Lithium battery renewal: co-culture using *Aspergillus niger* and *Arthrobacter nicotianae*

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Each year consumers dispose of billions of batteries containing toxic and corrosive materials. Some of these batteries contain lithium, a hazardous waste, that is harmful to the environment if not disposed of properly. The product proposed herein will extract the lithium from the batteries to be reused by taking a fungus that uses citric acid to release lithium from the battery and using bacteria to absorb the lithium, which will then be recycled. Bioleaching, using the fungus *Aspergillus niger*, will extract metals from waste using microorganisms to oxidize the metals. *Arthrobacter nicotianae* is a bacteria that absorbs the most amount of lithium, making it a practical approach to the fungus-bacterium co-culture. This system will help extract the lithium to be reused, rather than being thrown away. Bioleaching begins with metabolites, which dissolve the lithium by displacing the lithium ion from the battery with hydrogen ions or by the formation of soluble metal complexes and chelates. Lithium is then displaced and released from the battery. Co-culture is initiated through placing two populations of cells, in this case bacteria and fungi with a specific amount of contact between both. Segregated co-culture creates a physical barrier. Extracted lithium passes through the porous membrane. Adsorbed lithium is desorbed with citric acid using a segregated 2D co-culture system. Cells immobilized with polyacrylamide gel can be used again. Therefore, the lithium will be separated from the bacteria resulting in pure lithium for reuse.

Keywords: lithium, co-culture, *Aspergillus niger*, *Arthrobacter nicotianae*, bioleaching

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Background

Increasing demand for raw materials, such as lithium, is a result of an emerging mass market in electric cars, as well as any electronics which require lithium-ion batteries to function. Batteries which contain lithium are harmful to the environment if not disposed of properly. The reason batteries are not being repurposed regularly is the costly nature and limited regulation of the process. By employing battery renewal as a solution to the indirect polluting by electric cars, one can save raw materials that are ever more in demand while also saving the ailing planet.

The present means of extracting valuable materials from batteries through hydrometallurgical and pyrometallurgical renewal has a greater impact on climate change than landfill use owing to the copious amount of energy used in transportation and recycling (Swain 2017). Safety and financial complications have led to scientific research looking for a more natural way to recycle lithium. We propose a product that will extract the lithium from the batteries to be reused, by taking a fungus that uses citric acid to release lithium from the battery and then using a bacterium to absorb the lithium to be recycled. Our system uses a co-culture which is a cell cultivation set up that involves two or more populations of cells growing with a specific amount of contact between them. This method allows a variety of cells to be placed in close proximity or direct contact for observation. Our system would be more eco-friendly and perhaps more localized compared to current methods. This is better because it could avoid transportation between the site of the lithium to an outside source leading to fewer pollutants. The use of bacteria and fungi in the co-culture method would essentially be a more natural and safe means of lithium extraction and recycling.

Systems level

Our system uses a co-culture, allowing different cells to be placed in close proximity in order to examine one of the cell culture systems besides the other system of cells. One method of co-culture, known as mixed co-culture, allows for unrestricted contact between cells. Another method, known as segregated co-culture, controls the cell contact by establishing physical barriers, which allows for regulation of the space and temperature of the cell seeding patterns in the co-culture. This process of segregated co-culturing begins with the introduction of conditioned media from the fungus *A. niger* into the culture of the bacterium *A. nicotianae* (Bogdanowicz and Lu 2013). The benefit of the segregated method is that contamination between the fungus and bacterium is minimized as well as foreseeable competition for resources. Due to this advantage, this design will employ a segregated co-culture.

Device level

The fungal bioleaching begins with the powdered lithium-ion battery provided by a battery recycling plant and pre-culture which converts an insoluble metal compound into a water-soluble form or leach liquor (Figure 1). The main mechanisms involved in this process are acidolysis, complexolysis, redoxolysis, and bioaccumulation (Asghari, Mousavi, Amiri, et al. 2013). The first two processes concentrate on metal dissolution, in this case, dissolution of lithium. Acidolysis refers to the replacement of hydrogen ions with metal ions allowing for the formation of a second complex (Faraji, Golmohammadzadeh, Rashchi, et al. 2018). Complexolysis or ligand-induced metal solubilization stabilizes the metal ions. This is a natural reaction of microorganisms to limit incoming toxic metal ions. During redoxolysis, the oxidation-reaction assists the fungal leaching by producing a lithium ion (Horeh, Mousavi and Shojaosadati 2016) and slightly increasing the metal mobility. The metal solubility is made possible by highly oxidized metal compounds from enzymatic reduction (Asghari, Mousavi, Amiri, et al. 2013). Bioaccumulation occurs when the now soluble metals pass through the membrane and disturb the equilibrium allowing for the constant solubility of the metals. This mechanism may be the reason for the effectiveness (higher yield) of bioleaching compared to chemical leaching, for example.

By employing bioaccumulation, the fungus seems to play a direct role in the leaching process proving that it is not simply a chemical reaction but a catalyst, a substance which propels a chemical reaction without itself undergoing a permanent chemical change (Asghari, Mousavi, Amiri, et al. 2013). The percent yield from the lithium-ion battery after fungal bioleaching using

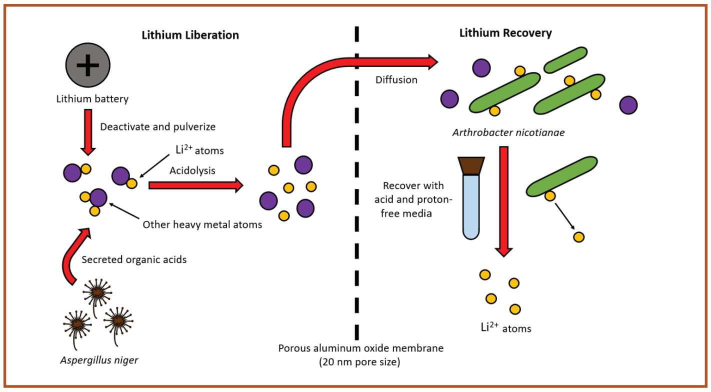

Figure 1

Aspergillus niger should equal around 100% Cu, 95% Li, 70% Mn, 65% Al, 45% Co, 38% Ni (Horeh, Mousavi and Shojaosadati 2016). Citric acid is the most prominent lixiviant, allowing for the extraction of the lithium. The initial reaction for lithium in the system is:

$$2Li+_{(aq)} + Na_2CO_3 \rightarrow Li_2CO_{3(8)} + 2Na^+_{(aq)}.$$

Segregated co-culture (Figure 1) begins with the inoculation of conditioned media — which includes necessary metabolites, matrix proteins, and growth factors — (Freshney 2010) into the culture of the bacterium *A. nicotianae* to determine soluble factor effects (Bogdanowicz and Lu 2013). A porous membrane is created closely following Chung's protocol and considering a controlled pore size (nanoporous), porosity, and membrane thickness (<1 μm) to ensure effective permeability without compromising the membrane's mechanical strength (Chung, Mireles, Kwarta, et al. 2018). Specifically, a porous aluminum oxide membrane (25 mm diameter, pore size on outer surface 20 nm, porosity 30%, from SPI Supplies, United States) will be used (Thøgersen, Melchiorsen, Ingham, et al. 2018). A 3D-layered hydrogel system could be made, creating an artificial matrix to mimic the native environment of the cells (Kombe, Vielle and Casquillas 2020). However, this is a relatively recent method so the team would attempt a 2D co-culture system which has the advantage of simpler recovery of cultured cells. The conditioned media biochemically communicates with *A. nicotianae* allowing for limited contamination, as mentioned above, with a <1 nm pore size and a thin membrane (Chung, Mireles, Kwarta, et al. 2018). Independently of the co-culture system, *A. nicotianae* cells are immobilized with polyacrylamide gel adsorb cells to surface (Tsuruta 2005). This is a gram-positive bacterium meaning that the cell wall contains teichoic acids. Perhaps dependently in the co-culture system, the ionized phosphate groups present in the bacteria of the teichoic acid surround the Li2CO3(8) and replace the carbonates, creating a chelate with 2Li+. Cells would then be treated with a strong acid or a combination such as an acidic medium (H2SO4/H2O) and proton-free medium (Br2/CH3CN) solvent to recover lithium ions from the output solution (Swain 2017).

Parts level

According to NCBI GenBank, the internal transcribed spacer (ITS) sequence for A. niger strain MM1 is MH091025 and MH091026 for strain SG1 (Biswal, Jadhav, Madhaiyan, et al. 2018). *A. nige*r MM1/SG1 cultures are currently the only strains tested in this context and are used quite interchangeably. See NCBI for further genetic information. The strain IAM12342 of *A. nicotianae* would be used as it exhibited high lithium accumulating ability (Tsuruta 2005).

Safety

A. niger has been reviewed by the FDA and is considered generally recognized as safe (GRAS). *A. nicotianae* is also considered safe. To ensure maximum safety, one should wear gloves, protective eyewear, and a mask. One should also use sterile technique to ensure no contamination occurs during the experiment. Hazards in transporting the lithium-ion batteries include contact with corrosive materials, heat, and the possibility of thermal runaway — the rupturing of the battery's cell casing leading to a release of toxic gases.

Discussions

Although the team has not performed any tests to see if the mechanics of the co-culture works, this fungus-bacteria system should effectually output pure lithium with an input of a powdered lithium-ion battery.

One of the team's main concerns is about cohabitation between fungi and bacteria such as contamination and competition for resources. This could possibly be solved by employing segregated co-culture as a means of maintaining separation between these two types of cells.

Other concerns to be evaluated would include the levels of acidity due to the citric acid production which could kill the bacteria. *A. nicotianae*, for example, can tolerate a pH only as low as 6; this is well above the pH level for the citric acid that the fungus releases (~ 3-5 pH). A possible follow up experiment would be trying different strains of fungus such as Penicillium simplicissimum and bacteria such as Brevibacterium helovolum and Bacillus licheniformis. These bacteria have demonstrated to recover the second and third largest amount of lithium (Tsuruta 2005). Penicillium simplicissimum is another choice for the fungi because it also produces high amounts of citric acid which would help extract the lithium from the battery (Franz, Burgstaller and Schinner 1991). This fungus produces penicillic acid however, which is a mycotoxin, dangerous to animals and humans. The team also has concerns about *A. nicotianae*, since it has been shown that this bacterium cannot be reused (Tsuruta, Burgstaller and Schinner 2005). Lastly, the team has a potential problem in trying to recover the lithium from the bacterium once the processes are done without having bacterial or fungal residue as well as contamination from other extracted metals.

The team intends to go further into researching and testing the co-culture of the fungus and bacterium to see if they can work together in a co-culture system. This device will help explore if it is possible to effectively extract and collect the lithium from the battery. Additionally, the team would also like to test two other bacteria to help create the most effective product. Brevibacterium helovolum is a bacterium that is a coryneform, which is isolated from milk and dairy products and are known colonizers of human skin. This bacterium would be beneficial because it was one of the top bacteria to draw in the lithium. The second bacterium the team has been researching is Bacillus licheniformis, this may be beneficial to the project because it is very safe for humans and is even used as a probiotic (Ul Hassan, Al Thani, Alnaimi, et al. 2019).

The process scalability, if successful, could be large considering the comparatively low cost of culturing the cells, which are also quite accessible. However, the culturing of the cells could take over a month. By employing a more natural process of lithium-ion battery renewal, this slower method of fungus-bacterium co-culture will be overshadowed by the lithium output being purer and an overall less polluting process

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