

Enrichment and adaptation of bioleaching consortia for recovery of critical metals from spent Lithium-ion batteries

Lalropuia Lalropuia¹, Jiri Kucera³, Klemens Kremser² and Georg M. Guebitz²

¹ K1-MET GmbH, Stahlstraße 14, 4020 Linz, Austria

² University of Natural Resources and Life Sciences Vienna BOKU, Dept. of Agrobiotechnology, IFA-Tulln, Institute of Environmental Biotechnology, Konrad-Lorenz-Straße 20, 3430 Tulln an der Donau, Austria

³ Department of Biochemistry, Faculty of Science, Masaryk University, Kotlarska 2, 61137 Brno, Czech Republic

*Correspondence: lalropuia.lalropuia@k1-met.com

Keywords: Bioleaching, consortia, lithium-ion batteries, black mass, enrichment, adaptation, recovery

ABSTRACT

Recovery of critical metals like Co, Ni, Mn, Cu, and Li has become a high priority due to their limited availability and environmental concerns. Lithium-ion batteries (LIB) contain substantial amounts of these metals, but the current recycling rates are low and have many challenges. Bioleaching can provide a promising alternative for metal recovery from wastes such as LIB, printed circuit board (PCB), and slags due to its economic advantage and environment friendliness. Nevertheless, high concentrations of toxic heavy metals inhibit the growth and metabolism of bioleaching microorganisms. In this study, three environmental samples from a highly acidic lake with elevated metal concentrations were used in enrichment and adaptation experiments to overcome this issue. Using a universal medium, cultures were enriched with iron, sulfur, or a mixture of iron/sulfur as the sole energy source. One promising culture was selected, enriched, and used for adaptation experiments with elevated concentrations of Li⁺, Co²⁺, Ni²⁺, Mn²⁺, and Cu²⁺. Cultures after enrichment and adaptation were identified using 16S rRNA metagenomic analysis indicating that the dominant genera included *Acidithiobacillus*, *Alicyclobacillus*, and *Sulfobacillus*. Finally, up to 100% recovery of Li, Co, Ni, and Mn was achieved by direct bioleaching using the adapted enriched culture.

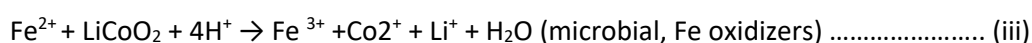
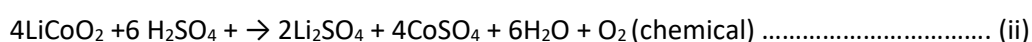
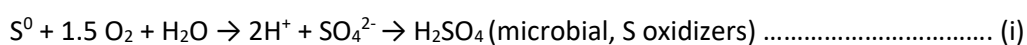
1. INTRODUCTION

Lithium-ion batteries (LIB) dominate the battery market with their use ranging from portable electronic devices to e-bikes and electric vehicles (EV) due to their high energy density and longevity. LIB's components include the anode (e.g., graphite), a lithium metal oxide (e.g., LiCoO₂) cathode, and a liquid electrolyte. The most common types of LIBs include Lithium Cobalt Oxide (LCO), Lithium Nickel Manganese Cobalt Oxide (LMC), and Lithium Iron Phosphate (LFP) (Boyden et al., 2016). The recent boom in LIB production is greatly influenced by the transition from exhaustible engine vehicles to EVs. This is the result of different countries especially developed countries like the USA, Japan, and the EU taking new initiatives to reduce carbon emissions and move towards green energy (Kim et al., 2012). It is predicted that 50% of the total vehicle production worldwide will be EVs by 2050 (Sonoc and Jeswiet, 2014). This increase in usage and production of LIB will result in the production of huge amounts of waste. It is estimated that around 200,000 tons of wastes from the LIB cathode alone was

produced as of 2020 (Ali et al., 2021). Most of these LIB will end up in landfills and toxic metals like Co, Ni along with other components pose a serious environmental threat as they could contaminate the soil and groundwater and could be harmful to human health (Bankole et al., 2013).

Spent LIB contains a substantial amount of critical metals like Co, Ni, Mn, and Li, they can be used as an important secondary source for such metals. There is a huge potential for the recycling of LIB whose current value is estimated to be \$860 per ton for LiMnO₄-based batteries while a staggering \$8900 per ton for LiCoO₂-based cathodes (Wang et al., 2014). As the resources required for LIB are limited and the production are concentrated only in a few countries like China, there arises a risk of supply disruptions of the critical metals essential for LIB production especially Li, Co, Ni, and Mn (Sun et al., 2019). Recycling of these LIB could help to mitigate the environmental impacts, minimize waste production, and to lower the mining of virgin mineral resources required for LIB production (Bankole et al., 2013). The current recycling process is mainly done through pyrometallurgical, mechanical, and hydrometallurgical methods and these processes have many disadvantages like the production of hazardous wastes (Boyden et al., 2016). To recover metals from the cathode material, nowadays inorganic acids like hydrochloric acid (HCl), sulfuric acid (H₂SO₄), and nitric acid (HNO₃) are mainly used to leach the metals from the LIB waste which offers high metal recovery efficiency but has a negative environmental impact and high energy consumption (Mossali et al., 2020). In recent years, research on the application of bioprocesses in the field of metal recycling has become an emerging topic. Organic acids such as citric acid, malic acid, and aspartic acids proved to be used as suitable leaching agents. For example, almost 100% of Li and Co were recovered from LIB in the presence of H₂O₂ using such organic acids (Li et al., 2013).

Bioleaching, a process by which metals are solubilized using microorganisms, could be a viable option for the recovery of critical metals from these LIB. Bioleaching provides several advantages over the conventional method of recycling. Bioleaching is more environment friendly as it produces lesser pollutants, is more cost-efficient, and has a lower energy consumption (Krebs et al., 1997). Bioleaching microorganisms are mainly acidophilic and thermophilic which means that they can thrive, tolerate, and grow at low pH and at high temperatures respectively. Most prominent examples are bacteria such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, or archaea such as *Sulfobacillus thermosulfidooxidans*. Bioleaching is mainly used for extracting metals from low-grade sulphidic ores in which the Fe/S oxidizing microbes help in solubilizing metal sulfides by producing Fe³⁺ and /or H₂SO₄ as shown in eqn (i) and (ii) and the H₂SO₄ produced helps in lowering the pH of the surrounding (Sajjad et al., 2019). It is also used for recycling of e-waste such as printed circuit boards (PCBs) and other waste streams such as ashes and slags. 96% Cu, 73% Ni, and 93% Co were able to be recovered from (PCBs) using *Leptospirillum ferriphilum* and *Sulfobacillus benefaciens* consortia in a bioreactor (Hubau et al., 2020). In a batch test bioleaching of ashes and slags from incineration residues, 100% leaching efficiency of Zn, Cu, and Mn was able to be achieved using Fe and S oxidizing bacteria (Kremser et al., 2021). As such, waste from LIB could be recycled using bioleaching resulting in increased research activities in this area in recent years. Jegan Roy et al. (2021) reported that they were able to leach 90% Ni, 82% Co and 92% Mn from spent NMC-based LIB using *A. ferrooxidans*. Another study demonstrated that up to 96% of Co and Ni could be recovered using a mixed culture of *Acidithiobacillus thiooxidans* and *Leptospirillum ferriphilum* from LIB collected from EVs (Y. Xin et al., 2016). The mechanisms involved in bioleaching of LIB using Fe and S oxidizers are given below: (Roy, Cao, et al., 2021)



Even though most acidophiles have a high tolerance to metals such as Co and Ni, LIB waste contains high concentrations of metals combined with an acid-consuming character which could be inhibitive for the growth of the acidophiles (Roy, Cao, et al., 2021). To improve their resistance and performance, these microbes are often adapted to high metal concentrations before inoculating them with substrates such as LIB, especially for the direct bioleaching approach. It involves direct cultivation of the microbes with the LIB waste different from the indirect leaching where the bio-lixiviant is produced and applied separately (Bosecker, 1997). In many of the studies, the adaptation process improves the growth of the cultures in the bioleaching consortia and as a result, improves the leaching efficiency of metals from the LIB (Bahaloo-Horeh et al., 2018; Ijadi Bajestani et al., 2014; Ilyas et al., 2007).

Acidophiles and thermophiles are usually found in harsh environments such as acid mine drainage or in hot springs where there is low pH (<3) or high temperature (>30°C) and with high metal concentration (Dopson et al., 2004; Salo-Zieman et al., 2006). Mixed cultures enriched from such environments often have higher bioleaching efficiency as compared to pure cultures (Retnaningrum et al., 2021; Xiang et al., 2010)

This study aims to recover critical metals like Li, Co, Ni, and Mn using a direct bioleaching process from the BM of spent LIB with a microbial consortium that was enriched from samples taken from an acidic lake. The enriched cultures were first pre-adapted to high metal concentrations to improve their tolerance to such metals and the leaching efficiency of both the adapted and non-adapted cultures were compared.

2. MATERIALS AND METHODS

2.1 Active material

Four types of black mass (BM) from spent LIB were received from a partner company after each has undergone a pre-treatment process of dismantling, crushing, and sorting. Inductively coupled plasma mass spectrometry (ICP-MS) analysis was done to obtain the elemental compositions for each type of BM, as previously described by Kremser et al. (2021) for characterization of the ashes and sludges.

Table 1: Concentration of different metals present in the BM (NMC, LFP, PSP and HL) after ICP-MS analysis.

		Concentration of metals (%)					
		Li	Co	Ni	Mn	Cu	Al
Type of BM	NMC	2.76	14.5	5.86	4.17	9.19	5.26
	LFP	2.46	0.08	0.35	0.12	5.84	1.69
	PSP	3.38	8.21	10.6	8.42	4.49	3.44
	HL	4.37	29.2	5.84	4.06	6.57	5.09

2.2 Media and Microorganisms

Three soil samples (sample 1, sample 2, and sample 3) were collected for enrichment from Hromnice Lake, Czech Republic which is an acidic lake formed on a previously pyrite mine (Hrdinka et al., 2013). For all experiments, a universal medium (UM) as described by Nancucheo et al was used for cultivation consisting of 7.5 g/l of Na₂SO₄·10H₂O, 22.5 g/l of (NH₄)₂SO₄, 2.5 g/l of KCl, 25 g/l of MgSO₄·7H₂O, 2.5 g/l of KH₂PO₄, 0.7 g/l of Ca(NO₃)₂·4H₂O along with trace element containing ZnSO₄·7H₂O, CuSO₄·5H₂O, MnSO₄·4H₂O, CoSO₄·7H₂O, H₃BO₃, Na₂MoO₄·2H₂O, NiSO₄·6H₂O, Na₂SeO₄·10H₂O, Na₂WO₄·2H₂O and NaVO₃ (Nancucheo et al., 2016). Three types of UM were prepared i.e., one for the cultivation of Fe oxidizers at pH 1.7 with 50 mM of FeSO₄ added (Fe media), one for S oxidizers at pH 3.5 with 1% w/v

of elemental sulfur (S media) and another one for mixed culture of Fe/S oxidizers at pH2 with both 50 mM of FeSO₄ and 1% w/v of S added (FS media). The UM was sterilized using 0.2µm pore sized filter (Thermo Fisher, Nalgene rapid flow) All experiments were carried out in triplicates and incubation at 30°C and 150 rpm.

2.3 Enrichment process

5g of each soil sample 1,2 and 3 collected from the acidic lake were enriched in a 250ml Erlenmeyer flask with 100 ml total volume using the Fe medium (1F,2F and 3F), S medium (1S,2S and 3S), and FS medium (1FS,2FS and 3FS). The experiment was carried out at 30°C, 150 rpm for 14 days for Fe medium and 21 days for S medium and FS medium. After the incubation, 10ml from each flask was taken and re-cultivated to be used for further experiments. The remaining cultures were first centrifuged at 1500 rcf for 1 min in a 50 ml falcon tube to remove soil and other solid residues. Afterwards, the supernatants were centrifuged in a fresh 50 ml falcon tube at 3700 rcf for 15 minutes to obtain cell pellets. The cell pellets were stored at -80°C until they were sent for 16S rRNA metagenomic analysis.

During the experiment, 1 ml samples from each flask was taken every day for the first week and on alternate days from the second week. To measure cell growth, samples to be measured were first centrifuged at 1500 rcf for 1 min, followed by measurement of the optical density (OD) at 660 nm. The samples were afterwards filtered through 0.45 µm pore sized syringe filter into a fresh tube followed by pH value, oxidation reduction potential (ORP) and Fe²⁺/Fe concentration were measured before storing them at -4°C.

2.4 Adaptation to elevated metal concentrations

After enrichment of the soil samples, sample 2FS was selected for the adaptation experiment. 2FS culture was adapted to high concentrations of Li⁺, Co²⁺, Ni²⁺, Mn²⁺ and Cu²⁺ metals. 1M synthetic solution of each metal was prepared using LiCl, CoCl₂, NiCl₂, MnSO₄ and CuSO₄.5H₂O respectively with ultra-pure water. The adaptation was done in 3 stages with increasing metal concentration after every stage. The adaptation experiment was performed in 100 ml volume using a 250 ml Erlenmeyer flask for 14 days for each stage at 30°C, 150 rpm. For the first adaptation (1A), second adaptation (2A) and third adaptation(3A), the inoculation was performed with metal concentration corresponding to 2.5 g/l, 5 g/l and 10 g/l of BM, respectively. Each time 10 ml of the inoculum from the previous stage were used for the next adaptation stage.

ORP, pH, OD and Fe²⁺/Fe were measured as discussed in section 2.3. Samples were collected after every stage for 16S metagenomic analysis. After the third stage of the adaptation experiment, the adapted consortia were used for the direct bioleaching of the BM.

2.5 Direct bioleaching

For the direct bioleaching experiment, the adapted and non-adapted 2FS consortia were pre-cultivated each in a sterilized 100 ml baffled Erlenmeyer flask in a volume of 50 ml for 7 days. Following the pre-cultivation, 1% of each BM was added to the flasks and the bioleaching experiment was carried out for 7 more days. Samples were taken on day 2,5 and 7 and pH, ORP, OD and Fe²⁺ were measured similar to the previous experiments. At the end of the experiment, the pregnant leach solution (PLS) from each flask was filtered using 50 ml syringe, 0.45 µm pore sized nylon filter and the clear PLS samples were sent for ICP-MS analysis. For calculation of the metal recovery % of the metals for the adapted cultures, the concentration of each metal from the synthetic solutions added to the medium during the 3 stages of the adaptation experiment were subtracted from the ICP-MS result to get the actual recovery.

2.6 Analytics and apparatus

Incubation was done using an Infors HT Multitron Pro shaker (Bartelt, Austria). For pH and ORP measurement, a Mettler Toledo S220 pH/ion meter with glass electrode was used. For OD measurement, a DR3900 spectrophotometer (Hach Lange, Austria) was used. All sterilization was done using a Systec VX-150 (Bartelt, Austria). Centrifugation was done using an Eppendorf 5427 R (Eppendorf, Germany).

For the measurement of Fe²⁺/Fe concentration, 228 µL of ferrozine was mixed with 12 µL of the sample in a 96 well plate. Measurement was done in a Tecan infinite M200 pro plate reader at 562 nm. After the addition of 45 µL of HONH₂-HCl and 15 µL of NH₄CH₃CO₂ to the plate and waiting time 20 mins, a second measurement was done again at 562 nm. The first measurement was for the Fe²⁺ concentration and the second measurement results in the total Fe concentration. A calibration curve was produced using 0 mM, 0.1 mM, 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, and 1 mM of FeSO₄ x7H₂O.

For the ICP-MS analysis, the samples were sent to Eurofins Umwelt Österreich GmbH and Co, Austria. The samples were diluted to 1:100 with MiliQ water and for the internal standard, a 400 µg/l solution of Sc was used. The 16S rRNA analysis was done as described in more detail in a previous study (Kremser et al., 2022).

The recovery % of each of the metals were calculated using the following formula:

$$\text{Recovery \%} = [(C_{\text{PLS}} - C_{\text{ME}}) / C_{\text{BM}}] * 100$$

where,

C_{PLS} → concentration of the metal in the PLS from ICP-MS result

C_{ME} → concentration of the metal in the added synthetic metal solution.

C_{BM} → concentration of the metal in the BM

3. RESULTS AND DISCUSSION

3.1 Enrichment of the Soil samples

Following the cultivation of the sample 1,2 and 3 in the Fe medium (1Fe,2Fe and 3Fe), S medium (1S,2S and 3S) and FS medium (1FS,2FS and 3FS) for 14/21 days, 16S rRNA metagenomic analysis was performed to identify the microbial consortia of the enriched cultures. The analysis showed that the dominant genera were *Acidithiobacillus* and *Alicyclobacillus* in both sample 1 and 2 while the genus *Sulfobacillus* were most representative in sample 3 (Fig 1). For sample 1, *Acidithiobacillus thiooxidans* and *Alicyclobacillus disulfidooxidans* species were identified in the S media, *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* in the FS media and a mixed culture of *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans* and *Acidiferrobacter thiooxydans* in the Fe media. *A.thiooxidans* uses elemental sulfur, thiosulfate or tetrathionate as a source of energy but cannot oxidize iron. *A. ferrooxidans* and *A. disulfidooxidans* (formerly *Sulfobacillus thermotolerans*) are acidophilic and mesophilic and can oxidise both iron and sulfur (Donovan P. Kelly and Ann P. Wood, 2000; Karavaiko et al., 2005). *L. ferrooxidans* is an iron oxidizing acidophilic and mesophilic bacterium (Hans Hippe, 2000) while *A. thiooxydans* is a thermo-tolerant facultatively anaerobic bacterium and can oxidizes both iron and sulfur (Hallberg et al., 2011). In sample 2, *Acidithiobacillus thiooxidans* and *Alicyclobacillus disulfidooxidans* species were identified in both the S media and the FS media while a mixture of *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans* and the extremely acidophilic Fe oxidizing heterotrophic bacteria *Ferrithrix thermotolerans* were found in the Fe media. The species identified in sample 3 were mainly of the genus *Sulfobacillus* where *S. thermotolerans* and *S. harzensis* were found in both the S media and FS media whereas *S. thermotolerans* and *A. ferrooxidans* were

found in the Fe media. *S. thermotolerans* and *S. harzensis* are thermophilic and acidophilic and can oxidizes iron and sulfur in the presence of small amount of yeast extract (Bogdanova et al., 2006; Zhang et al., 2021).

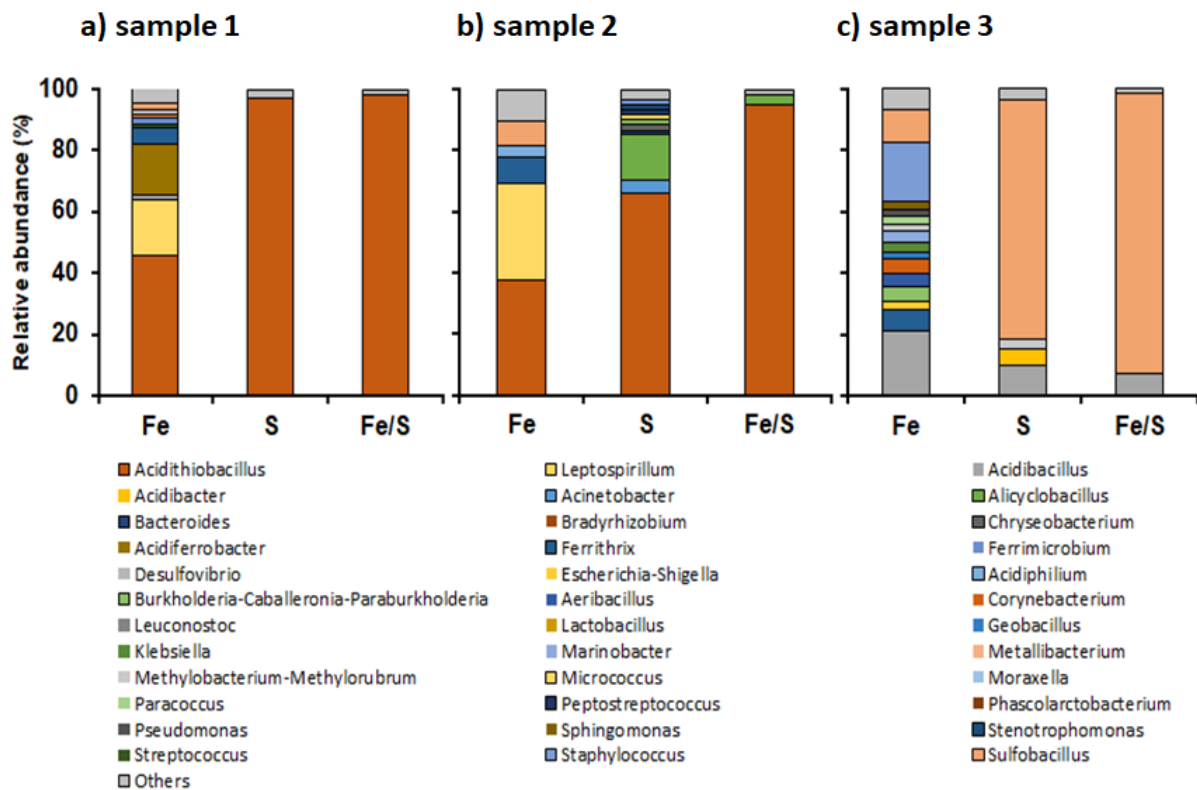


Figure 1: Result of 16S metagenomic analysis of microbial communities a) samples 1, b) sample 2 and c) sample 3 resulting from an enrichment experiment in Fe and S containing media.

For the enrichment in S media, the OD in 1S and 2S (Fig 2a) started to increase after day 7 and continued to increase until day 21, whereas the pH for 1S and 2S (Fig 2b) decreased to 0.5 and 0.47, respectively, at day 21. No increase in OD or decrease in pH was observed for 3S. (Fig 2a and b). The decrease in pH in 1S and 2S is the result of the S metabolizing bacteria *Acidithiobacillus thiooxidans* and *Alicyclobacillus disulfidooxidans* oxidizing the added elemental S in the media to H_2SO_4 as shown in eqn (i) (Dopson and Johnson, 2012). *A. thiooxidans* and *A. disulfidooxidans* have been reported to occur in a bioleaching industrial heap of copper sulphides where the abundance of *A. thiooxidans* was constant through the different leaching cycles (Remonsellez et al., 2009). The Fe^{2+} in the experiment 1FS and 2FS (Fig 2c) and 1Fe and 2Fe (Fig 2e) were almost completely oxidized by the Fe oxidizing bacteria after day 10 which was observed by the ferrozine method and at the same time the ORP reaches more than 600 mV due to the Fe redox reaction (Hansford and Vargas, 2001). This is most likely due the presence of Fe oxidizing bacteria like the *A. ferroxidans* and *L. ferrooxidans* in both sample 1 and 2. The lower activity i.e. lower OD, lower Fe oxidation rate and stable pH for sample 3 cultures in all of the 3 media as compared to sample 1 and 2 (Fig 2) could be attributed to *Sulfobacillus* being the dominant species in sample 3 which are thermophilic with optimum temperature of $<40\text{ }^\circ\text{C}$ and usually grown with yeast extract. The cultivation medium has no added yeast extract, and the experiments are carried out at $30\text{ }^\circ\text{C}$ and hence the experimental condition and set up may not be optimal for these cultures to grow and oxidize Fe or S substantially.

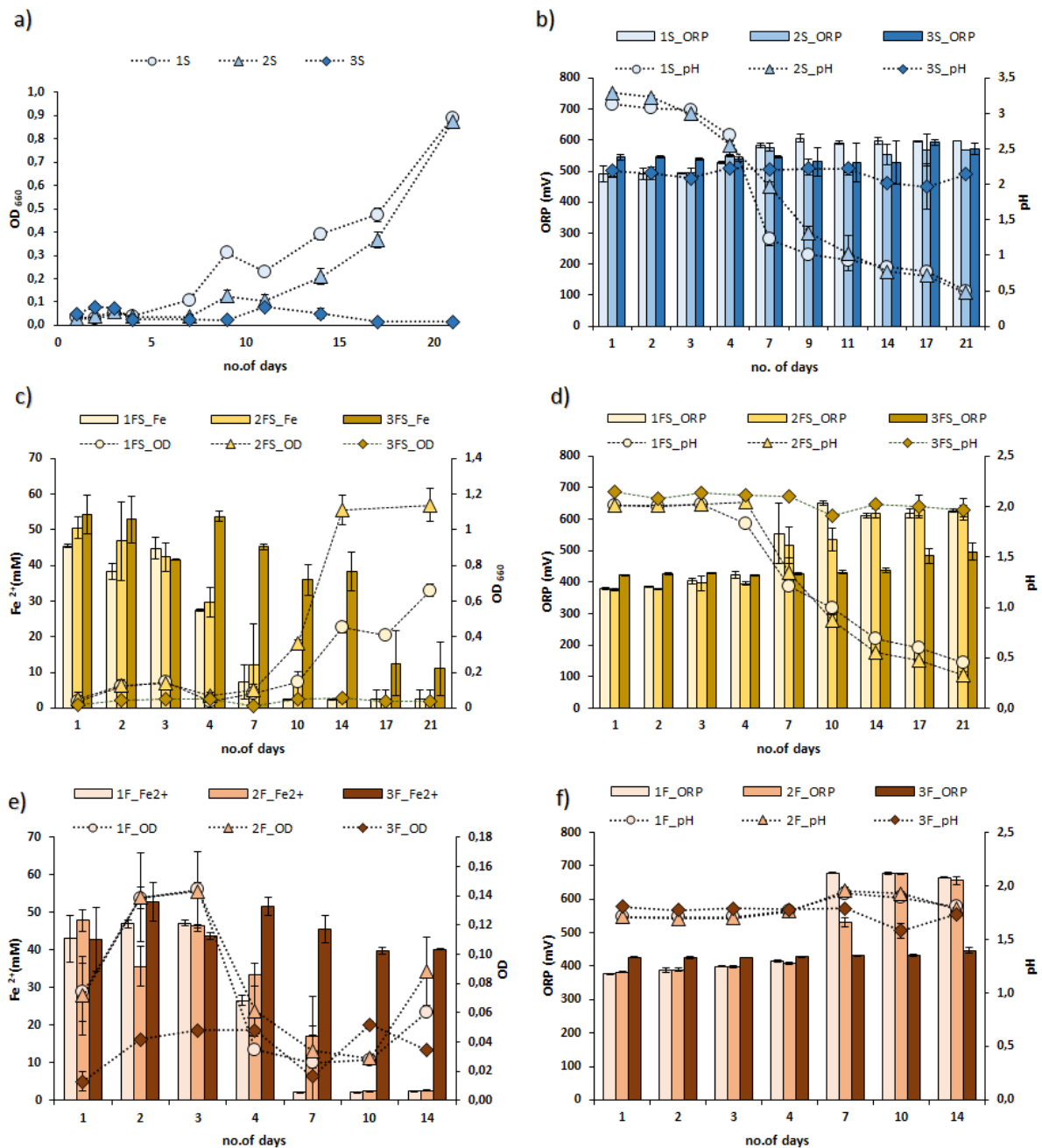


Figure 2: [a,c & e] Measurement of OD and Fe(II) using ferrozine solution [b,d & f] pH and ORP measurement, during the enrichment experiment of microbial communities (samples 1,2 and 3) in S media (a & b), FS media (c & d) and Fe media (e & f).

3.2 Adaptation to metal solution

Direct bioleaching of LIB has many challenges such as the inhibition of the microbial community due to the toxicity and acid consuming nature of the LIB which increases the pH (Roy, Madhavi, et al., 2021). Therefore, the culture enriched in the FS media is expected to be more promising for the direct bioleaching of the LIB. Furthermore, mixed cultures of Fe/S oxidizers proved to be more efficient in most bioleaching experiments as compared to pure Fe or pure S oxidizing culture (Akcil et al., 2007; Qiu et al., 2005). The H₂SO₄ production through oxidation of S could also stabilize or even lower the pH of the media when the cultures are mixed with the BM. Sample 2FS was chosen as the direct bioleaching culture since it showed the highest OD, lowest pH, and highest Fe²⁺ oxidation rate compared to the other two samples (Fig 2c and 2d). To improve the tolerance of the 2FS cultures to

the toxicity of the metals in the LIB, a series of 3 adaptation experiments (1A,2A and 3A) were performed using synthetic solutions of Li, Co, Ni, Mn, and Cu for 14 days for each stage with increase in concentration after each stage. There have been numerous studies of pre-adaptation of the bioleaching microorganisms to high metal concentrations or to the LIB waste. In the study made by Heydarian et al. (2018), a mixed culture was adapted to LIB in different cycles, and it was reported that the cultures stop growing above 40 g/l of pulp density and the pH was required to be controlled due to the alkaline nature of the LIB. From Fig 3a, the cell growth of 2FS is not inhibited by increasing metal concentrations in fact, the OD of the 1A,2A and 3A are higher than the non-adapted cultures. The cultures were also able to oxidize the Fe^{2+} to Fe^{3+} almost completely even during the third adaptation (3A) and the decrease in pH further indicate that S oxidation was taking place at the same time (Fig 3a and 3b).

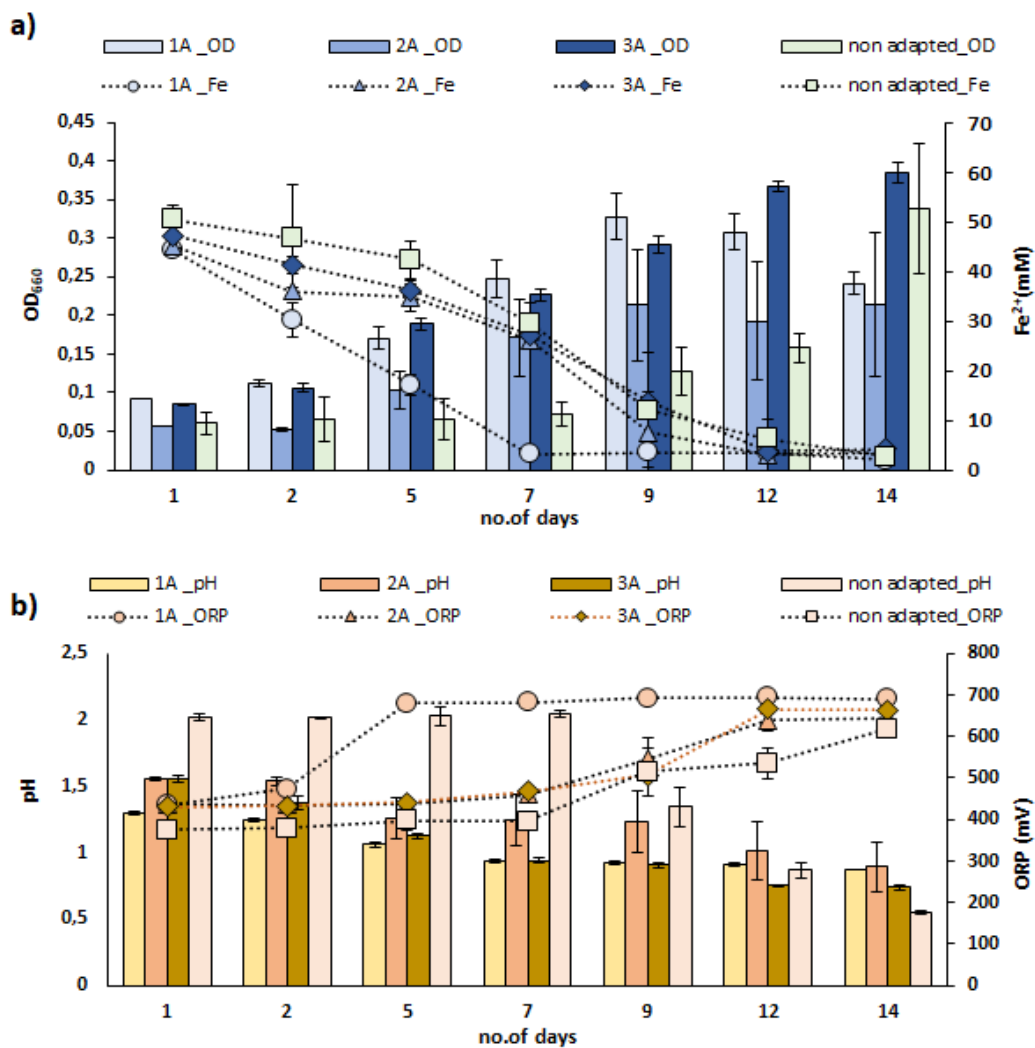


Figure 3: a) OD and Fe(II) measurement b) pH and ORP measurement during the first (1A), second (2A) and third (3A) adaptation experiments of the enriched sample 2 in FS media using different concentrations of Li, Co, Ni, Mn and Cu.

3.3 Direct bioleaching of the LIB

It has been reported that the direct bioleaching of LIB at high pulp density results in lower recovery of metals (Niu et al., 2014). Therefore, for the direct leaching experiment using the adapted and non-adapted 2FS, 1 % pulp density of LIB (NMC and HL type) was used. The adapted and non-adapted 2FS consortia were pre-cultivated for 7 days with just the medium at 30°C, 150 rpm and different parameters like pH, ORP, OD and Fe²⁺/Fe were measured at the end of the pre-cultivation. Afterwards, the two BM types NMC and HL were added to each of the cultures i.e., adapted, and non-adapted

separately and the bioleaching was continued for 7 more days. From Fig 4a, the adapted cultures have higher OD in both the BM type (NMC and HL). The Fe²⁺ were almost completely oxidized (Fig 4a) at day 0 i.e., after the pre cultivation, but after the LIB were added, the Fe²⁺ concentration increased to 40-50 mM which indicate that the Fe in the LIB was leached out. Fig 4a show that the adapted cultures were able to thrive more in both the BM type. The pH in all of experiments were below 1.5 which is ideal for leachability of the metals and prevents possible precipitation of metals such as Fe which precipitates at around pH 3 (Nurmi et al., 2010). In the study made by Mishra et al. (2008), they were able to leach 65 % of Co from LIB using adapted *A. ferrooxidans*. Up to 94% of Co and 60% Li were recovered in 3 replenishing cycles using *A. ferrooxidans* as inoculum in 72 hrs with 10% pulp density (Roy, Madhavi, et al., 2021). Cultures enriched from soil samples were used for bioleaching of LIB where 62.83% of Li were recovered after 15 days (Hartono et al., 2017). To the best of the author’s knowledge, there has not been any study where complete recovery of combination of metals like Li, Co, Ni and Mn has been achieved during direct bioleaching of LIB.

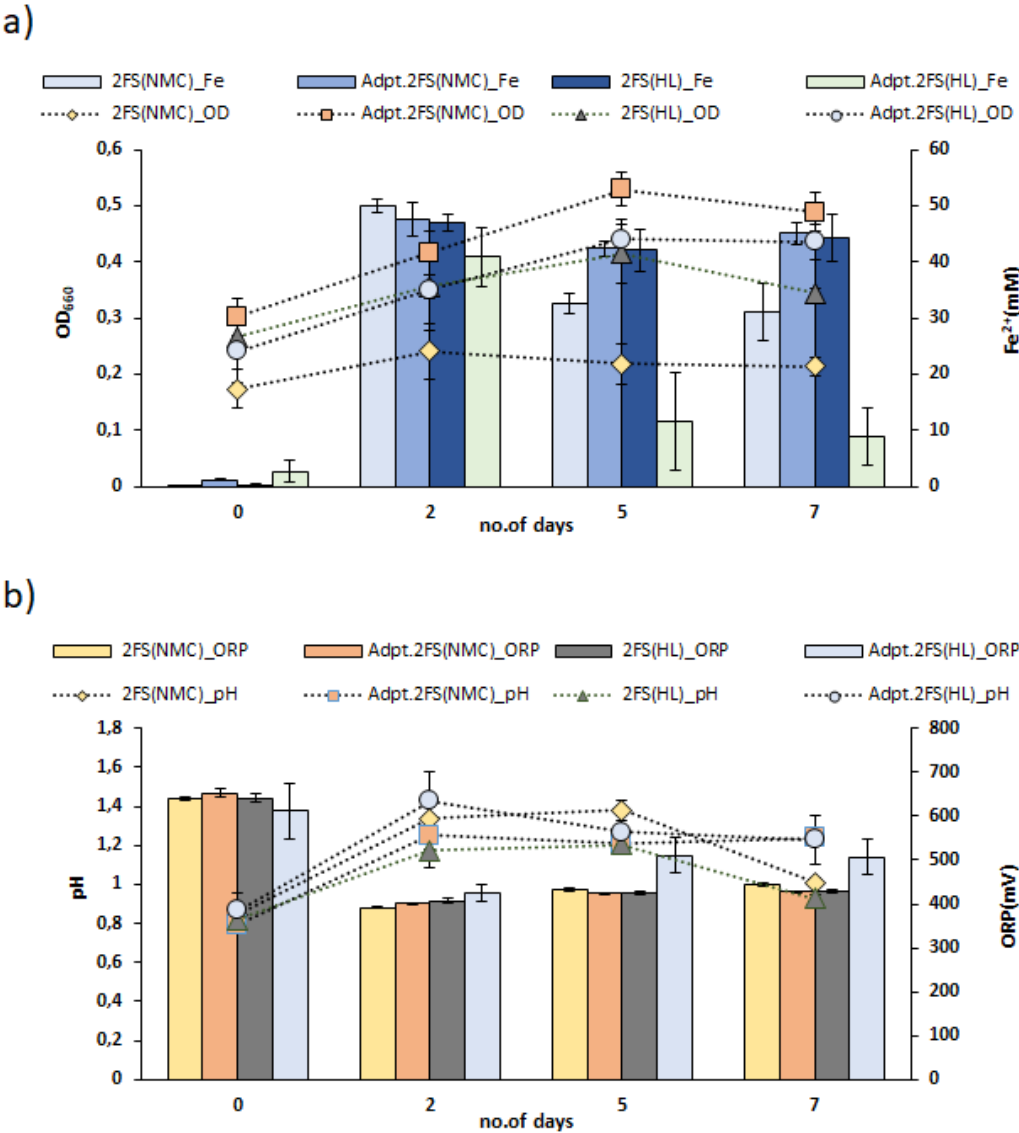


Figure 4: [a] Fe (II) and OD measurement [b] pH and ORP measurement during the direct bioleaching of BM (NMC and HL type) using both non-adapted and adapted microbial cultures (sample 2 consortia) in FS media for 7 days at 30°C, 150 rpm.

3.3.1. Metal recovery

The ICP-MS analysis of the PLS after the leaching experiments indicated that a high concentration of Li, Co, Ni, Mn, Cu and Al were leached from the LIB as shown in table 2

Table 2: Concentration of the different metals in the PLS after the direct bioleaching of the BM

Culture(BM type)	Metal concentration (mg/l)					
	Li	Co	Ni	Mn	Cu	Al
2FS(NMC)	303.6	1609.6	643.9	490.6	348.6	581.3
adpt 2FS(NMC)	377.5	1986.4	805.6	606.1	443.8	653.6
2FS(HL)	329.8	2305.1	480.7	342.3	440.	372.3
adpt 2FS(HL)	482.1	3186.4	709.6	508.1	679.8	507.5

After the recoveries of each metal were calculated, some exceeded 100% which may be due to the inhomogeneity of the BM. Normalization was done for the metal recovery according to the highest recovery rate of each metal (Fig 5). For both BM types, the adapted cultures had higher recovery rates for all the metals. For NMC, the adapted cultures improved the yield from 80% to 100% for Li, 81% to 100% for Co, 79% to 100% for Ni and 80% to 100% for Mn as compared to the yield of the non-adapted cultures (Fig 5).

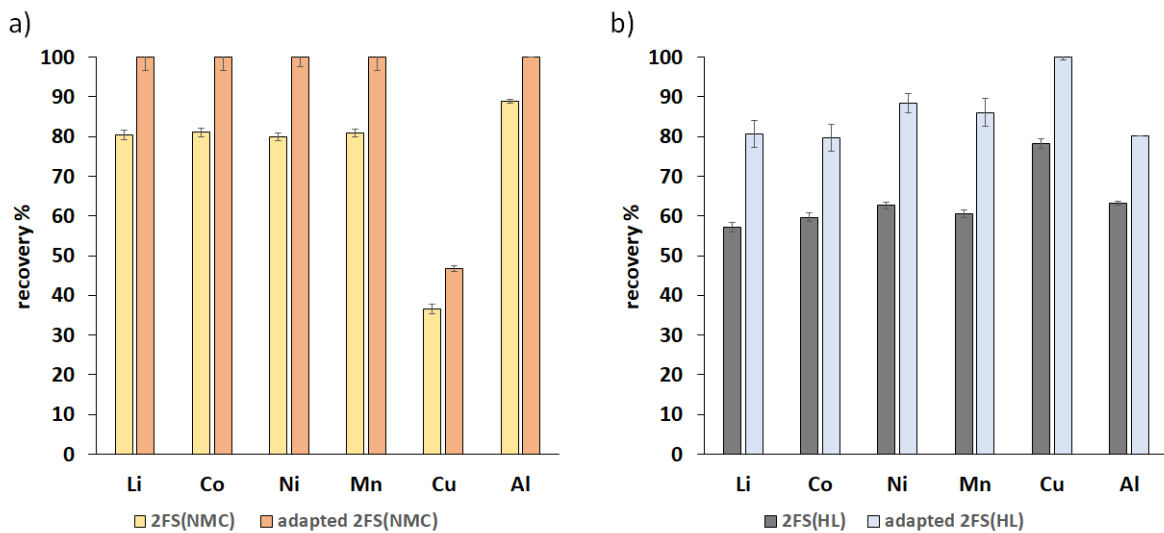
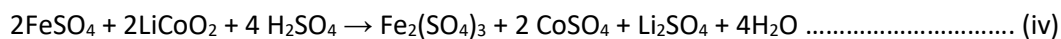


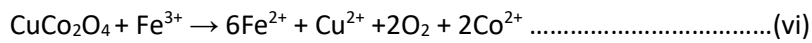
Figure 5: Recovery (%) of metals from the LIB after direct leaching experiment using adapted and non-adapted 2FS culture for 7 days. The recovery % is normalized according to the highest recovery % (which is above 100%) for each metal.

Studies have suggested that the leaching of Li^+ is mainly by acid dissolution and leaching of metals like Co^{2+} and Ni^{2+} were by a combination of Fe^{2+} reduction and the acid attack. (B. Xin et al., 2009). Co and Ni are mostly present as Co^{3+} and Ni^{3+} respectively in the LIB which is less soluble as compared to the more soluble Co^{2+} and Ni^{2+} . A study by Wu et al., 2019 show that the presence of pyrite and Fe^{2+} improves the bioleaching efficiency of Co and Ni where Fe^{2+} act as a reducing agent to convert the less soluble Co^{3+} and Ni^{3+} to the soluble Co^{2+} and Ni^{2+} . The equation for Co dissolution in the presence of Fe^{2+} is given below.



There have been studies for the effect of metal catalysts on bioleaching of spent LIB. For example, Ag^+ could promote the dissolution of Co from LiCoO_2 using *A. ferrooxidans* with the formation of AgCoO as intermediate product (Zeng et al., 2013). There has also been a report where the bioleaching efficiency

of Co greatly increased from 43.1% to 99.9% by using Cu^{2+} as a catalyst. The equation is as given below. (Zeng et al., 2012)



Similarly, in the current direct leaching experiment, the dissolution of Li, Co, Ni and Mn could follow the pathway as shown in eqn (iv). The Li was leached by the H_2SO_4 produced as a result of the S oxidation by *A. thiooxidans* while Co, Ni and Mn were solubilized by the combination of the acid attack and Fe reduction. Furthermore, the BM has high concentration of Cu and Fe which could be leached out from the BM. The leached Cu could act as a catalyst and further promotes the dissolution of Co, Ni and Mn as shown in eqn (v) and (vi). The Fe oxidizing bacteria *A. disulfidooxidans* produces the Fe^{3+} by oxidizing the leached Fe^{2+} from the BM which further helped in the dissolution of Co/Ni/Mn.

4. CONCLUSION

A mixture of *Acidithiobacillus thiooxidans* and *Alicyclobacillus disulfidooxidans* identified in enriched samples collected from the acidic lake proved to be a very promising culture for the bioleaching of LIB. The cultures could tolerate a high metal concentration and the adaptation step greatly improved the recovery of metals during the bioleaching of the BM. A leaching efficiency of up to 100% of Li, Co, Ni and Mn from NMC and up to 100% of Cu from HL was accomplished by the adapted culture. The high leaching efficiency could be attributed to the leached Cu from the BM acting as a catalyst for the dissolution of the metals from the BM.

5. ACKNOWLEDGEMENTS

This study is under the K1-MET GmbH project FuLIBatter which is supported by COMET (Competence Center for Excellent Technologies), the Austrian programme for competence centres. COMET is funded by the Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology, the Federal Ministry for Labour and Economy, the Federal States of Upper Austria, and Styria as well as the Styrian Business Promotion Agency (SFG). Furthermore, Upper Austrian Research GmbH continuously supports K1-MET. Beside the public funding from COMET, this research project FuLIBatter is partially financed by the scientific partners acib GmbH, Coventry University, Montanuniversitaet Leoben, University of Natural Resources and Life Sciences, UVR-FIA GmbH, and the industrial partners AUDI AG, BRAIN Biotech AG, Ebner Industrieofenbau GmbH, RHI Magnesita GmbH, Saubermacher Dienstleistungs AG, TÜV SÜD Landesgesellschaft Österreich GmbH and Voestalpine High Performance Metals GmbH.

REFERENCES

- Akcil, A., Ciftci, H., & Deveci, H. (2007). Role and contribution of pure and mixed cultures of mesophiles in bioleaching of a pyritic chalcopyrite concentrate. *Minerals Engineering*, 20(3), 310–318. <https://doi.org/https://doi.org/10.1016/j.mineng.2006.10.016>
- Ali, H., Khan, H. A., & Pecht, M. G. (2021). Circular economy of Li Batteries: Technologies and trends. *Journal of Energy Storage*, 40, 102690. <https://doi.org/https://doi.org/10.1016/j.est.2021.102690>

- Bahaloo-Horeh, N., Mousavi, S. M., & Baniasadi, M. (2018). Use of adapted metal tolerant *Aspergillus niger* to enhance bioleaching efficiency of valuable metals from spent lithium-ion mobile phone batteries. *Journal of Cleaner Production*, *197*, 1546–1557. <https://doi.org/10.1016/j.jclepro.2018.06.299>
- Bankole, O. E., Gong, C., & Lei, L. (2013). Battery Recycling Technologies: Recycling Waste Lithium Ion Batteries with the Impact on the Environment In-View. *Journal of Environment and Ecology*, *4*(1), 14. <https://doi.org/10.5296/jee.v4i1.3257>
- Bogdanova, T. I., Tsaplina, I. A., Kondrat'eva, T. F., Duda, V. I., Suzina, N. E., Melamud, V. S., Tourova, T. P., & Karavaiko, G. I. (2006). *Sulfobacillus thermotolerans* sp. nov., a thermotolerant, chemolithotrophic bacterium. *International Journal of Systematic and Evolutionary Microbiology*, *56*(5), 1039–1042. <https://doi.org/10.1099/ijms.0.64106-0>
- Bosecker, K. (1997). Bioleaching: metal solubilization by microorganisms. *FEMS Microbiology Reviews*, *20*(3–4), 591–604. <https://doi.org/10.1111/j.1574-6976.1997.tb00340.x>
- Boyden, A., Soo, V. K., & Doolan, M. (2016). The Environmental Impacts of Recycling Portable Lithium-Ion Batteries. *Procedia CIRP*, *48*, 188–193. <https://doi.org/10.1016/j.procir.2016.03.100>
- Donovan P. Kelly and Ann P. Wood. (2000). Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov. and *Thermithiobacillus* gen. nov. *International Journal of Systematic and Evolutionary Microbiology*, *65*(10), 511–516. <https://doi.org/10.1099/ijsem.0.000468>
- Dopson, M., Baker-Austin, C., Hind, A., Bowman, J. P., & Bond, P. L. (2004). Characterization of *Ferroplasma* Isolates and *Ferroplasma acidarmanus* sp. nov., Extreme Acidophiles from Acid Mine Drainage and Industrial Bioleaching Environments. *Applied and Environmental Microbiology*, *70*(4), 2079–2088. <https://doi.org/10.1128/AEM.70.4.2079-2088.2004>
- Dopson, M., & Johnson, D. B. (2012). Biodiversity, metabolism and applications of acidophilic sulfur-metabolizing microorganisms. In *Environmental Microbiology* (Vol. 14, Issue 10, pp. 2620–2631). <https://doi.org/10.1111/j.1462-2920.2012.02749.x>
- Hallberg, K. B., Hedrich, S., & Johnson, D. B. (2011). *Acidiferrobacter thiooxydans*, gen. nov. sp. nov.; an acidophilic, thermo-tolerant, facultatively anaerobic iron- and sulfur-oxidizer of the family *Ectothiorhodospiraceae*. *Extremophiles*, *15*(2), 271–279. <https://doi.org/10.1007/s00792-011-0359-2>
- Hans Hippe. (2000). *Leptospirillum* gen. nov. (ex Markosyan 1972), nom. rev., including *Leptospirillum ferrooxidans* sp. nov. (ex Markosyan 1972), nom. rev. and *Leptospirillum thermoferrooxidans* sp. nov. (Golovacheva et al. 1992). *International Journal of Systematic and Evolutionary Microbiology*, *50*(2), 501–503. <https://doi.org/10.1099/00207713-50-2-501>
- Hansford, G. S., & Vargas, T. (2001). Chemical and electrochemical basis of bioleaching processes. *Hydrometallurgy*, *59*(2), 135–145. [https://doi.org/10.1016/S0304-386X\(00\)00166-3](https://doi.org/10.1016/S0304-386X(00)00166-3)
- Hartono, M., Astrayudha, M. A., Petrus, H. T. B. M., Budhijanto, W., & Sulistyono, H. (2017). Lithium recovery of spent lithium-ion battery using bioleaching from local sources microorganism. *Rasayan Journal of Chemistry*, *10*(3), 897–903. <https://doi.org/10.7324/RJC.2017.1031767>

- Heydarian, A., Mousavi, S. M., Vakilchah, F., & Baniasadi, M. (2018). Application of a mixed culture of adapted acidophilic bacteria in two-step bioleaching of spent lithium-ion laptop batteries. *Journal of Power Sources*, 378, 19–30. <https://doi.org/10.1016/j.jpowsour.2017.12.009>
- Hrdinka, T., Šobr, M., Fott, J., & Nedbalová, L. (2013). The unique environment of the most acidified permanently meromictic lake in the Czech Republic. *Limnologica*, 43(6), 417–426. <https://doi.org/10.1016/j.limno.2013.01.005>
- Hubau, A., Minier, M., Chagnes, A., Joulian, C., Silvente, C., & Guezennec, A.-G. (2020). Recovery of metals in a double-stage continuous bioreactor for acidic bioleaching of printed circuit boards (PCBs). *Separation and Purification Technology*, 238, 116481. <https://doi.org/https://doi.org/10.1016/j.seppur.2019.116481>
- Ijadi Bajestani, M., Mousavi, S. M., & Shojaosadati, S. A. (2014). Bioleaching of heavy metals from spent household batteries using *Acidithiobacillus ferrooxidans*: Statistical evaluation and optimization. *Separation and Purification Technology*, 132, 309–316. <https://doi.org/10.1016/j.seppur.2014.05.023>
- Ilyas, S., Anwar, M. A., Niazi, S. B., & Afzal Ghauri, M. (2007). Bioleaching of metals from electronic scrap by moderately thermophilic acidophilic bacteria. *Hydrometallurgy*, 88(1–4), 180–188. <https://doi.org/10.1016/j.hydromet.2007.04.007>
- Jegan Roy, J., Srinivasan, M., & Cao, B. (2021). Bioleaching as an Eco-Friendly Approach for Metal Recovery from Spent NMC-Based Lithium-Ion Batteries at a High Pulp Density. *ACS Sustainable Chemistry and Engineering*, 9(8), 3060–3069. <https://doi.org/10.1021/acssuschemeng.0c06573>
- Karavaiko, G. I., Bogdanova, T. I., Tourova, T. P., Kondrat'eva, T. F., Tsaplina, I. A., Egorova, M. A., Krasil'nikova, E. N., & Zakharchuk, L. M. (2005). Reclassification of “*Sulfobacillus thermosulfidooxidans* subsp. *thermotolerans*” strain K1 as *Alicyclobacillus tolerans* sp. nov. and *Sulfobacillus disulfidooxidans* Dufresne et al. 1996 as *Alicyclobacillus disulfidooxidans* comb. nov., and emended description. *International Journal of Systematic and Evolutionary Microbiology*, 55(2), 941–947. <https://doi.org/10.1099/ijs.0.63300-0>
- Kim, T.-H., Park, J.-S., Chang, S. K., Choi, S., Ryu, J. H., & Song, H.-K. (2012). The Current Move of Lithium Ion Batteries Towards the Next Phase. *Advanced Energy Materials*, 2(7), 860–872. <https://doi.org/https://doi.org/10.1002/aenm.201200028>
- Krebs, W., Brombacher, C., Bosshard, P. P., Bachofen, R., & Brandl, H. (1997). Microbial recovery of metals from solids. *FEMS Microbiology Reviews*, 20(3), 605–617. [https://doi.org/https://doi.org/10.1016/S0168-6445\(97\)00037-5](https://doi.org/https://doi.org/10.1016/S0168-6445(97)00037-5)
- Kremser, K., Maltschnig, M., Schön, H., Jandric, A., Gajdosik, M., Vaculovic, T., Kucera, J., & Guebitz, G. M. (2022). Optimized biogenic sulfuric acid production and application in the treatment of waste incineration residues. *Waste Management*, 144(March), 182–190. <https://doi.org/10.1016/j.wasman.2022.03.025>
- Kremser, K., Thallner, S., Strbik, D., Spiess, S., Kucera, J., Vaculovic, T., Vsiansky, D., Haberbauer, M., Mandl, M., & Guebitz, G. M. (2021). Leachability of metals from waste incineration residues by iron- and sulfur-oxidizing bacteria. *Journal of Environmental Management*, 280, 111734. <https://doi.org/10.1016/j.jenvman.2020.111734>
- Li, L., Dunn, J. B., Zhang, X. X., Gaines, L., Chen, R. J., Wu, F., & Amine, K. (2013). Recovery of metals from spent lithium-ion batteries with organic acids as leaching reagents and environmental

- assessment. *Journal of Power Sources*, 233, 180–189.
<https://doi.org/https://doi.org/10.1016/j.jpowsour.2012.12.089>
- Mishra, D., Kim, D. J., Ralph, D. E., Ahn, J. G., & Rhee, Y. H. (2008). Bioleaching of metals from spent lithium ion secondary batteries using *Acidithiobacillus ferrooxidans*. *Waste Management*, 28(2), 333–338. <https://doi.org/10.1016/j.wasman.2007.01.010>
- Mossali, E., Picone, N., Gentilini, L., Rodríguez, O., Pérez, J. M., & Colledani, M. (2020). Lithium-ion batteries towards circular economy: A literature review of opportunities and issues of recycling treatments. *Journal of Environmental Management*, 264.
<https://doi.org/10.1016/j.jenvman.2020.110500>
- Ñancucheo, I., Rowe, O. F., Hedrich, S., & Johnson, D. B. (2016). Solid and liquid media for isolating and cultivating acidophilic and acid-tolerant sulfate-reducing bacteria. *FEMS Microbiology Letters*, 363(10), 1–6. <https://doi.org/10.1093/femsle/fnw083>
- Niu, Z., Zou, Y., Xin, B., Chen, S., Liu, C., & Li, Y. (2014). Process controls for improving bioleaching performance of both Li and Co from spent lithium ion batteries at high pulp density and its thermodynamics and kinetics exploration. *Chemosphere*, 109, 92–98.
<https://doi.org/https://doi.org/10.1016/j.chemosphere.2014.02.059>
- Nurmi, P., Özkaya, B., Sasaki, K., Kaksonen, A. H., Riekkola-Vanhanen, M., Tuovinen, O. H., & Puhakka, J. A. (2010). Biooxidation and precipitation for iron and sulfate removal from heap bioleaching effluent streams. *Hydrometallurgy*, 101(1), 7–14.
<https://doi.org/https://doi.org/10.1016/j.hydromet.2009.11.004>
- Qiu, M., Xiong, S., Zhang, W., & Wang, G. (2005). A comparison of bioleaching of chalcopyrite using pure culture or a mixed culture. *Minerals Engineering*, 18(9), 987–990.
<https://doi.org/https://doi.org/10.1016/j.mineng.2005.01.004>
- Remonsellez, F., Galleguillos, F., Moreno-Paz, M., Parro, V., Acosta, M., & Demergasso, C. (2009). Dynamic of active microorganisms inhabiting a bioleaching industrial heap of low-grade copper sulfide ore monitored by real-time PCR and oligonucleotide prokaryotic acidophile microarray. *Microbial Biotechnology*, 2(6), 613–624. <https://doi.org/10.1111/j.1751-7915.2009.00112.x>
- Retnaningrum, E., Wilopo, W., & Warmada, I. W. (2021). Enhancement of manganese extraction in a biochar-enriched bioleaching column with a mixed culture of indigenous bacteria. *Biodiversitas*, 22(5), 2949–2955. <https://doi.org/10.13057/biodiv/d220560>
- Roy, J. J., Cao, B., & Madhavi, S. (2021). A review on the recycling of spent lithium-ion batteries (LIBs) by the bioleaching approach. In *Chemosphere* (Vol. 282). Elsevier Ltd.
<https://doi.org/10.1016/j.chemosphere.2021.130944>
- Roy, J. J., Madhavi, S., & Cao, B. (2021). Metal extraction from spent lithium-ion batteries (LIBs) at high pulp density by environmentally friendly bioleaching process. *Journal of Cleaner Production*, 280, 124242. <https://doi.org/https://doi.org/10.1016/j.jclepro.2020.124242>
- Sajjad, W., Zheng, G., Din, G., Ma, X., Rafiq, M., & Xu, W. (2019). Metals Extraction from Sulfide Ores with Microorganisms: The Bioleaching Technology and Recent Developments. *Transactions of the Indian Institute of Metals*, 72(3), 559–579. <https://doi.org/10.1007/s12666-018-1516-4>
- Salo-Zieman, V. L. A., Sivonen, T., Plumb, J. J., Haddad, C. M., Laukkanen, K., Kinnunen, P. H. M., Kaksonen, A. H., Franzmann, P. D., & Puhakka, J. A. (2006). Characterization of a thermophilic sulfur oxidizing enrichment culture dominated by a *Sulfolobus* sp. obtained from an

- underground hot spring for use in extreme bioleaching conditions. *Journal of Industrial Microbiology and Biotechnology*, 33(12), 984–994. <https://doi.org/10.1007/s10295-006-0144-x>
- Sonoc, A., & Jeswiet, J. (2014). A review of lithium supply and demand and a preliminary investigation of a room temperature method to recycle lithium ion batteries to recover lithium and other materials. *Procedia CIRP*, 15, 289–293. <https://doi.org/10.1016/j.procir.2014.06.006>
- Sun, X., Hao, H., Hartmann, P., Liu, Z., & Zhao, F. (2019). Supply risks of lithium-ion battery materials: An entire supply chain estimation. *Materials Today Energy*, 14. <https://doi.org/10.1016/j.mtener.2019.100347>
- Wang, X., Gaustad, G., Babbitt, C. W., & Richa, K. (2014). Economies of scale for future lithium-ion battery recycling infrastructure. *Resources, Conservation and Recycling*, 83, 53–62. <https://doi.org/https://doi.org/10.1016/j.resconrec.2013.11.009>
- Wu, W., Liu, X., Zhang, X., Li, X., Qiu, Y., Zhu, M., & Tan, W. (2019). Mechanism underlying the bioleaching process of LiCoO₂ by sulfur-oxidizing and iron-oxidizing bacteria. *Journal of Bioscience and Bioengineering*, 128(3), 344–354. <https://doi.org/10.1016/j.jbiosc.2019.03.007>
- Xiang, Y., Wu, P., Zhu, N., Zhang, T., Liu, W., Wu, J., & Li, P. (2010). Bioleaching of copper from waste printed circuit boards by bacterial consortium enriched from acid mine drainage. *Journal of Hazardous Materials*, 184(1–3), 812–818. <https://doi.org/10.1016/j.jhazmat.2010.08.113>
- Xin, B., Zhang, D., Zhang, X., Xia, Y., Wu, F., Chen, S., & Li, L. (2009). Bioleaching mechanism of Co and Li from spent lithium-ion battery by the mixed culture of acidophilic sulfur-oxidizing and iron-oxidizing bacteria. *Bioresource Technology*, 100(24), 6163–6169. <https://doi.org/https://doi.org/10.1016/j.biortech.2009.06.086>
- Xin, Y., Guo, X., Chen, S., Wang, J., Wu, F., & Xin, B. (2016). Bioleaching of valuable metals Li, Co, Ni and Mn from spent electric vehicle Li-ion batteries for the purpose of recovery. *Journal of Cleaner Production*, 116, 249–258. <https://doi.org/10.1016/j.jclepro.2016.01.001>
- Zeng, G., Deng, X., Luo, S., Luo, X., & Zou, J. (2012). A copper-catalyzed bioleaching process for enhancement of cobalt dissolution from spent lithium-ion batteries. *Journal of Hazardous Materials*, 199–200, 164–169. <https://doi.org/10.1016/j.jhazmat.2011.10.063>
- Zeng, G., Luo, S., Deng, X., Li, L., & Au, C. (2013). Influence of silver ions on bioleaching of cobalt from spent lithium batteries. *Minerals Engineering*, 49, 40–44. <https://doi.org/10.1016/j.mineng.2013.04.021>
- Zhang, R., Hedrich, S., Jin, D., Breuker, A., & Schippers, A. (2021). *Sulfobacillus harzensis* sp. Nov., an acidophilic bacterium inhabiting mine tailings from a polymetallic mine. *International Journal of Systematic and Evolutionary Microbiology*, 71(7). <https://doi.org/10.1099/ijsem.0.004871>