adaptation of the acidophilic mixed culture with a 0.1% LiB sample, a significant decrease in Fe^{2+} concentration was observed, from 9 g L^{-1} down to 0.1 g L^{-1}, along with a reduction in pH from 1.8 to 1.3. These changes were caused by the activity of microorganisms that oxidize Fe^{2+} into Fe^{3+} and elemental sulfur into sulfuric acid. The maximum ORP reached 546 mV. Similar results were observed with 0.25% and 0.5% pulp densities, where the final pH was 1.5 for both cases. The maximum ORP values were 572 mV for 0.25% and 571 mV for 0.5% pulp density. The adapted culture from the 0.5% LIB sample was subsequently utilized to investigate its tolerance towards a 1% LIB sample. Figure 5(a), (b) shows the change in ORP, Fe^{2+} and pH. The ferrous oxidation rate stayed constant after a 4-day incubation period, as indicated in Fig. 5(b). Based on this result, it can be assumed that the toxic effect of the LiB sample may have affected the microbial activity, resulting in a slow ferrous oxidation at 1% pulp density. Therefore, all subsequent bioleaching experiments were conducted using a 0.5% adapted culture as a consequence of the decreased mixed culture activity at 1% of LIB.

Similar findings have been reported elsewhere. Bajestani *et al.*,[26] reported that acidophilic microorganisms such as *A. ferrooxidans* are not able to survive in 1% pulp density of spent household batteries after several serial adaptations. Similar findings have been reported elsewhere. In one of the studies conducted by Heydarian *et al.*,[27] the stepwise cell adaptation was investigated using a mixed culture of *A. ferrooxidans* and *A. thiooxidans* in the presence of LiB powder. The microbial growth was monitored by cell-counting. The same adaptation procedure was adopted by Ghassa *et al.*[20] with mixed moderate thermophilic microorganisms consisting of

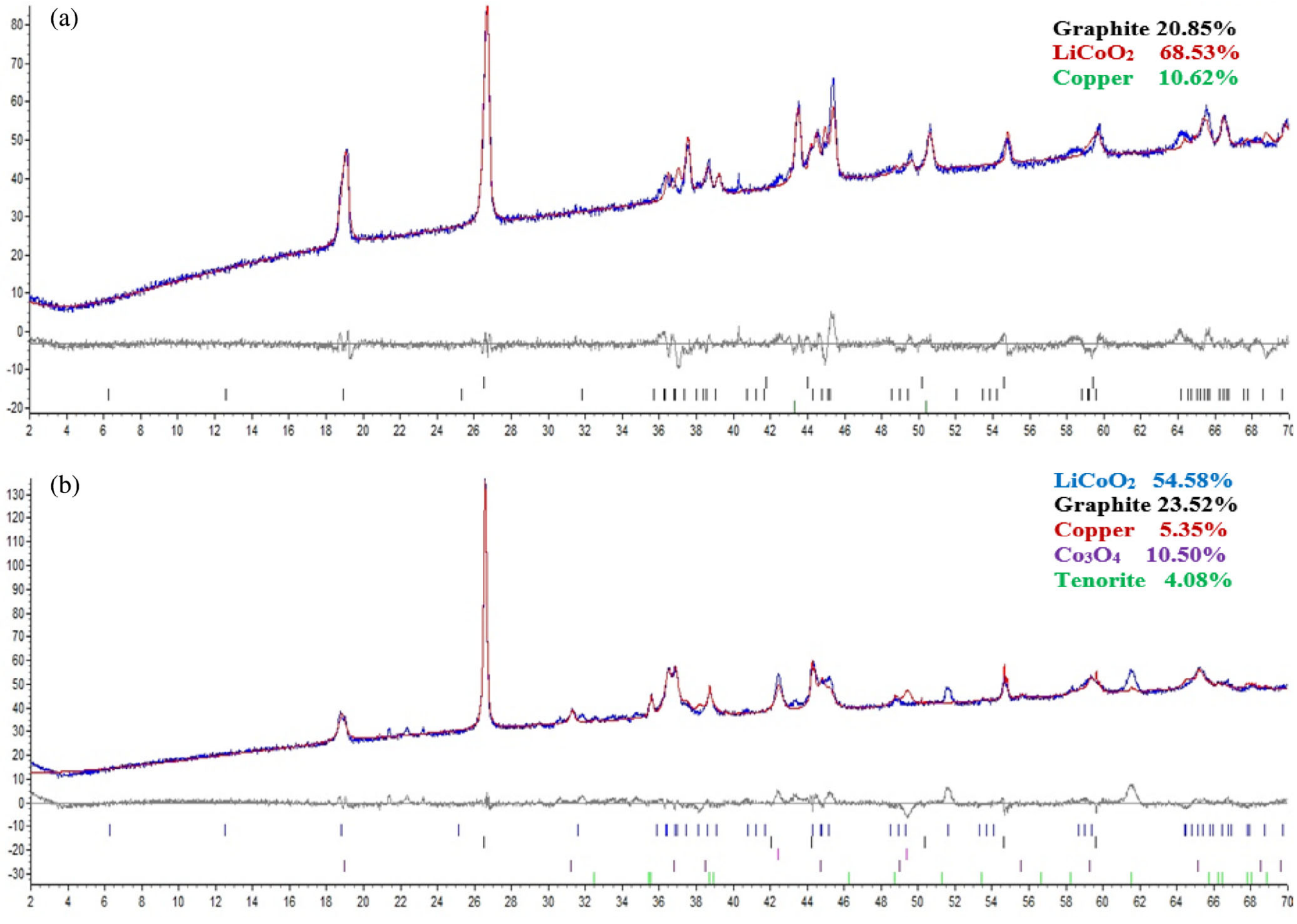
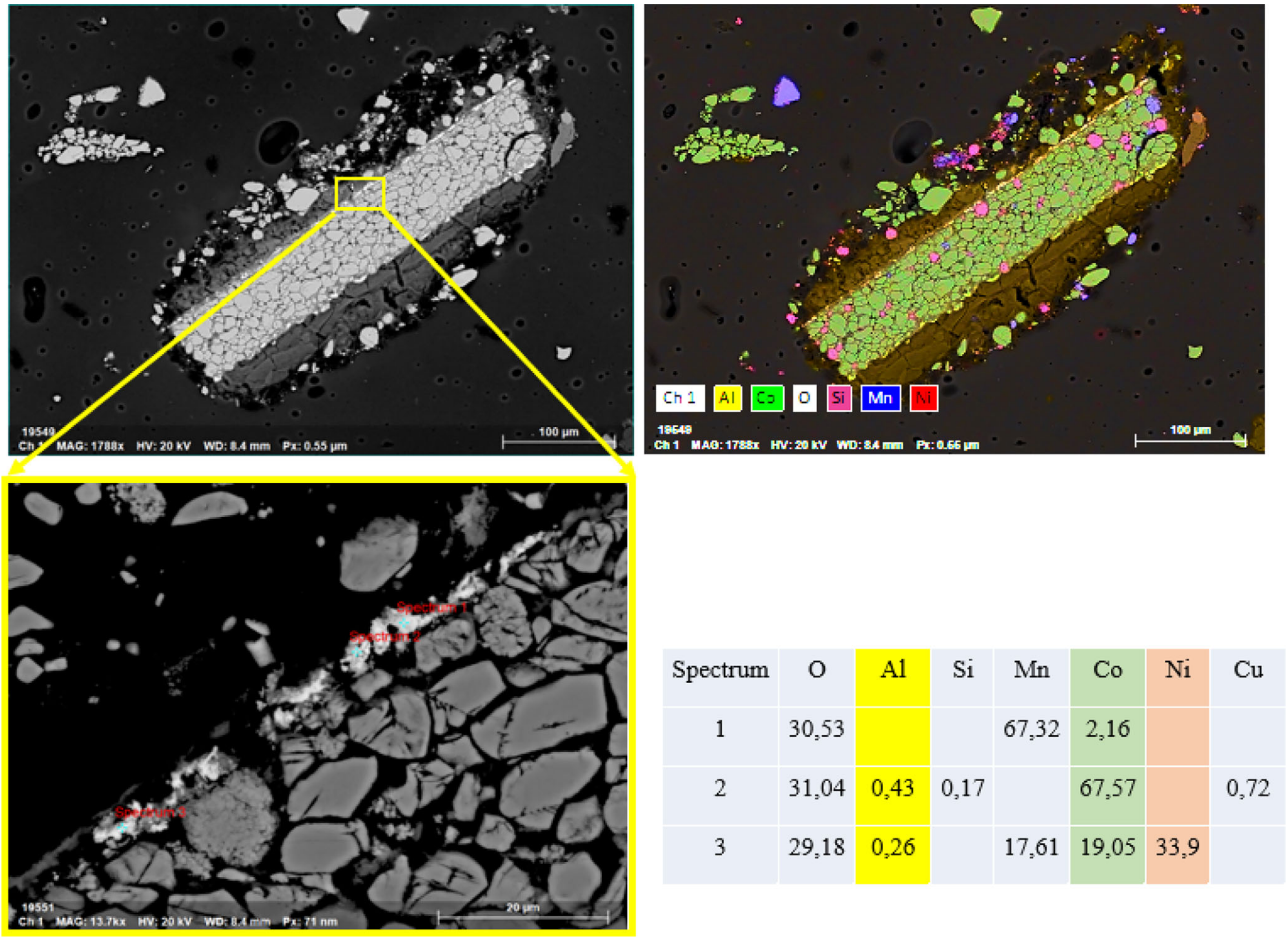


Figure 3. (a) X-ray diffraction (XRD) pattern of as-received black mass and (b) black mass treated at 700 °C.



Spectrum	O	Al	Si	Mn	Co	Ni	Cu
1	30,53			67,32	2,16		
2	31,04	0,43	0,17		67,57		0,72
3	29,18	0,26		17,61	19,05	33,9	

Figure 4. Back scattered electrons view of an untreated black mass with major metal-oxide phases and electron dispersive X-ray (EDX) spectrum of characteristics spots.

Acidithiobacillus caldus, Leptospirillum ferriphilum, Sulfobacillus spp. and *Ferroplasma* spp. before bioleaching of LiB samples. In both studies, a pulp density of 1% was found to affect microbial growth and activity. It has been shown that the most toxic cation for the acidophilic microorganisms is Co, among other heavy metals,[28] which can be explained by the high concentration of Co in the present sample (see Chemical analysis of the LiB sample section). Higher concentrations of metal ions result in bacteria extinction as a result of the cytotoxicity of metal ions, the greater stress induced by osmotic pressure, higher oxidative stress, etc. In addition, higher pulp densities during LiB bioleaching lead to a rise in the solution viscosity, which affects the dissolved oxygen and air distribution. Consequently, the microbial metabolism is affected and there is a decrease in the metal extraction efficiency.[29] Highly

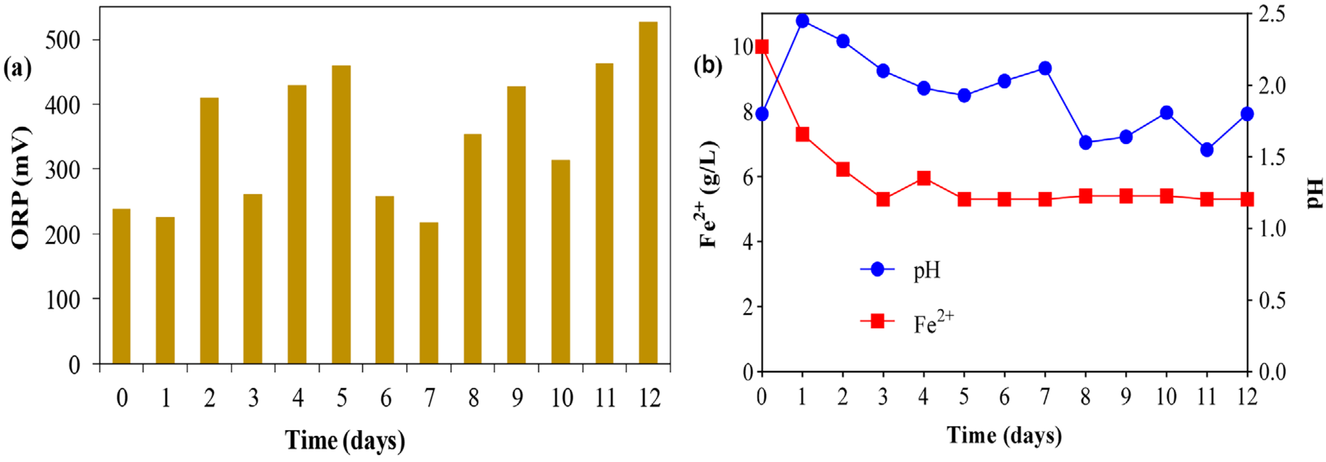


Figure 5. Adaptation of the mixed culture at 1% pulp density of lithium-ion batteries: (a) redox potential (ORP; mV) and (b) Fe^{2+} concentration (g L^{-1}).

acidic environments also are responsible for oxidative stress in acidophilic microorganisms. Reactive oxygen species (ROS) produced inside the cell act to provoke a defence mechanism in response to oxidative stress. However, it has been reported that proper regulation of the ROS by the external addition of glutathione leads to higher recovery of Li and Co at a pulp density of 5%.[30] the metal toxicity of LiBs towards microorganisms is governed by the concentration of heavy metals, and the contact time. In addition, the alkaline nature of the LiB sample demands a lower system pH for proper growth and function of the acidophilic microorganisms. Therefore, the adaptation of microbes through slow and gradual exposure of the microorganisms to an increased concentration of the toxic LiB becomes essential to maintain the metabolic growth and activity of the microbes in a bioleaching environment. In this regard, serial adaptation of the microbes through a gradual increase in pulp density leads to higher metal tolerance by the microbes.[20]

Laboratory-scale bioleaching tests

Effect of bioleaching time

As the total time of any process plays a vital role in determining the overall time required to achieve maximum yield, bioleaching tests were performed to evaluate the effect of test duration on metal dissolution. Solid density of 0.5% of the nontreated 500-µm LIB sample was used in the experiment. The effect of bioleaching time on the dissolution each metal is shown in Fig. 6. The maximum dissolution of Li, Co, Ni and Mn was 65.28%, 75.53%, 92% and 80%, respectively. In the study by Mishra *et al.*[28] at a similar pulp density and pH 2.5, Li dissolution was <10% and Co dissolution reached 65% in bioleaching of LIBs using single *A. ferrooxidans*. The results of the present study indicated that 6 days was the optimum bioleaching time, which resulted in maximum metal extraction, after which no significant improvement in leaching efficiency was observed. Different bioleaching times have been observed in some laboratory studies including: Mishra *et al.*,[28] 20 days; Zeng *et al.*,[31] 6 days; Xin *et al.*,[32] 9 days; Heydarian *et al.*,[27] 16 days; and Ghassa *et al.*,[20] 2 days.

Effect of size fraction on metal dissolution

The size fraction or particle size of the sample is an important parameter in determining the microbial efficiency for LiB bioleaching. It also sheds light on the accessibility of microbial metabolites for metal extraction from the sample. In the current study, different size fractions such as 500-µm, 250-µm, 150-µm and 75-µm were investigated to evaluate their effect on metals dissolution (Li, Co, Ni and Mn) for 6 days of leaching time. The effect of particle size on the metal dissolution is shown in Fig. 7. From the figure, it can be seen that for three metals (Li, Co and Ni) the metal dissolution during the first 2 days was high for the lowest size (75-µm), whereas for Mn the high value was obtained with 150-µm at the same period. In general, the leaching efficiency increased with decrease in particle size within 2 days. However, after this period it changed as 500-µm presented the highest metal dissolution for Li and Co. The Li dissolutions for distinct size fractions were 65%, 63%, 60% and 69% for 500-µm, 250-µm, 150-µm and 75-µm size fractions, respectively. For Co, the dissolutions for size fractions were respectively, 76% (500-µm), 67% (250-µm), 72% (150- and 75-µm). For Ni, the dissolutions were 90%, 87%, 83% and 88% for 500-µm, 250-µm, −150-µm and 75-µm size fractions, respectively. The dissolutions for Mn were 74%, 73% 73% and 76% for the -500-µm, 250-µm, 150-µm and 75-µm size fractions, respectively.

Recently, Liao *et al.*[33] used a fine particle size of 200 mesh (74-µm) in mixed culture bioleaching of LiBs. High metal dissolution was obtained in the study as the authors combined gallic acid and mixed culture in the process. Based on the data in this present study, it was observed that almost similar results were obtained with 500-µm and 75-µm size fractions and these two size fractions were more amenable to microbial attack. This indicated that grinding the sample to a lower size fraction of 75-µm was not necessary from an economic point of view. While considering scale-up aspects or industrial applications, it is important to note that there should be minimum possible fragmentation steps for the feed to make the process economically feasible.

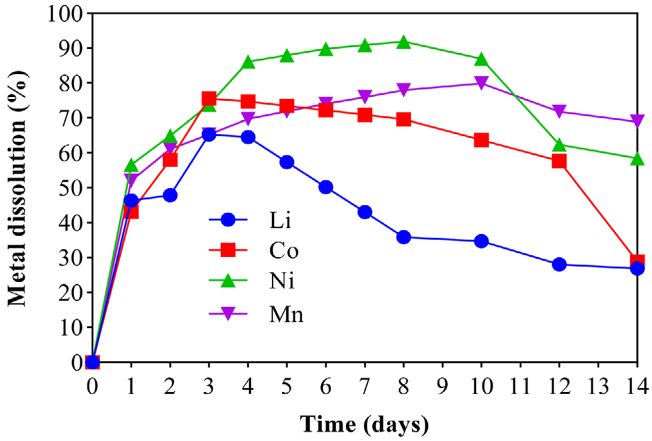


Figure 6. Effect of bioleaching time on lithium (Li), cobalt (Co), nickel (Ni) and manganese (Mn) dissolution; particle size 500-µm and leaching time 14 days at pH 1.8, Pulp density (0.5%), Fe^{2+}(9 g L^{-1}), sulfur (1 g L^{-1}), 150 rpm and 30 °C.

pH, Fe^{2+} and solution redox potential

Figure 8(a), shows the variation in Fe^{2+} concentration (g L^{-1}) for different particle sizes at the same pulp density. It can be seen that the Fe^{2+} concentration decreased considerably with time. In this case, the particle size does not have a significant effect on Fe^{2+} oxidation rate, because the Fe^{2+} concentration is similar in all the tested particle size fractions. The decrease in the Fe^{2+} to Fe^{3+} is due to oxidation by iron oxidizer bacteria in the mixed culture [Eqn (1)]. The biogenic Fe^{3+} is known as a strong oxidizing agent capable of attacking the valuable metals in the LiBs [Eqn (2)]. As the LiBs do not contain energy sources; the addition of this source helps the microorganism to grow and ensures the biogenic Fe^{3+} production.[34]

$$2Fe^{2+} + 0.5O_2 + 2H+ \overset{\text{Bacteria}}{\rightarrow} 2Fe^{3+} + H2O \tag{1}$$

$$Fe^{3+} + M^{\circ} \rightarrow Fe^{2+} + M^{2+} \tag{2}$$

It is important to adjust the pH of the system to find out the optimum value suitable for proper microbial growth and bioleaching conditions.[35] In this test, the initial pH was maintained at 1.8. The pH variation is presented in Fig. 8(b). The pH increased on Day 1 of the process and started to decrease for all the particle sizes. The decrease after Day 1 indicates the production of H_2SO_4 by elemental sulfur oxidizing microorganisms in the mixed culture [Eqn (3)]. The produced H_2SO_4 acts as a leaching agent for the

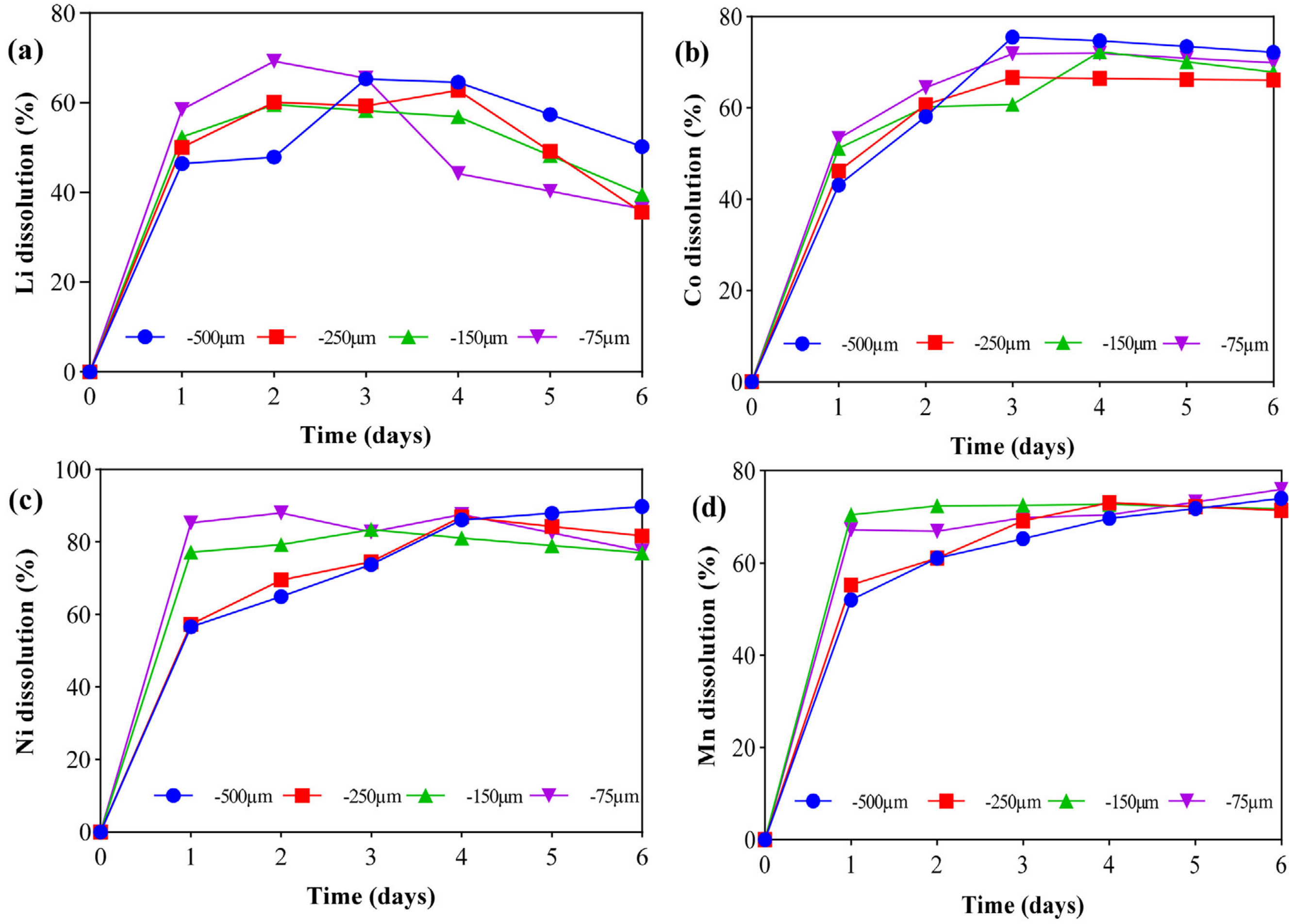


Figure 7. Variation in particle size and time, for (a) lithium (Li), (b) cobalt (Co), (c) nickel (Ni) and (d) manganese (Mn) at pH 1.8, pulp density (0.5%), Fe^{2+}(9-g L^{-1}), sulfur (1 g L^{-1}), 150 rpm and 30 °C.

dissolution of metals from the LIBs samples as indicate in the Eqn (4).[18,20]

$$S^{\circ} + H_2O + 1.5O_2 \rightarrow H_2SO_4 \text{ (biogenic product of } \textit{A. thiooxidans}\text{)} \quad (3)$$

$$H_2SO_4 + M \rightarrow MSO_4 + 2H^+ \quad (4)$$

Figure 8(c) presents the variation of redox potential (ORP) for different LIBs sizes. In general, the ORP decreases as the pH of the bioleaching system increases and *vice versa*. In this case, it can be seen that the ORP decreased in the first few days and then started to increase. This initial decrease can be attributed to the increase of the pH at the beginning with following start decrease [Fig. 8(b)]. The final ORPs were 509, 512, 505 and 507 mV for 500-µm, 250-µm, 150-µm and 75-µm size fractions, respectively. The redox potential of a system is a fundamental factor in ferrous sulfate-mediated leaching. The redox potential of the bioleaching solution is governed by the Nernst equation [Eqn (5)].[36] A higher redox potential indicates the presence of a higher amount of Fe^{3+} ions in the system and the process is driven by the Fe^{3+}/Fe^{2+} redox couple. The oxidation of ferrous ions to ferric ions leads to an increase in the redox potential of the system. Higher pulp densities have negative effect on the redox potential and lead to a decrease in the ORP.[20,29]

$$E = E^{\circ} + \frac{RT}{F} + \ln\left(\frac{Fe^{3+}}{Fe^{2+}}\right) \quad (5)$$

Effect of initial pH on metal dissolution

The initial pH variation study was conducted to evaluate its effect on metal leaching. The pH was varied as 1.5, 1.8 and 2, while all other conditions were kept constant. Figure 9 illustrates the effect of initial pH variation on metal leaching efficiency. The leaching of all metals increased slightly when the pH was decreased from 2 to 1.5. When the pH was maintained at 1.5, the leaching rate of Li, Co, Ni and Mn reached 68%, 99%, 98% and 97%, respectively. Likewise, at pH 1.8, the leaching of the Li, Co, Ni and Mn reached 66%, 97%, 97% and 97%, respectively. Finally, the leaching of Li, Co, Ni and Mn reached 65%, 97%, 97% and 99%, respectively, when the pH was maintained at 2. This study indicated that a pH variation in the range of pH 1.5–2 did not have a significant effect on the bioleaching process and almost similar metal extraction efficiency was observed at distinct pH values. This may be a result of the fact that the pH was only adjusted on Day 1 (it was not kept constant after the first day).

In a study conducted by Biswal *et al.*,[18] a single acidophilic sulfur oxidizing species of *A. thiooxidans* was used to leach Li and Co from the LiBs sample. Despite the pulp density (0.25% LiBs) being

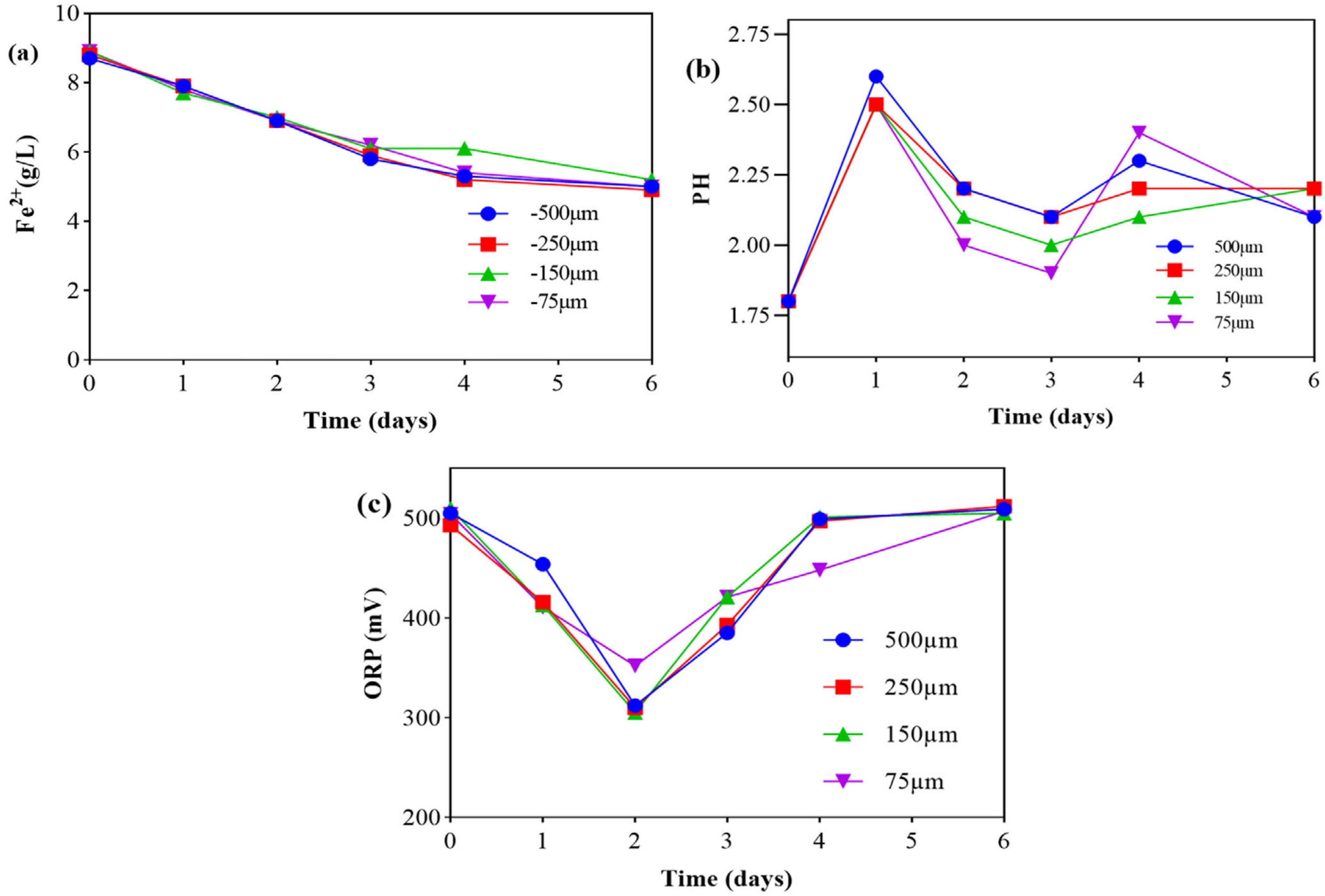


Figure 8. (a) Fe^{2+} concentration, (b) pH and (c) Redox potential, using different sizes of lithium-ion batteries.

low when compared to the present work (0.5%), the maximum dissolutions were 2.7% Co and 22.8% Li in a one-step bioleaching achieved after 40 days of incubation at an initial pH 3.3. The authors highlighted that the reason behind the very low metal dissolution in the one-step bioleaching was that the microorganisms were in direct contact with the sample during the bioleaching process. As mentioned in the Adaptation results section, the toxicity of LIBs affected the growth of microorganisms and then the biogenic H_2SO_4 production was reduced. Another reason was the nonadaptation of the microorganisms to the LiB sample. In another investigation by Naseri *et al.*,[29] *A. ferrooxidans* was used to leach Li, Co and Mn from coin cell batteries at high pulp density (40 g L^{-1}). At an initial pH of 2.0–2.5 the metal dissolutions were 100%, 88% and 20% for Li, Co and Mn, respectively. In their investigation, the authors were able to use high pulp density to obtain high metal dissolution because the microorganisms grew in the nutrient medium without LiBs in the first step. When the bacteria enter the logarithmic growth phase, the LiB sample was added for the bioleaching step. The initial pH of the system is considered to be a vital parameter in Li bioleaching, as the extraction of Li primarily proceeds via acid dissolution. LIBs are considered to be a common secondary source of Li, consuming a large amount of acid and having a normal pH value of 9.6. The leaching efficiency of acidophilic microorganisms generally decreases above a pH of 2.5. Lower pH media solutions are favorable for Li bioleaching from LiBs. Besides, the system pH also lowers gradually owing to the production of inorganic acid (sulfuric acid) as a result of microbial growth and activity, which further favors metal extraction. Several other studies have been carried out to study the effect of initial pH on metal bioleaching.[28,37] and some studies have highlighted the role of jarosite formation at higher pH, which affects the overall metal leaching efficiency.[38] In addition, higher pH values lead to inhibition of microbial cell growth, as they can only tolerate a specific range of pH.[46]

Effect of initial ferrous concentration on metal dissolution

The initial ferrous (Fe^{2+}) concentration variation tests were undertaken at pH 1.8 at three different concentrations (3, 5 and 9 g L^{-1}) within 6 days. In LiB bioleaching, the ferrous ions can play the role of electron donors to increase the metabolism and activity of acidophilic bacteria. The ferric produced by ferrous oxidation is a powerful biogenic reagent for metal leaching.[19] The main role of iron-oxidizing microorganisms is to regenerate the essential Fe^{3+} ion, which provides an oxidative attack on the material and leads to the bio-dissolution of metals.[39] Because e-wastes such as LIBs are noncarbon-based materials and contain low amounts of sulfur and iron,[40] a proper nutrient medium consisting of Fe^{2+}, S^0 and other vital nutrients is important for the proper growth and metabolism of the microorganisms during LIB bioleaching. However, it also is important to determine the optimum ferrous iron concentration in the medium, as higher Fe^{2+} concentrations reduce bioleaching efficiency owing to the toxic effects of iron.[41]

In the current study, the effect of Fe^{2+} ion concentration on metal leaching was investigated and the results are presented in Fig. 10. In these experiments, the lowest ferrous concentration

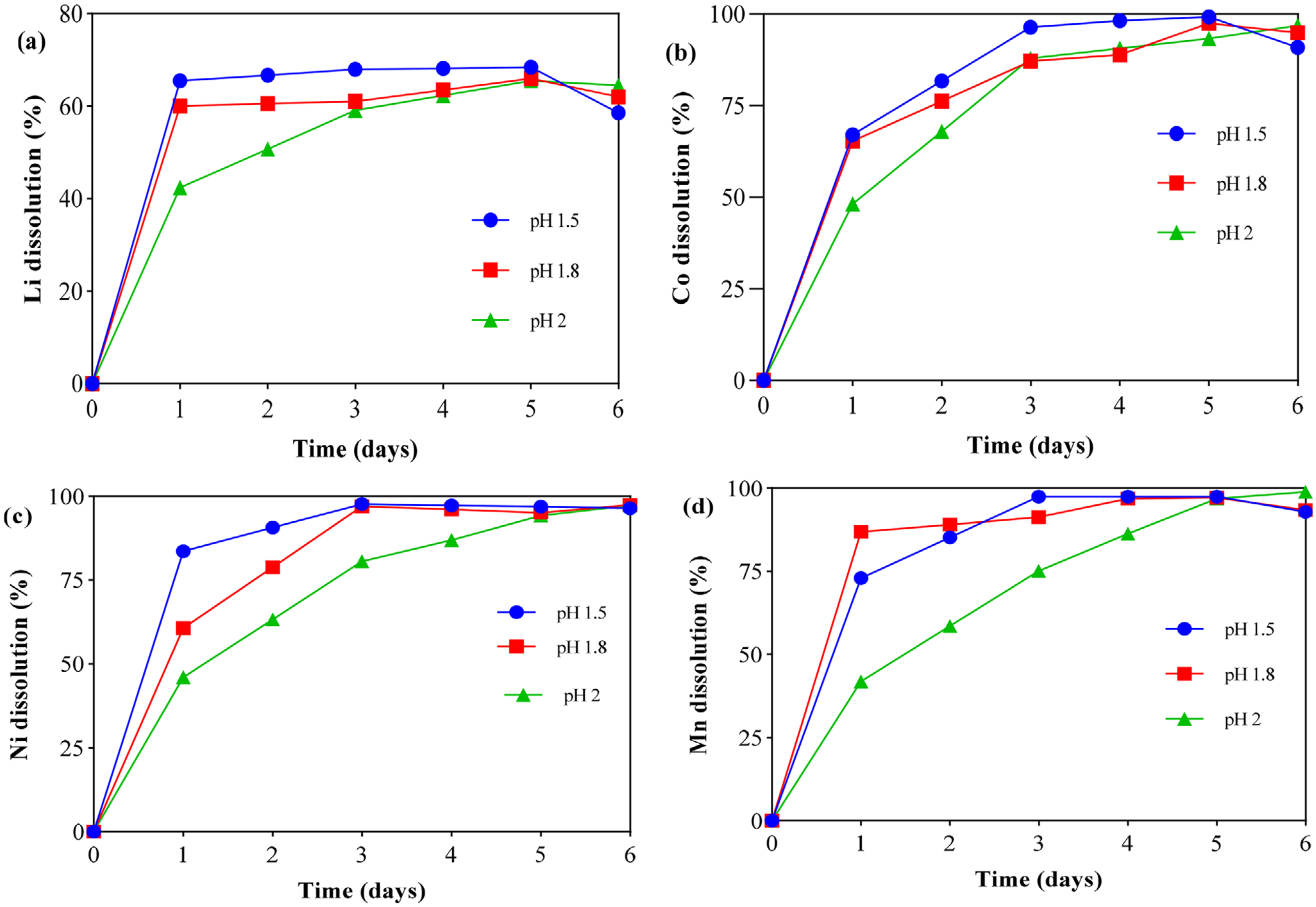


Figure 9. Variation of pH for (a) lithium (Li), (b) cobalt (Co), (c) nickel (Ni) and (d) manganese (Mn). Particle size (500-μm), time (6 days), pulp density (0.5%), Fe^{2+} (9 g L^{-1}), sulfur (1 g L^{-1}), 150 rpm and 30 °C.

(3 g L^{-1}) showed superior results for all the metals leaching. The maximum leaching efficiencies were 82%, 98%, 98% and 99% for Li, Co, Ni and Mn, respectively.

A similar study carried was out by Niu *et al.*[43] on the beneficial effects of appropriate amount of nutrients on metal bioleaching from LiBs, where three predetermined concentrations of mixed energy substrates – sulfur and pyrite – were studied. The results of the study showed that the bioleaching of Li and Co was higher when a concentration of 4 g L^{-1} of energy substrate was used compared to 2 and 8 g L^{-1}, indicating that 4 g L^{-1} was the appropriate concentration of energy substrate to achieve maximum bioleaching efficiency. In another study by Liu *et al.*[42] tested $LiCoO_2$ sample using pyrite as Fe^{2+} donor by using acidophilic mixed culture (*Leptospirillum ferrooxidans* and *Sulfobacillus thermosulfdooxidans*). The process consisted of growing the microorganisms in 10% FeS_2 to generate Fe^{2+} via oxidation process in a first step. By adding 5% of the $LiCoO_2$ after 2 days bacteria growth, the leaching efficiencies reached 97.1% of Li and 96.1% of Co.

Bioreactor leaching experiments

Various acidophilic iron and sulfur oxidizing microorganisms have been reported to have bioleaching abilities. The Fe^{3+} acts as a chemical oxidant and is converted to Fe^{2+} during the bioleaching of sulfide minerals. The continuous supply of Fe^{3+} by microbial reoxidation of Fe^{2+} ensures the continuity of the bioleaching process, which is otherwise a chemically slow process. İn the presence of microbes, the rate of the process is increased 105–106-fold. In addition, the acidity of the solution is maintained by the oxidation of sulfur compounds. In this regard, it can be considered that the redox reactions carried out by the acidophilic microorganisms are the key factors in the bioleaching process.

The treatment of spent batteries and battery wastes requires a higher acidity in the bioleaching solution and if Co and Mn are present in the battery waste, it is necessary to first reduce these metals to soluble redox states as follows:

$$Co^{3+} + e^{-} \rightarrow Co^{2+} \tag{6}$$

$$Mn^{4+} + 2^{e-} \rightarrow Mn^{2+} \tag{7}$$

However, the oxidation of Ni is a direct process, where Ni is oxidized to Ni^{2+} through proton attack.[3] Several studies have been conducted with respect to the bioleaching of Li, Co, Ni and Mn from spent LIBs.[32,37] Some studies have suggested the role of acid in the bioleaching of Li from spent batteries,[32] while others have indicated the importance of both acid and Fe^{3+}/Fe^{2+} redox cycle in the bioleaching of metals such as Co from spent batteries.[43] One of the study carried out by Wu *et al.*[44] describes about the role of EPS in the bioleaching of spent batteries, where it was suggested that the Fe^{3+}/Fe^{2+} redox cycle produced in the EPS was responsible for the leaching of Li, Co, Ni and Mn. In the current study, the bioreactor leaching experiments were performed with untreated and thermally treated LIB samples using the optimal

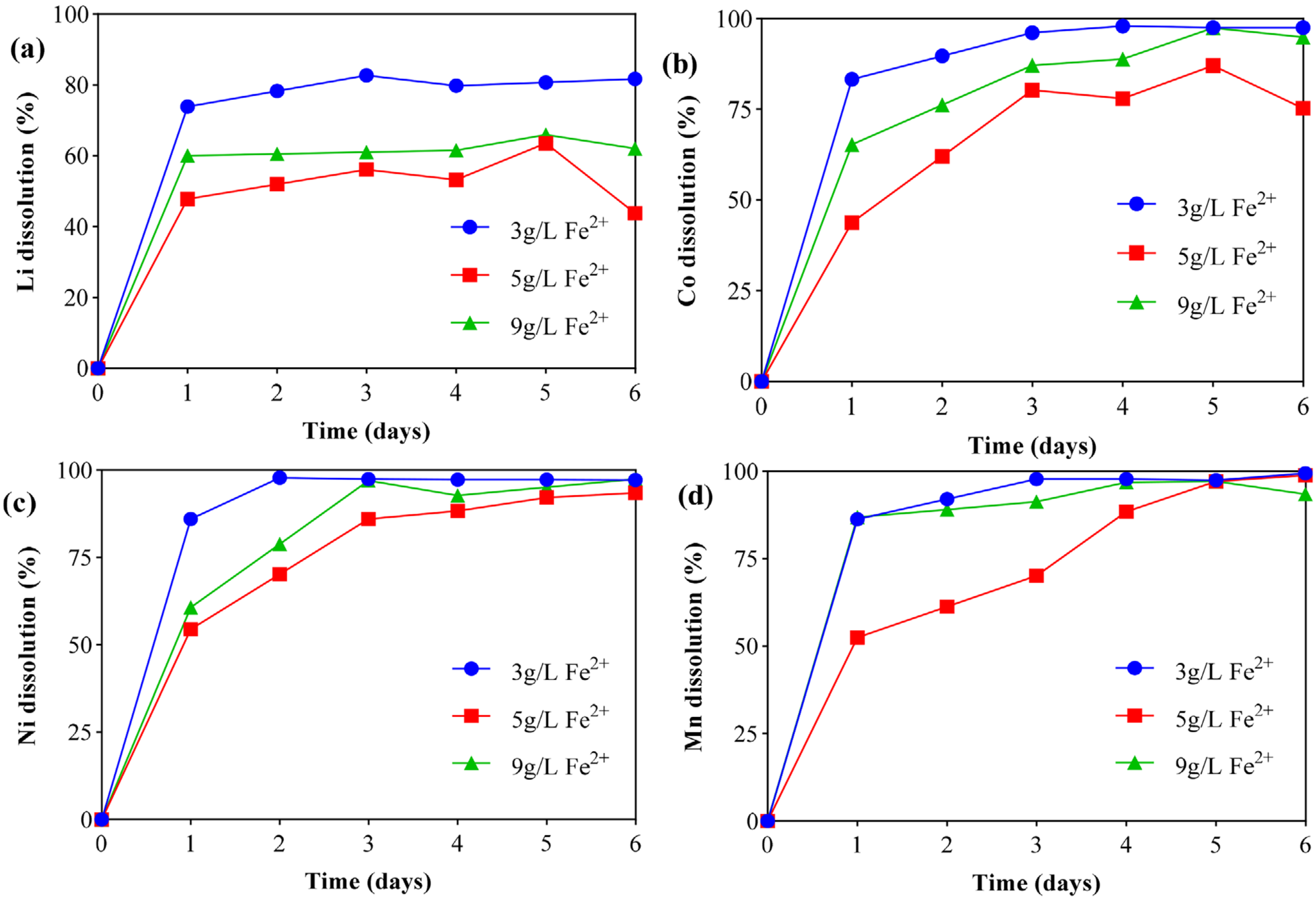


Figure 10. Variation of Fe^{2+}, for (a) lithium (Li), (b) cobalt (Co), (c) nickel (Ni) and (d) manganese (Mn). Particle size (500-μm), time (6 days), pulp density (0.5%), pH 1.8, sulfur (1 g L^{-1}), 150 rpm and 30 °C.

parameters obtained in shake-flask experiments. All the operating conditions were kept the same with the laboratory-scale experiments except the leaching time, which was kept at 6 days for 1-L bioreactor tests and 4 days for 10-L bioreactor tests. The bioleaching time was fixed at 4 days for the 10-L reactor because metal dissolution in the 1-L bioleaching experiments reached its maximum value at this process duration. As mentioned in pH, Fe2+ and solution redox potential section, a slight difference in bioleaching efficiency was observed in the shake-flask experiments when the pH was reduced from 2 to 1.5. Therefore, the pH was maintained at 2 in the bioreactor experiment as to avoid an increased acid consumption during large-scale operations. The results of the bench-scale batch 1-L bioreactor test for nontreated and treated samples are shown in Fig. 11(a), (b), respectively. Higher dissolution of Ni and Li was observed with the untreated sample, whereas the Co and Mn dissolution was slightly enhanced in the treated sample compared to the untreated sample. The maximum dissolution of Li (67%), Co (81%), Ni (99%) and Mn (86%) was achieved with the nontreated sample. In the case of thermally treated samples, 49% Li, 86% Co, 87% Ni and 85% Mn dissolution was achieved.

The results from the 10-L bioreactor test are presented in Fig. 11(c), (d). The maximum metal dissolutions obtained with the nontreated LIBs sample were 80%, 75%, 91% and 63% for Li, Co, Ni and Mn, respectively. In the case of thermally treated LiB samples, the metal dissolutions were 67%, 75%, 88% and 75% for Li, Co, Ni and Mn, respectively. According to a recent study by Moosakazemi *et al.*,[3] it has been concluded that based on the Gibbs free energies of alternative reactions, Fe^{2+} can act as a reductant for Co^{3+} and reduce it to Co^{2+}. Consequently, the Fe redox cycle was involved in both the microbial oxidation of Fe^{2+} and the reduction of Co^{3+} as follows:

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

$$Co^{3+} + e^{-} \rightarrow Co^{2+}$$

In a bioleaching system, there are several factors which might be responsible for low metal dissolution such as the toxicity of metals or organics that influence the oxidation activity, the pulp density in the solution that can affect the oxygen transfer rate in the microbe, the collision of particles with the cells causing shear in the cell membranes.[3] As mentioned in the Mechanism of microbial action section, both direct and indirect mechanisms are involved in the extraction and dissolution of various metals from spent LIBs. Apart from that, protein-related molecules in the EPS also are responsible for higher acid production, which results in higher metal extraction from spent LIBs. The thermal treatment of the sample might have been responsible for some structural and compositional changes in the sample, which might have affected the microbial cell/cell attachment and acid production. In addition, changes in the physical/chemical properties of the sample after thermal treatment might influence the Fe^{2+}/Fe^{3+}

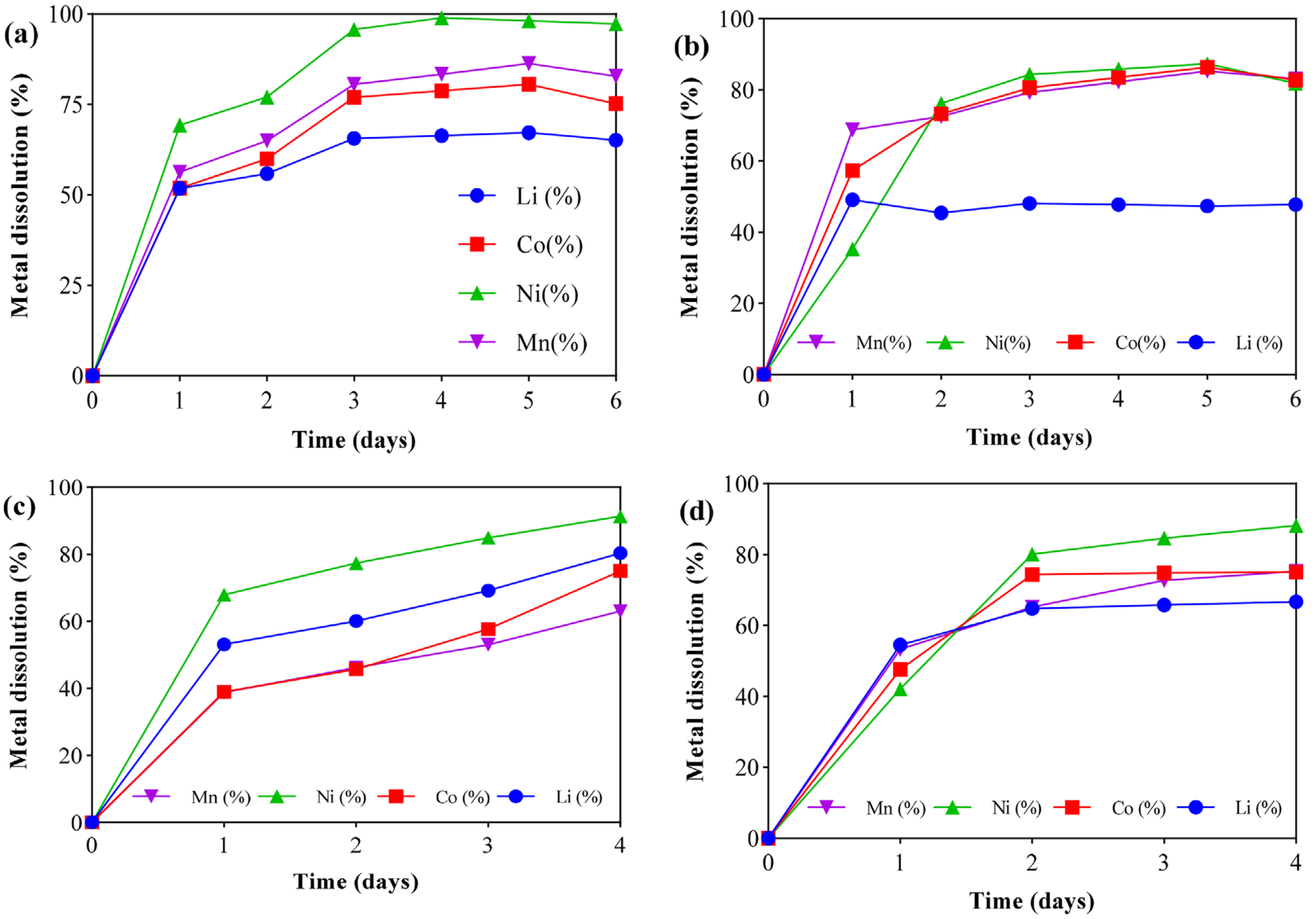


Figure 11. Bench scale and semi-pilot bioreactor leaching of metals from (a) untreated 1-L and (b) treated 1-L; and (c) untreated 10-L and (d) treated 10-L.

redox cycle, subsequently leading to lower metal extraction. As mentioned in the Mechanism of microbial action section, Li bioleaching is primarily based on the noncontact mechanism and is linked with acid action. As per reports, the oxidation of Ni also is a direct process, which proceeds via proton attack (mentioned in above paragraph). However, the Co and Mn dissolution depends both on contact and noncontact bioleaching mechanisms. This might be the reason behind the marginal difference that was observed in the treated and untreated samples in case of these two metals.

Mechanism of microbial action

The extracellular polymeric substances secreted by the acidophilic microorganisms in response to environmental factors is responsible for various cell interface activities such as cell aggregation, cell surface attachment, biofilm formation, cell-to-cell recognition, cell sorption of external organic and inorganic ions. The EPS primarily comprise proteins, lipids and polysaccharides. A recent study carried out by Wu *et al.*[44] has indicated the role of extracellular polymeric substances in the enhancement of bioleaching efficiency of Li and Co from LiBs. In the study, protein, polysaccharide and uronic acid were selected for analysis to determine which component was mainly responsible for the improvement in bioleaching efficiency. It was observed that addition of protein extracted from the EPS led to an enhancement in the dissolution of Li^+ and Co^{2+}, whereas the addition of polysaccharide had no profound effect. Furthermore, a similar comparison made by the chemical method using cysteine and glucose as representative monomers of proteins and polysaccharide, respectively, led to the conclusion that cysteine enhanced the leaching of Co^{2+} and Li^+. It was suggested that the sulfhydryl groups present in cysteine might have been responsible for the enhanced leaching efficiency. A different study carried out by Xin *et al.*[45] indicates the importance of acid dissolution and Fe^{2+}-catalyzed reaction in Li^+ and Co^{2+} leaching. In order to carry out their metabolic activities, the acidophilic microorganisms derive energy via the oxidation of inorganic elements or compounds. According to this study, although both contact and noncontact mechanisms are involved in the bioleaching of metal sulfides, the bioleaching of spent LiBs entirely proceeds via the noncontact mechanism. Consequently, the findings of the study indicate the evident differences that exist between the bioleaching of metal sulfides and spent LiBs. However, a recent study carried out by Moazzam *et al.*[46] highlights the importance of both direct and indirect mechanisms in the dissolution of various metals from spent LiBs. This study also indicates the significance of proteomics linked research, which imply the involvement of protein-related molecules for higher acid production and subsequent enhancement in bioleaching. The hydrophobic and electrostatic interactions mediated by the extracellular polymeric substances are primarily involved in cell attachment to the solid surfaces or e-wastes. The electrostatic interactions result from the positive charge that is formed on the cell surface due to the chelated Fe^{3+} in the extracellular polymeric substances. The role of exopolymeric substances is vital in case of direct or contact bioleaching. However, the indirect/noncontact mechanism does not require the direct microbial attachment to solid surfaces, as they are mainly responsible for the production of leaching agents such as sulfuric acid for dissolution of metals. Apart from that, another agent that influences the indirect bioleaching process is the complexing agent, which is responsible for chelation of metal ions and formation of stable complexes.[47] Although the indirect bioleaching mechanism does not require the attachment of the microbes to the solid surface, the efficiency of the process can be improved by locally increasing the concentration of the leaching agents such as sulfuric acid. Indirect bioleaching is more suitable from industrial point of view, because it offers a greater flexibility in terms of optimising process parameters compared to the direct method. In addition, the process parameters such as pH, temperature and dissolved oxygen concentration, along with the toxic metals concentration, also affect the microbial growth and metabolism in the indirect approach.[48] The bioleaching mechanisms of valuable metals from low grade ores and spent LiBs are quite different. The bioleaching mechanism of Li and other metals present in LiBs (Ni, Co and Mn) also is different. As per the study carried out by Xin *et al.*,[32] the EPS play an important role in the leaching of Ni, Co and Mn present in LiBs, however, the leaching of Li proceeds via the production of biogenic sulfuric acid and is not dependent on the production of extracellular polymeric substances. The leaching of insoluble forms of Ni, Co and Mn occurred through the redox reactions carried out by ferrous iron oxidation. Apart from that, the effective mobilization of these metals also occurred through acid dissolution. This study provides the evidence for the role of both direct and indirect bioleaching in the extraction of various metals from spent LiBs.

CONCLUSION

In this study, initial investigations on nontreated samples in laboratory-scale experiments helped to determine the optimum parameters. These optimum parameters were used in bench-scale leaching and semi-pilot scale leaching for both nontreated and treated samples. The results of the study showed that bioleaching using the acidophilic mixed culture resulted in high metal dissolution for the nontreated sample than for the thermally treated sample. The highest leaching results for Li and Ni were achieved with the untreated samples, at 80% and 91%, respectively. The leaching of Co and Mn was only marginally improved in the treated sample, which was 75% for both metals. The lower leaching efficiency achieved in the treated samples might have been a consequence of the higher concentration of metals in the treated sample, structural/compositional changes in the sample following thermal treatment. Further studies to improve the metal extraction efficiency through identification of novel and highly metal-tolerant microbial strains, cheaper nutrient source, design of improved bioreactors and genetic modification of microbes (genetically engineered microbes) to produce highly efficient strains could open new horizons in the area of spent LiBs leaching.

CONFLICT OF INTEREST STATEMENT

There are no conflicts to declare.

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ETHICAL APPROVAL

Not Applicable.

CONSENT TO PUBLISH AND PARTICIPATE

All authors approve this submission.

AUTHOR CONTRIBUTIONS

- **Sandeep Panda:** Conceptualization, Methodology, Validation, Investigation, Supervision Writing-Original Draft
- **Seydou Dembele:** Validation, Investigation, Writing-Original Draft
- **Srabani Mishra:** Methodology, Validation, Investigation, Writing-Original Draft, Visualization
- **Ata Akcil:** Funding Acquisition, Supervision, Resources, Project Administration
- **İsmail Agcasulu:** Validation, Investigation
- **Edris Hazrati:** Validation, Investigation
- **Aysenur Tuncuk:** Project Administration
- **Pierre Malavasi:** Validation, Investigation
- **Stoyan Gaydardzhiev:** Funding Acquisition, Supervision, Resources, Writing-Original Draft, Project Administration

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