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URBAN BIOMINING: LITHIUM RECOVERY FROM SPENT BATTERIES THROUGH MULTI-STEP BIOPROCESSES

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Abstract

Multi-step design to evaluate the bio-recovery of lithium from spent batteries was studied. The first step consisted of lithium extraction from spent batteries, using bacterial and fungal acid extrolites. The second step explored lithium recovery in the form of carbonate salts by using MICP (Microbial Induced Carbonate Precipitation) bacteria from *Sporosarcina* species. For lithium extraction (Step 1) sulfuric acid produced by sulphur oxidizing bacteria *Acidithiobacillus thiooxidans* was evaluated for its capacity to leach lithium. Extraction with biogenic sulfuric acid and with fungal bio- produ-cts (from *Aspergillus sp*. and *Simplicillum sp*. isolated at our facilities) were compared with commercial sulfuric acid. For biorecovery processes (Step 2), two type strains of *Sporosarcina sp.* were tested due to their capacity to precipitate lithium carbonate. Results showed fungal bioextracts gave a lithium leaching yield close to 60% and a global recovery yield of 27%. These observations are reported for the first time and lay the foundations for continuing the study and scaling up of this combined process for lithium recovery.

Keywords: biomining, lithium, *Acidithiobacillus thiooxidans*, fungi, *Sporosarcina sp*.

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Introduction

A suitable match between physicochemical properties and industrial needs has made lithium one of the most commercialized metals of the last 20 years. Between 2015 and 2019 lithium Annual Accumulated Growth Rate was calculated at 13.6% and it is expected to reach an extraordinary 18.5% value by 2030, due to the emerging commercialization of electric vehicles and fossil fuel transitions (Jones, 2021). While demand for construction (glass, ceramics, and lubricants), pharma and electronics (mobile telephones, computers, and tablet batteries) remained stable, lithium carbonate and hydroxide for mobility batteries increased from 20,000 to 176,000 tons in 2019, being China and Japan the major consumers (Jones, 2021, Eyzaguirre, 2020). Furthermore, lithium is considered a Critical Raw Material (CRM) as part of an energetic transition plan to achieve net zero CO_2 global emissions by the year 2050 (IEA, 2021).

According to market tendencies, Foster et.al (Foster, 2014) predicted 3.78 million exhausted lithium batteries discarded by the year 2035, which will need an end-of-life management plan. Major lithium reserves are placed in Australia, Germany, Canada, Mexico, Perú, China and the so-called "lithium triangle" in the Andean region of South America, composed by Argentina, Chile, and Bolivia (CEPAL, 2021).

Lithium deposits can be divided into three forms, with decreasing difficulty of processing and purification: pegmatites or mineral rocks (e.g., spodumene) which are distributed in various geographical locations; sedimentary rocks; salars and brines with 66% of abundance, strategically found in the "Lithium Triangle" which satisfies almost 50% of world lithium demand (Jones, 2021, MDM, 2020, MEM, 2017). Brines are also rich in other metals of economic interest such as boron, potassium, sodium, and magnesium, used in batteries, fertilizers, ceramics, detergents, and other industrial products (Marazuela, 2019). Particularly, the battery industry is focused on lithium carbonate of 99.5% purity (MDM, 2020). As purifying lithium from rocks comprises classical open pit exploitation -digging, grinding, lixiviation and purification processes- extraction from brines implies a simplification of these steps by working directly with aqueous raw material. Thus, soluble lithium is harvested by brine pumping, sequenced solar evaporation and final carbonate precipitation (Bustos-Gallardo, 2021). This process takes advantage of special climatic conditions found at 3000- 4600 m.a.s.l. which happens particularly in the lithium triangle in South America.

Regarding the sustainability of brine pumping, studies in the Atacama Desert have demonstrated a shift in the natural balance of brines through the damping capacity, that is, through the loss of the level of the underground water table caused by a negative mass balance between pumping and recharge that causes an overall reduction of evaporation rate (Marazuela, 2019). Furthermore, depletion of water basins and native species loss were also reported (Gajardo, 2019).

Thus, considering the latest evidence, major facts regarding the sustainability of lithium batteries' life cycle -from mining to depleted battery management- are claimed to be regulated (Editor, 2021, Gajardo, 2019).

Spent battery recycling typical process takes at least 4 well differentiated steps (Tawonezvi, 2023):

1. Pre- treatment: to completely discharge the batteries and separate components (plastic cover, graphite, anode, and cathode). This step also increases overall recovery efficiency. Includes chemical, mechanical, and thermal methods.
2. Physical treatment: focused on the recovery of valuable metals. Typically, pyro and hydrometallurgical processes are executed.
3. Chemical treatment: extractions and dissolution of metals, following precipitation and recovery. Includes classical leaching with acidic solutions, such as hydrochloric, nitric, sulfuric, and organic acids; extraction with solvents and ionic liquids; chemical precipitation for recovery. Biological processes are taking relevance specially in this step to replace or complement the process and increase the overall yield.
4. Metal recovery and reuse: finally, to achieve the complete recycling process, the recovery of metals of economic interest - such as lithium, nickel, and cobalt, between others- is done by chemical means. Ultimate objective is to insert them in the commercial environment as raw materials for energy, chemical and construction industries.

Important disadvantages of the above-mentioned processes include emission of toxic or greenhouse gases, especially in those steps including thermal treatments. Thus, effective methods for metal recovery and reuse will contribute to reducing the carbon and water footprints of brine exploitation (Jones, 2021, Amato, 2021).

As research on effective recovery processes is taking relevance, biomining - i.e. biological systems for extraction of metals from minerals and waste materials (Rawings, 2007; Johnson, 2014) - shows up as an attractive discipline to achieve recovery and sustainability goals. Such processes are characterized by their robustness, cost-effectiveness, low toxicity or risk for workers and the environment, and comparable yields to traditional chemical processes, among others (Johnson, 2007).

In this research two biologically mediated processes were investigated to be applied at the treatment, recovery, and reuse of lithium from spent batteries. The first step evaluated the capacity of bacterial and fungal acid extrolites (fungal bioextractants) for lithium extraction from crushed spent batteries. Specifically, the use of sulfuric acid generated by the bacterial strain *Acidithiobacillus thiooxidans* and acidic organic extrolites produced by fungal species *Aspergillus sp*. and *Simplicillum sp*. was explored.

Biogenic sulfuric acid from *A. thiooxidans* and its application in leaching processes is well described (Johnson, 2007, Steudel, 1989). It has been used for uncountable metal bioleaching applications (Brierley, 2013, Gentina, 2016) including lithium present in similar residues (Boxall, 2018[1]).

Environmental molds (filamentous fungi) can segregate organic acids and other chelating compounds with various ecological functions and industrial applications, also including metal leaching processes (Boxall, 2018[1], Jain, 2004, Frisvad, 2015, Frisvad, 2018, Lambert, 1997). In the present research two environmental fungal isolations were tested for acid extrolites production and its leaching capacity.

In both cases, acid leaching means a typical solubilization of metals by hydrolysis in acidic conditions, as shown in Equation 1:
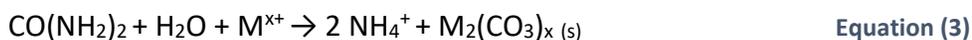
$$M(OH)_x + H^+ \rightarrow M^{x+} + H_2O \qquad\qquad \text{Equation (1)}$$

Where:
M: metal element
x: metal valence – stochiometric coefficient

The second step approached to lithium recovery in the form of carbonate salts by using MICP (Microbial Induced Carbonate Precipitation) bacteria from *Sporosarcina* species.

Microbial Induced Carbonate Precipitation species couple the hydrolysis of urea to extracellular carbonate precipitation in a well-described pathway (Kumari, 2016, Zhang, 2021). This ubiquitous pathway leads to calcite ($CaCO_3$) precipitation due to a shift in the extracellular carbonate-bicarbonate equilibrium. Such process has been of industrial interest for applications in architecture, cementation, sculpture restoration and bioremediation (Zhang, 2021). Equation 2 shows the overall chemical reaction involving *Sporosarcina sp.* ureolitic metabolism and its contribution to calcium carbonate precipitation. Alternatively, calcium can be replaced by any other metal (Equation 3):

$$CO(NH_2)_2 + H_2O + Ca^{2+} \rightarrow 2\ NH_4^+ + CaCO_{3\ (s)} \qquad\qquad \text{Equation (2)}$$

$$CO(NH_2)_2 + H_2O + M^{x+} \rightarrow 2\ NH_4^+ + M_2(CO_3)_{x\ (s)} \qquad\qquad \text{Equation (3)}$$

Where:
M: metal element
x: metal valence – stochiometric coefficient

Interestingly, *Sporosarcina* species are believed to be part of the brine environment (Bakermans, 2011) so future processes could be designed using native isolations, already adapted to extreme conditions, instead of collection strains. Testing their capacity to recover lithium in an economical valuable carbonate salt opens new possible industrial applications to these species.

Materials and methods

Processing and characterization of spent batteries

A batch of 20 units of Lithium spent batteries (LIBs) complete discharging was tested before processing, manually, using a digital tester. Not being a usual industrial pre-treatment but following mandatory institutional procedures, LIBs were frozen in liquid nitrogen before been grounded in a cutting mill (RETSCH SM300). This measure was taken to prevent possible increases in temperature in the shredding process that could generate some type of explosion.

The crushed material was sieved through ASTM 10 series (particle size 2 mm) in order to eliminate part of the plastic material and homogenize the sample. This fraction was identified as "LiB" and used for all the experiments.

Multi-acid digestion and subsequent reading by inductively coupled plasma emission spectrometry (ICP – OES Perkin Elmer OPTIMA 8.300) was accomplished to characterize lithium composition of the spent battery batch. LiB batch used for leaching experiments had a concentration of 24.835 g Li/Kg.

Microorganisms

Bacterial and fungal cultures and its by-products were used for indirect metal recovery experiments in batch conditions.

Acidithiobacillus thiooxidans DSM 11478 inoculum was grown in a 250 mL flask in an orbital shaker at 200 rpm, 30 °C. Culture media was 0K medium (per liter: 0.5 g K_2HPO_4; 2 g $(NH_4)_2SO_4$; 0.5 g MgSO4- 7H_2O; 0.001 g $CaCl_2$; 0.1 g KCl; pH 2.5) with elemental sulfur as energy source (1% w/v). Culture volume was 100 ml. Successive subcultures were made until cultures reached pH ⩽ 1,00 and cellular density achieved a value beyond 1.00E+09 cells/mL, entire flask was used as inoculum for a 5-liter (useful volume) sulfur packed air-lift reactor (Buonomo, 2022). Biogenic sulfuric acid of 0.10N (titrated against NaOH 0.01N) and pH 1.5 was collected after 6 days of retention time, collected, and kept at 4 °C until use.

Two fungal strains were isolated from a fungi- contaminated bacterial culture with high lithium and other metals content. In these cultures, the carbon source for the fungi corresponded to carbon compounds fixed by acidophilic bacteria. Mycelium isolation followed a previously

described method (Gargarello, 2008). Briefly, mycelium was picked up from the bacterial culture and plated onto Potato-Dextrose-Agar (PDA) supplemented with 0.25 g/L of chloramphenicol. After five days of growth on PDA at 30 °C, two different fungal hyphae morphologies were visualized and separately re-plated on PDA supplemented with antibiotics by taking a 5 mm agar plug cut out from the colony margins. After two subculturing processes, the axenic stock cultures were kept on PDA medium at 4°C, and fresh specimens were sent to molecular sequencing. According to ITS1 and ITS4 region sequencing (Macrogen Inc., Corea), specimens were identified as *Aspergillus sp*. and *Simplicillium sp*. both uncultured strains. DNA sequencing results were confirmed by growth observation at nude eye and at optic microscope of conidiophores: green mold, separated, branched radiated conidiophores for *Aspergillus*; white mold, late sporulation for *Simplicillium*. Acid production was confirmed by culturing on PDA supplemented with 0,015 g/L of bromocresol purple.

For liquid culturing and bioextractant solution recovery, conidial suspensions in sterile modified P5 liquid medium were prepared from the agar plates, quantified in a Neubauer chamber (1,00 E+07 spore/ mL) and used for inoculating 250 ml Erlenmeyer flasks with 100 ml liquid medium. Modified P5 composition (per liter): 0.3 g $(NH_4)_2SO_4$; 1.28 g K_2HPO_4; 0.5 g $MgSO_4$-$7H_2O$; 20 g D-glucose; 0.1 g thiamine- HCl; pH 5.5). As studied by Buonomo, A. (2022) after 6 days, when cultures arrived to a final pH of 2.5 – 3.00, they were filtrated through Whatman 41 paper and the recovered solution filtered through 0.22 μm membrane in sterile conditions. Solutions with biogenic acids were kept at 4 °C until use.

For Microbial Induced Carbonate Precipitation (MICP), two type strains of *Sporosarcina sp.*, provided by the culture collection of the Servicio Geologico Minero Argentino, were used. *S. saromensis* (SS) and *S. newyorkensis* (SN), were cultivated in Luria Broth (LB) enriched with 500 mM urea, 1%v/v inoculum.

For metal biorecovery assays, grown cultures of OD 620 nm higher than 1 unit were harvested by centrifugation (7100 G), washed twice with sterile distilled water, and finally resuspended in a minimal volume. If needed, cells were inactivated by sterilization with hot stream at 120 °C, 1atm pressure. All solutions were handled and preserved in sterile conditions.

Multi-step biorecovery assays.
Graphic summary of the global process is shown in Figure 1. All experiments were done in batch conditions and by duplicate. LiB mass was put in sterilized flasks, sprayed with 70% ethanol, and left under UV radiation for 30 minutes to diminish microbial charge and subsequent crossed contamination. All experiments were conducted in sterile conditions.
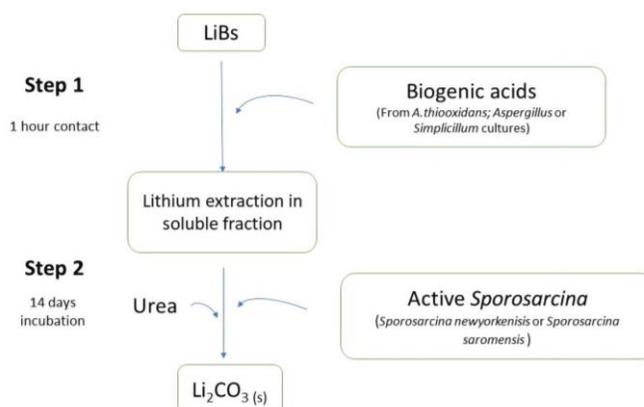
Figure 1. Graphic summary of multi-step lithium biorecovery.

For indirect lithium extraction assays (Step1) 2% w/v of LiB fraction was put in contact with biogenic acids, for 1 hour at 200 rpm and 30 °C. Initial and final pH value was registered. For pH-controlled experiments, pH value was adjusted to 3.00 or 6.00 with NaOH 0.1 N or HCl 0.1N.
After contact time, systems were filtered through Whatman 41 paper and liquid fraction recovered. Extracted lithium was quantified by emission method at λ= 670.8, slit 0.2 as indicated by equipment handbook (Perkin Elmer, Analyst 100).

Lithium extraction yield was calculated considering LiB elemental composition and lithium concentration in solution after Step 1 through Equation 4:

$$Lithium\ Extraction\ (mg/g)= Mr / Mi \qquad \textbf{Equation (4)}$$

Where:
Mr: total mass (mg) of Lithium recovered in solution after 1 h contact time (Concentration measured/ volume of contact solution).
Mi: total mass (g) of Lithium in the LiB present in each system.

For Li_2CO_3 recovery assays (Step 2) minimal volume of *Sporosarcina* resuspended in sterile distilled water was inoculated to reach initial OD 620 nm of 0.1 units. Systems were also enriched with 500 mM final concentration of urea and incubated at 200 rpm and 30 °C. OD 620 nm, pH, ammonia (Urea color 2, Wiener) and lithium in solution were followed for at least 7 days. If necessary, enrichment with a concentrated solution of Li_2CO_3 was made to each system to accomplish a minimum concentration of Lithium equal to 800 mg/L and guarantee Ksp concentrations (Ksp = 2.5 E-02).

Control systems for Step 1 were made of commercial sulfuric acid 0.1N solution and water. For Step 2, a set of systems were inoculated with heat-inactivated *Sporosarcina* solutions (grown culture of the microorganism was autoclaved at 100°C - 1atm for 20 minutes) to confirm that lithium depletion was metabolically mediated (indicated as "inactive").

Another set of flasks identical to the study systems but without urea was incubated to confirm urea metabolism coupling to lithium depletion (indicated as "metabolic control"). A third set of flasks consisted of culture media with urea but without microorganisms ("abiotic control").

To facilitate comparison between these experiments, results are shown in relative lithium concentration remaining in solution, obtained by dividing all results by the highest value measured at each time (Figures 4 to 8).

Results

Lithium leaching with bioextractants.

Lithium recovery with biogenic sulfuric acid showed to be more effective when system pH value initiated at 1.5. Results are summarized in Figure 2.
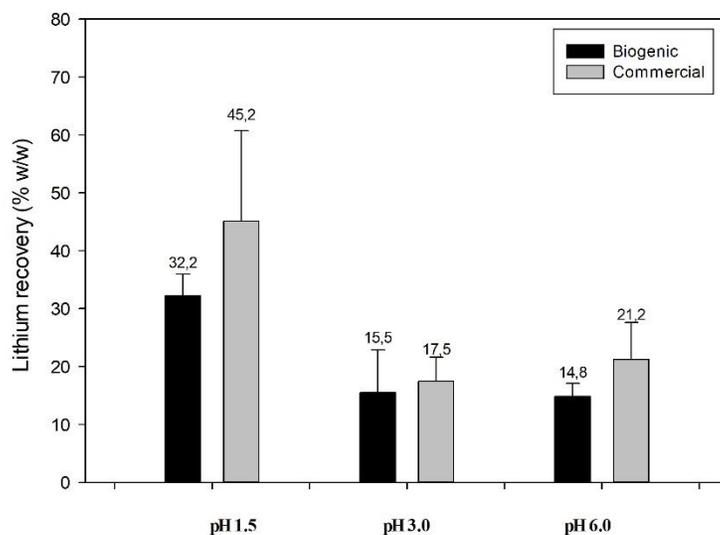

Figure 2. Lithium recovered in solution (leaching) with biogenic sulfuric acid and commercial sulfuric acid solutions. Numbers indicate final value in mg/g. Percentage of recovery is also indicated in bold font.

Systems at pH 1.5 did not need addition of acid or alkali. Variation of pH was 0.22 units for systems with biogenic acid and 0.03 units for systems with commercial acid. Recovery at pH 1.5 meant a 32.2% for biogenic acid compared with 45.2% of commercial sulfuric acid.

Systems at pH 3.00 and pH 6.00 needed constant adjustment with acid solution to remain stable (volume variation < 1%). As recovery observed was good at pH values lower than 3.0 for next experiments only conditions below this value were assayed.

Lithium recovery with fungal bioextractants showed to be more effective when working with *Aspergillus sp.* solutions. Results are summarized in Figure 3.
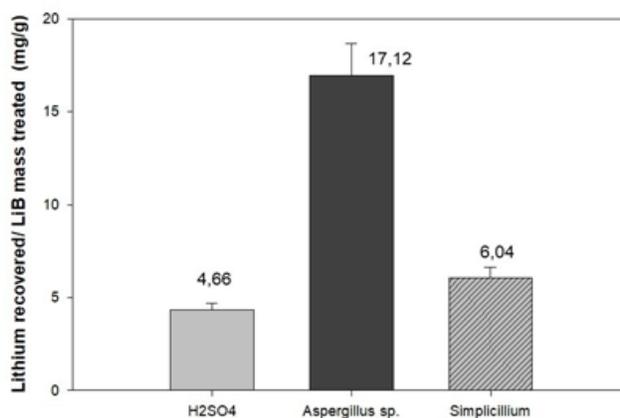

Figure 3. Lithium recovery in solution (leaching) with fungal bioextractants.

pH variation remained between 0.10 units for all systems and did not need adjustment. Initial values were 2.6 ± 0.10 for fungal bioextractans (matching with typical final pH of cultures (Buonomo, 2022). H_2SO_4 initial pH was set at 2.5 units.

Percentage of recovery for these systems were: 45% for commercial H_2SO_4; 68.26%, *Aspergillus sp.*; 24.34% *Simplicillium*. Lithium recovery in direct extraction experiments (i.e., in situ growing fungi) was not comparable to indirect extraction assays, despite visible fungal growth and evidenced tolerance to high metal concentrations (data not shown).

Lithium recovery through bio- precipitation with Sporosarcina sp.
Lithium carbonate precipitation was assayed with *Sporosarcina* cultures in Step 2, to complete a multi- step biological process of recovery.

When Step 1 was accomplished with biogenic sulfuric acid, no evidence of lithium fading was observed in Step 2 (Figure 4). Variation of lithium remaining in solution obtained after fourteen days of incubation seemed to have the same behavior of the control systems. Furthermore, no significant ammonia production was evidenced (Figure 5). and pH values remained acidic, ranging from an average of 1.80 ± 0.1 to 4.50 ± 0.1 in all systems which would not favor any precipitation process. These set of experiments could be held for 14 days before contamination was evidenced.
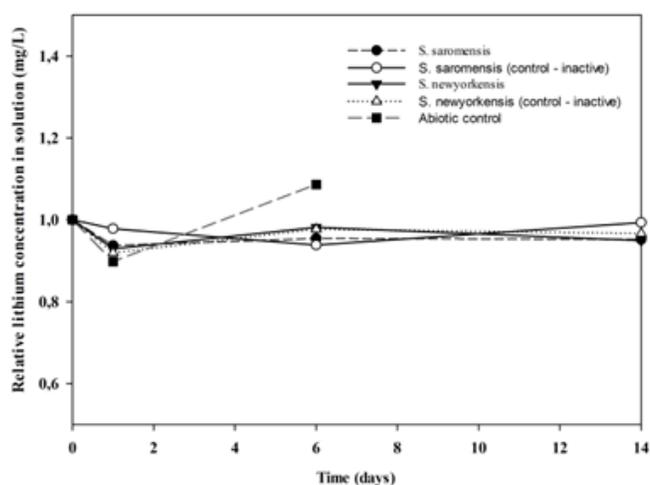

Figure 4. LiBs treated with biogenic sulfuric acid and *Sporosarcina sp*. to evaluate recovery as Li_2CO_3.
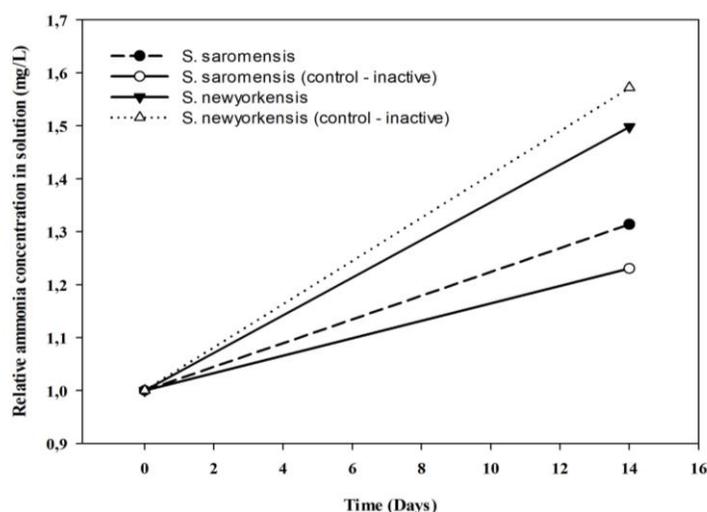

Figure 5. Variation of ammonia in biogenic sulfuric acid put into contact with *Sporosarcina sp.* to evaluate recovery as Li_2CO_3.

On the other hand, when lithium was leached with fungal bioextractants in Step 1, a depletion of 40% in solution after 7 days of incubation (Figure 6) was evidenced in Step 2. No relevant differences were observed between *Sporosacina saromensis* or *Sporosarcina newyorkensis* performance.
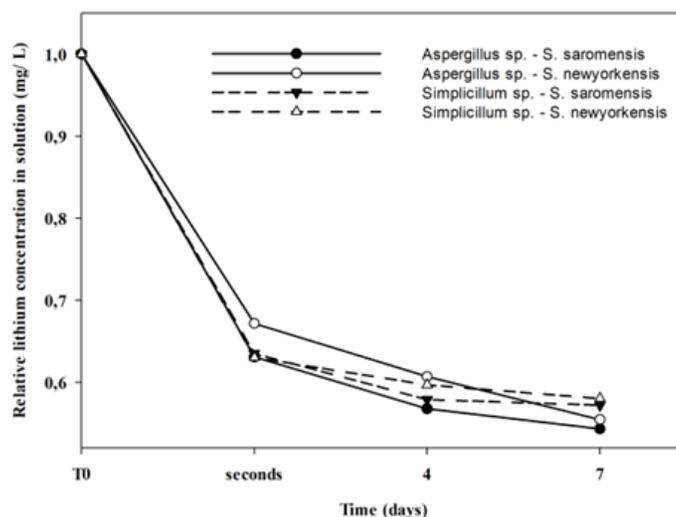

Figure 6. Relative lithium concentration in solution for systems previously treated with fungal bioextractants in contact with *Sporosarcina sp.*

Lithium depletion can be exclusively attributed to urease metabolism of *Sporosarcina,* as ammonia in solution tendency is to increase (Figure 7), evidence of metabolic ureolitic activity.

On the other hand, control systems did not evidence metal decrease: nor dead cells superficial phenomena or physicochemical factors that include urea oxidation are related (Figure 8).

Although lithium carbonate as a precipitated solid was not evidenced at nude eye, carbonate and lithium were confirmed by semi- quantitative analysis on one set of systems for Step 2. At day 7, *Aspergillus sp*. and *Sporosarcinas* system was filtrated through 0.22 μm membrane. The retentate was resuspended in HCl 10% and bubbling was observed, typical of carbonate dissociation in CO_2. Investigation of the presence of lithium by measuring the suspension by atomic emission revealed a positive result. However, no mass balances could be done due to the lower quantities recovered.
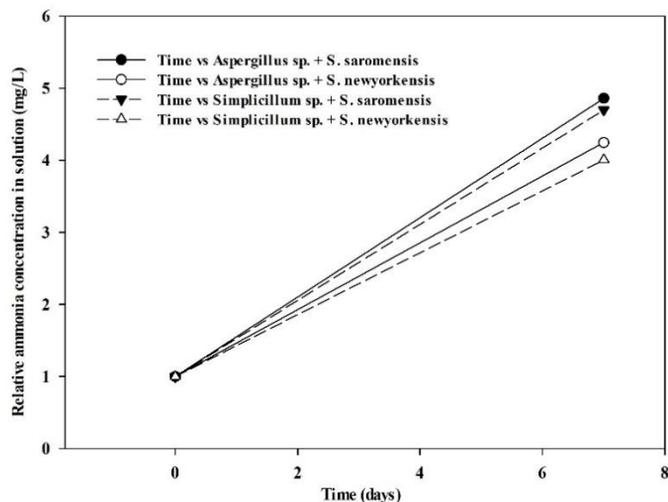
Figure 7. Relative ammonia concentration in solution for systems previously treated with fungal bioextractants in contact with *Sporosarcina sp.*
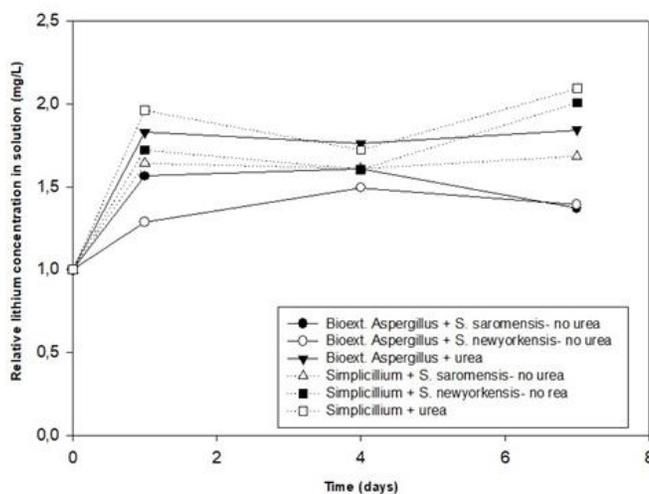

Figure 8. Relative lithium concentration in solution for control systems.

These set of experiments could be held only for 7 days before contamination was evidenced. According to different performances through Step 1 and 2, global yields are summarized in table 1:

Table 1. Lithium leaching and recovery yields at different steps and global

System	Step 1- Leaching % mg/g	pH Step 1	Step 2- Recovery % mg/g	pH Step 2	Global yield % mg/g
Commercial H_2SO_4	45.2	1.5	N. A[3]	N. A	N. A
Commercial H_2SO_4	18.9	2.5	N. A	N. A	N. A
Biogenic H_2SO_4	32.2	1.5	N. D	5.5[4]	N. D
Aspergillus sp. bioextractant	68.26	2.5	40 %[1]	5.5	27.30
Simplicillium bioextractant	24.34	2.5	40 %	5.5	8.54

[1]*As no differences between S. newyorkensis and S. saromensis were observed, this recovery value is an average.*

[2] *Commercial sulfuric acid solutions were tested at the same pH of the biogenic acid set of experiments.*

[3] *Abbreviations: Not Assayed (N. A); Not Detected (N. D).*

[4] *Average values between sets of experiments. pH value did not change during the experiment.*

Discussion

For lithium extraction (Step 1) biogenic sulfuric acid produced by sulfur oxidizing bacteria and biogenic organic acids from fungi were evaluated and compared with commercial sulfuric acid extraction yields.

Results showed comparable leaching yields of both bio-products compared with commercial acid (table 1). Even more, extraction with fungal bioproducts gave higher recovery yields, reaching a 68.26% for *Aspergillus sp*. in contrast to a 45% for commercial sulfuric acid, at a pH value of 2.5 (Figure 3). These results are lower of that obtained with commercial organic acid leaching – 90% with citric acid at high temperature filtration (Gao, X. 2019). However, they appear as promising and feasible to be improved by exploring more variables in future studies as: mass: volume ratios, contact time, batch or semi continuous (recirculation) process.

Between previously reported biological processes, Boxall (2018[1]) obtained around 50% of lithium extraction using biogenic compounds from *Acidithiobacillus ferrooxidans*, improved to 70% when 10mM H_2SO_4 was added, after 4 steps of 1 hour of contact each. Considering the results of this manuscript, *Aspergillus sp*. biogenic compounds appear to be an advantageous option, as similar yields can be achieved in similar time, without commercial acid manipulation.

Based on general information of the genus *Aspergillus* (Samson, 2019) the diversity of compounds segregated under different culturing conditions open the possibility of existence of coordination phenomena added to acid- base equilibrium. Siderophores, carboxylic acids, quinolones, between others are part of an extensive list of possible metal chelating agents that can be involved and act synergically in lithium extraction (Buonomo, 2022, Frisvad, 2015, Kocsubé, 2016). Full characterization of the chemical profile of the strains is an aspect that exceeded the main initial objectives but is being considered to predict chemical interactions and kinetics to improve the hole extraction process.

For lithium precipitation and recovery from solution (Step 2), two *Sporosarcina sp*. strains were examined (*S. newyorkensis* and *S. saromensis*), with very similar interaction with lithium depletion from solution.

However, the identity of the bio-extractant used at Step 1 did influence the activity of *Sporosarcina* at step 2. As can be seen in figure 4, systems where biogenic sulfuric acid was used on Step 1 for lithium leaching, did not lead to any *Sporosarcina* activity on Step 2, i.e. no lithium harvesting was measured. On the other hand, lithium leaching using fungal bio- extractants in Step 1, did lead to considerable lithium depletion in the following step with MICP bacteria, as is shown in Figure 6. Global yield for the configuration *Aspergillus sp*. bioextract – *Sporosarcina sp*. reached 27.3%, comparable to previously reported multi- stage chemical processes (Boxall, 2018[2]).

For the observed differences in *Sporosarcina sp*. development with fungal bioextractans over biogenic sulfuric acid, bulk buffered pH and molecular identity of the matrix can be two distinctive aspects that promote/ prevent cellular activity, giving the conditions for urease activity.

The absence of lithium carbonate crystals precipitation may be due to the lack of enough alkalinization or to concentrations that still do not acieve its Ksp values. If chelating agents are present in solution, thus they may be responsible for the increased lithium solubility. Additionally, acidic to neutral bulk pH does not favor any $LiCO_3$ precipitation (highest pH achieved at step 2 was 5.5 and carbonate salts precipitation leads to a pH value near 8.00), even though lithium and carbonate presence was evidenced in filtered systems. These process variables in Step 1, as well as chemical equilibriums with other metals, need further studies for improving the bio-mediated lithium recovery.

Even though all experiments and sampling were done in sterile conditions, experiments coming from fungal bioextractant leaching at Step 1, showed a lower capacity to remain without contamination beyond day 7. For systems coming from biogenic sulfuric acid at step 1, this occurred at day 14. The fact that systems showed contamination, even manipulated in sterile conditions, can be attributed to an inefficient sterilization process onto the LiBs fraction. The selected method (UV exposure) was preferred among others available at the institute facilities (i.e. heat sterilization) to avoid any physical or chemical change due to this treatment. Even though at the laboratory scale sterile conditions are crucial to follow and characterize *Sporosarcinas* activity, for industrial scale this specification is impossible to achieve. Thus, future research must be done to design a system where *Sporosarcina* activity is not affected by other biological contaminants.

Conclusions

Basis of a completely bio-mediated process for lithium recovery from spent batteries was stablish, consisting on two steps: one of indirect bioleaching, and the second of bio- precipitation.

The best performance for lithium recovery from solution was observed when combining *Aspergillus sp.* bioextractant for step 1 and any *Sporosarcina sp*. strain for step 2, leading to a global yield of 27.3%, comparable with previously reported multi-step chemical processes.

Further studies on *Aspergillus* chemical profile characterization, bulk conditions control and equilibria between generated compounds, lithium, and other metals are needed to improve the process for a potential scaling up.

Acknowledgments

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