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Mining e-waste and coal ash in landfills for rare earth elements using bioleaching methods:

Analysis of bioleaching data

A thesis submitted in partial satisfaction of the
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in Civil and Environmental Engineering

by

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ABSTRACT OF THE THESIS

Mining e-waste and coal ash in landfills for rare earth elements using bioleaching methods:

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Master of Science in Civil and Environmental Engineering

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Professor Sanjay K. Mohanty, Chair

Bioleaching is an economical process to extract rare earth elements, which requires less energy and produces less toxic gases than chemical extraction methods. However, the extraction efficiency varies with different parameters. Bioleaching mechanisms, as well as factors controlling the process, were comprehensively analyzed in this study. The quantitative analysis will help optimize bioleaching process parameters for the high co-extraction of metals. Analyzing 61 peer-reviewed articles on bioleaching, we identified three main factors that affect the bioleaching mechanism — microorganism type, source type, and specific REE. Extraction efficiency varies between 0 to 100% based on the condition used in the study. Among different types of microorganisms used to extract REE, fungi were found to have a competitive advantage over other microorganisms such as chemolithoautotrophs or heterotrophs. Primary and secondary

sources of REE are typically used for extraction. Among them, electronic waste leaches more REE per unit mass of source reacted, irrespective of the microorganism type. Extraction efficiencies of transition metals were higher than those of REEs.

The thesis of Samiha Karim is approved.

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Table of Contents

List of Figures	vi
List of Tables	vii
List of Acronyms	viii
Acknowledgments.....	ix
1. Introduction.....	1
2. Bio-extraction of REE.....	4
2.1. Microorganisms capable of leaching REE.....	4
2.2. Mechanisms of REE extraction:	6
2.2.1. Mobilization of REEs from solid phase	7
2.2.2. Immobilization of REEs from liquid phase.....	9
2.3. Methods of REE extraction.....	11
2.4. Parameters influencing REE bioleaching	12
3. Data Collection	14
4. Data Analysis	21
4.1. Effect of microorganism type on REE leaching	21
4.2. Extraction efficiencies in a mixture of REEs with transition metals.....	24
4.3. Variation of efficiency with source type.....	28
5. Challenges and Opportunities	32
6. Conclusions.....	34
7. References.....	35

List of Figures

Figure No.		Page
1.	REE sources and metal-microbe interaction.....	6
2.	REE mobilization and immobilization techniques	7
3.	REE extraction efficiency varies with microorganism type: fungi, autotrophic, and chemolithotrophic. Fungi appear to have higher efficiency than heterotrophic, and chemolithotrophic microorganisms. There are 196 total data points on this graph gathered.....	22
4.	REE extraction efficiencies of 6 different microorganism genera. Acidithiobacillus appears to be most effective, and Acetobacter is least effective. There are 158 total data points on this graph gathered from 43 reference papers. Under each genus is written the number of data points that contribute to the corresponding box plot.	23
5.	Extraction efficiencies of various metals, categorized by material group (HREE, LREE, transition metals). Transitional metals have higher leaching efficiency than REEs. There are 115 total data points on this graph, gathered from 35 reference papers. Under each type of metal is written the number of data points that contribute to the corresponding box plot.	26
6.	REE bioleaching levels are lower than their non-REE counterparts. There is a total of 92 data points, gathered from 28 reference papers. Under each metal is written the number of data points that contribute to the corresponding box plot.	27
7.	REE extraction efficiencies from 5 different groups of sources. Leaching of REE is maximum for WEEE and lowest in multi-metallic solutions. There are 218 total data points on this graph gathered from 61 reference papers.	29
8.	REE extraction efficiencies of secondary sources by different groups of microorganisms. There are 218 total data points on this graph gathered from 61 articles.	30

List of Tables

Table No.	Page
1. List of studies that examined bioleaching of REE.....	16

List of Acronyms

REE	Rare earth element
HREE	Heavy rare earth element
LREE	Light rare earth element
EPS	Extracellular Polymeric Substances

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1. Introduction

Seventeen metals are classified as rare earth elements. They include scandium (Sc), yttrium (Y), and 15 lanthanides: lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu) (Corbett et al., 2018). A rare earth element can be categorized into two groups based on its atomic weight: a heavy rare earth element (HREE) or a light rare earth element (LREE). HREEs consist of Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, and Y; and LREEs consist of La, Ce, Pr, Nd, Pm, and Sm. Both groups have different characteristics. The LREEs have lower nuclear charge numbers, lower atomic mass numbers, larger ionic radius, higher and alkalinity. In contrast, HREEs have higher nuclear charge numbers and masses but a smaller ionic radius and lower alkalinity (Fathollahzadeh et al., 2019).

There are multiple sources from where REEs are extracted. The earth's crust contains a large quantity of rare earth elements, but they are rarely concentrated into ore deposits that can be mined (Balaram, 2019). They are mostly found as minor components in a variety of ores such as monazite, xenomite, bastnaesite, loparite, ion-adsorption clays, and gadolinite (Brisson et al., 2016). REEs also exist in a variety of secondary sources and wastes (Gaustad et al., 2021). The REEs are crucial raw materials for a wide range of technological applications such as cell phones, fluorescent lamps, electric vehicles, computer hard drives, and essential raw materials for the defense industry. The demand for REEs is continuously increasing because of their numerous chemical, catalytic, X-ray scattering, electrical, magnetic, and optical properties (Y. Chen & Zheng, 2019). At present, China has almost 60% of total REE available globally,

whereas the USA owns only 12% of them. Also, India, Australia, Russia, and Brazil are among the top supplier (Barmettler et al., 2016; Dev et al., 2020). Therefore, REE supply in the USA is limited, which is a primary concern for the industry. To keep up with the current demand for REEs, secondary sources such as electronic waste and fly ash are being explored alongside primary sources like mineral ore (Zhang et al., 2020). Several chemical and physical strategies such as acid leaching, electroslag refining, liquid media extraction, the glass slag method, direct melting, and gas-phase extraction have been adopted for extracting REEs from source materials. However, these methods require very high temperatures, and a large number of harsh chemicals and yield large amounts of toxic waste as a byproduct. Hence, these approaches are not sustainable in the long run.

As an environmentally conscious solution, biological approaches could supplement or replace the current extraction methods at ambient temperatures while utilizing biodegradable and aqueous chemicals. The major advantage of bioleaching over chemical leaching is that the necessary reagents for metal mobilization are biologically produced and there is no need for continuous delivery to the plant; this implies obvious advantages both for the process economics and environmental impact in terms of carbon emissions (Beolchini et al., 2012) Bioleaching is one of the emerging biological technologies that facilitate REE extraction by using different microorganisms. It has a higher metal specificity and better efficiency of extraction.

The extensive use of REEs in different technological industries has made it very important to investigate efficient and environmentally friendly extraction methods. Hence, understanding different aspects of the biological leaching process has great significance. Despite the importance of bioleaching, no review to date has analyzed its optimal REE extraction

conditions. The analysis aims to answer the following questions: (1) What microorganism groups are currently in use for REE extraction? (2) What physical factors affect the total leaching process? (3) What is the efficiency of a particular microorganism in leaching REEs? (4) What type of metal is being leached? and (5) What is the primary or secondary source material for the experiment? This review aims to provide an understanding of the possible application of microorganisms in the extraction of REEs from various sources as well as fundamental knowledge of how REEs interact with microorganisms and how they mobilize REEs. Also, we analyzed the effects of source material, microorganisms, and rare earth element types based on data synthesis. The results demonstrate help to understand the uncertainty factor during leaching and try to point toward optimal leaching conditions.

2. Bio-extraction of REE

2.1. Microorganisms capable of leaching REE

Microorganisms can extract metal cations, including REEs, from different sources. Microbes produce organic acids, proteins or metal-binding compounds to dissolve the metallic elements in minerals or waste (Gavrilescu, 2022). The microbial cell must first come into contact with the source material and then get attached to the surface (Figure 1). Microorganisms, when stuck on the mineral surfaces, form the exopolysaccharides (EPS) layer. In this EPS layer, bio-oxidation reaction takes place and therefore EPS serves as reaction space (Yaashikaa et al., 2022). Once microbes are attached, they secrete organic acids. Negatively charged organic acids produced by the microbial cell bind with positively charged metal ions. Organic acids provide protons and ligands that dissolve metals (Jadhav et al., 2016). The rate of metal leaching depends on acid production (Li et al., 2021).

Based on the current literature, REE extracting microorganisms are divided into three major groups such as (i) chemolithotrophic bacteria, (ii) heterotrophic bacteria, and (iii) fungi (Abhilash et al., 2021; Balaram, 2019; Dev et al., 2020; Işıldar et al., 2019; Rasoulnia, Barthen, & Lakaniemi, 2021; Srichandan et al., 2019). Few factors were taken into account during their classification such as O₂ source and requirement, acidity, and type of nutrition necessary for growth (Coram & Rawlings, 2002; Plumb et al., 2008; Rohwerder et al., 2003). In chemolithotrophic bacteria, CO₂ is used as a carbon source, and it is capable of oxidizing iron or sulfur present in the mineral. Sulfuric acid and ferric iron are produced due to oxidization. This leads to the dissolution of sulfidic minerals (Mowafy, 2020; Rasoulnia, Barthen, & Lakaniemi, 2021). In most cases, heterotrophic microorganisms are used when a source contains carbonates, oxides, and silicates. REE can be bioleached without sulfur or iron or maintain a high pH when

this group of microorganisms is used. (Mowafy, 2020, Das & Das, 2013). Heterotrophic microorganisms rely mainly on organic carbon sources such as glucose, and sucrose. While fungi are also heterotrophic organisms, they are evaluated separately due to their significant physiological difference from bacteria and distinct mechanisms in leaching REE (Işıldar et al., 2019). When heterotrophic bacteria and fungi use glucose as a carbon source for growth, they produce a variety of metabolites, including organic acids, carbohydrates, and proteins. Comparatively to chemolithotrophic microorganisms, heterotrophs in bioleaching might seem more viable at first because they produce higher outcomes, regardless of the need for organic carbon sources constantly. (J Abdullah et al., 2017). Microorganisms are chosen based on the source material or the type of metal to be leached (Dev et al., 2020; Li et al., 2021).

Based on the current literature, it was evident that there is no mention of what type of microorganism has advantages to extract REE. Different strain types were used in different experiments based on the source or process performed, or the abundance and availability of the strain. By improving and modifying the bioengineered systems, any type of microorganism can selectively leach REE (Park et al., 2017).

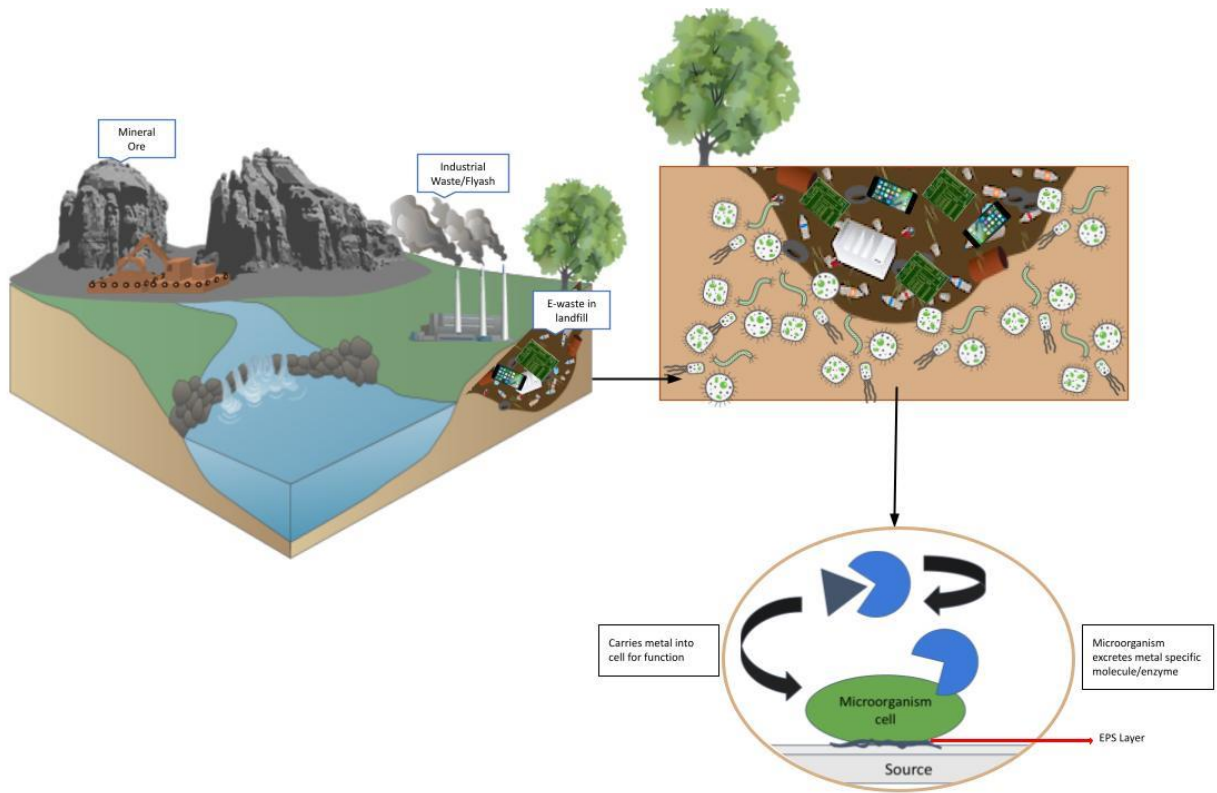


Figure 1: REE sources and metal-microbe interaction

2.2. Mechanisms of REE extraction

The dynamics of metal ion mobilization and immobilization control the REE extraction process. Mobilization of metals occurs through microbial solubilization from solid matrices, such as ores, solid waste like electronic waste, spent catalysts, and others (Gavrilescu, 2022). The mobility of metals can be influenced by biofilm formation and the presence of extracellular polymeric substances (EPS) such as polysaccharides and proteins. Metal forms and the chemical composition of source materials determine the impact of mobilization. Mobilization of metals can occur through leaching mechanisms, complexation with metabolites and siderophores, or volatilization because of methylation. On the other hand, the immobilization process controls the microbial cells in a particulate form in order to make them an ideal adsorbent for REEs.

Microbial cells can be adsorbed or embedded into materials by various methods. Immobilization can be achieved by sorption of solubilized metal ions to biomass or exopolymers of microorganisms, or transport and precipitation as organic and inorganic compounds (Jing & Kjellerup, 2018). The balance between mobilization and immobilization is determined by the organisms, their environment, and the physicochemical conditions.

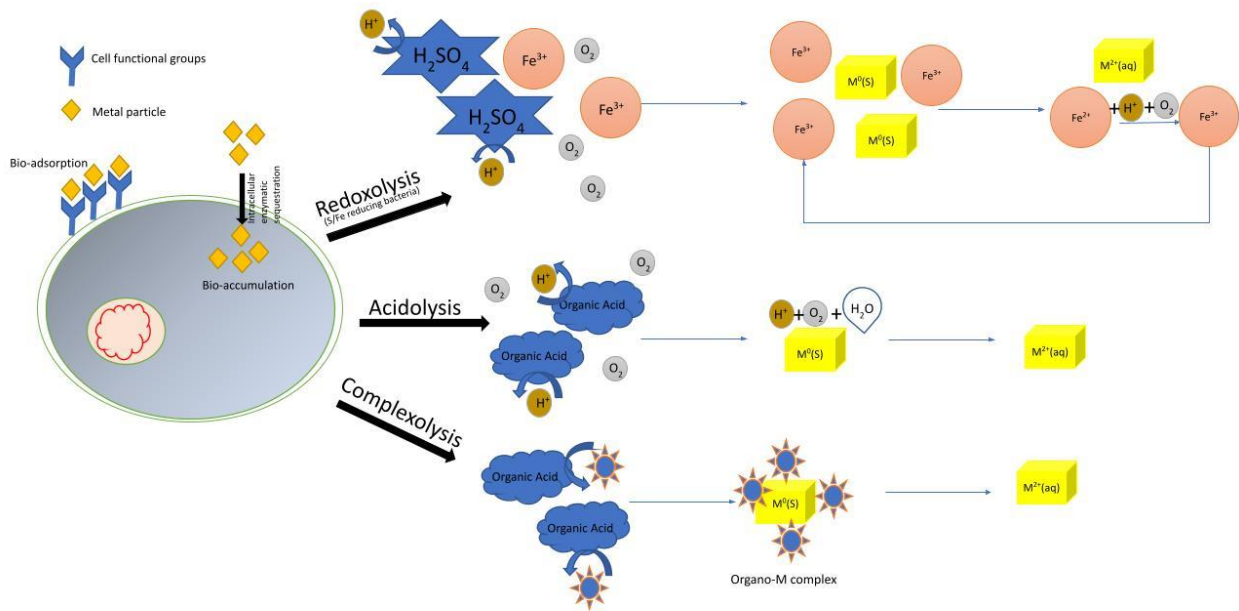


Figure 2: REE mobilization and immobilization techniques

2.2.1. Mobilization of REEs from solid phase

The mobilization of REEs from the solid to the aqueous phase involves three mechanisms: acidolysis, redoxolysis, and complexolysis (Figure2) (Barnettler et al., 2016; Dev et al., 2020; Nancaraiah et al., 2016).

Acidolysis refers to the replacement of minerals by protons. In acidolysis, microorganisms produce organic acids such as acetic, citric, formic, gluconic, and pyruvic acids

or inorganic acids such as sulphuric acid. (Gavrilescu, 2022). The oxygen atoms on the surface of the metal oxide get protonated by the acid produced by the microorganism. Protons attach and react to the source surface by reducing the strength of bonds. Metal solubilization occurs when protonated oxygen atoms can interact with water and promotes the release of the metal compound in the solution. (Desmarais et al., 2020). Inorganic acids such as sulphuric acid (H_2SO_4) which can be formed by sulfur-oxidizing bacteria from externally added sulphate compounds such as ferric sulphate and ferrous sulphate can also similarly protonate the metal surface coating, although the compound does not fully originate from microorganisms (Desmarais et al., 2020). The Acidolysis mechanism works for both autotrophic microorganisms and heterotrophic fungal and bacterial cells. This is the most used technique to leach REE from electronic waste.

Redoxolysis refers to the transport of electrons from metals to microbes through oxidation and reduction reactions (Dev et al., 2020). The metal compounds are oxidized by an oxidizing agent and thus become water-soluble. There are several redoxolysis agents used in leaching systems. A common example is Fe^{3+} . Besides, KMnO_4 , dissolved oxygen can also be a potent oxidizing agent depending on the environment and source material. It is produced by iron oxidizers. Most iron reduction is carried out by specialized acid generation bacteria that use Fe^{3+} as a terminal electron acceptor (D. Mishra & Rhee, 2014). It is a two-step process where initial electron transfer happens by oxidation of metal sulfides by Fe^{3+} , which then mobilizes REEs from the solid to the aqueous phase. In the process, Fe^{3+} is reduced to ferrous iron (Fe^{2+}) which is then recycled or oxidized back to Fe^{3+} . It is the most common process to use for mineral ore containing sulfur and iron. It can still be used for electronic waste. Generally, ferrous sulfate and

elemental sulfur are added to e-waste as they are devoid of any energy source (S. Mishra et al., 2021)

In complexolysis, the metal solubilization takes place by a ligand-induced and chelate formation mechanism. Acetate, formate, gluconate, citrate, oxalate, malate, and succinate are some of the naturally occurring organic ligands secreted by microorganisms, and they play a crucial role in the mobility of REEs. Microorganisms do not directly interact with the elements, but instead produce metal-complexing anions or chelating agents with organic acids that increase the metal solubility (S. Mishra et al., 2022). The solubilization of metal ions depends on the complexing capacity of the ligands (Li et al., 2021). If metal ions bond strongly to ligands instead of solid particles, the metal can be leached from the solid particles (Gopikrishnan et al., 2020). The acidolysis mechanism complements complexolysis through the stabilization of metal ions produced during acidolysis (Dusengemungu et al., 2021). Mostly Fungi and cyanogenic bacteria leach REE using this mechanism.

2.2.2. Immobilization of REEs from liquid phase

Immobilization techniques to recover REEs from the aqueous phase involve bioadsorption, bioaccumulation and bio precipitation techniques (Figure 2) (Castro et al., 2020; Nancharaiah et al., 2016).

Bioadsorption is considered a separation method for recovering REEs and is based on the adhesion of REE molecules onto microbial cells due to surface forces. Both living and non-living cells of microorganisms can be used for the biosorption of REEs (Gavrilescu, 2022). Metabolic activities are the primary factor when living organisms are used to extract REEs. In case of, nonliving cells metal ions retain through precipitation, ion exchange, van der waals forces,

electrostatic interactions, complexation, or by a combination of several of these processes (Rasoulnia et al., 2021a). During living cell adsorption, positively charged metal ions are separated from the liquid phase by the negatively charged cell membranes and polysaccharides secreted on the surface of bacteria (Prakash et al., 2013). For non-living bioadsorption, several functional groups such as —COOH , —NH_2 , and HPO_4^{2-} bind metal cations to the microbe surface through electrostatic interactions (Yu et al., 2020; Zhuang et al., 2015). Functional group —PO_4^{3-} shows specificity toward Gd, —COOH is specific for Yb, Er, and Sm. (Dev et al., 2020). The light lanthanides such as La and Nd show affinity towards —PO_4^{3-} , whereas middle (Sm and Gd) and heavy (Er and Yb) lanthanides show similar specificity toward both —COOH and —PO_4^{3-} functional groups (Ngwenya et al., 2009). The selectivity is determined mainly by pH, molecular weight, and REE concentrations (Chang et al., 2020). LREEs adsorption is significant at pH of more than 4, whereas HREEs adsorption is significant at a pH of less than 4 (Hosomomi et al., 2013). Increasing the density of functional groups would increase the adsorption capacity (Yu et al., 2020).

Bioaccumulation involves the intracellular uptake of REEs by living biomass. It is a two-step process. The REEs are first adsorbed on the cell surface, then translocated into intracellular spaces by the importer complex in the membrane lipid bilayer (Maleke et al., 2019). Microbial cells are capable of undergoing biochemical reactions between metals and organic acids. Several factors control the rate of bioaccumulation such as the metal's intrinsic structure, adaptations to genetics and physiological processes, environmental modification as well as the metal's availability and toxicity. Additionally, metal accumulation is affected by cell density. Highly dense cells in suspension are more likely to link up, reducing the number of metal-binding active sites (Timková et al., 2018).

Microorganism-mediated precipitation is employed to recover dissolved REEs as phosphate (Liang & Gadd, 2017). The extracellular polymeric matrix of a microorganism contains enzyme phosphate, and when this enzyme is supplied with enough organic phosphate, it secretes inorganic phosphate. Metal ions are attracted to the liberated phosphate and precipitation ensues (Feng et al., 2011).

2.3. Methods of REE extraction

Bioleaching method can be classified into two groups depending on whether microorganisms come in contact with the source material- contact bioleaching and non-contact or spent medium bioleaching. Contact bioleaching can occur by one-step and two-step processes depending on microbial growth conditions (Barnett et al., 2018; Qu & Lian, 2013; L. Zhang et al., 2018). In the contact bioleaching process, the attached microorganisms produce extracellular, macromolecular compounds that act as oxidation reaction sites (Jia et al., 2019; Tao & Dongwei, 2014). For the contact mechanisms, the production of EPS is very essential because it fills up the void space between the microbial cell wall and the surface of the source material (Gopikrishnan et al., 2020). In one step contact method, when microorganisms begin to grow, they are combined with an REE source material in the same medium. During two-step contact bioleaching, the microbial culture grows in a separate vessel, followed by the addition of REE source material to previously cultivated microorganisms. In non-contact bioleaching, it is first ensured that the microorganisms are given proper nutrition for their optimal growth in absence of the REE source material in order to produce as much leaching agent as possible. Then the material containing REEs is mixed with the cell-free medium containing only biologically produced leaching agents. There are advantages and disadvantages to each of these methods. For

example, contact bioleaching prevent the microbial growth and metabolic activity due to the toxicity of the extracted metals and other toxic compounds present in REE-containing materials. Using non-contact bioleaching, it is possible to separately optimize the growth of the microorganisms as well as the steps involved in leaching REE, regardless of the toxicity of the leaching material (Hassanien et al., 2014).

2.4. Parameters influencing REE bioleaching

REE bioleaching is a complex process that is often controlled by both biotic and abiotic factors. Characteristics and type of microorganisms used are the primary biotic factors, whereas abiotic factors include aeration, temperature, growth environment, pulp density, pH, redox potential, and metal toxicity (Dev et al., 2020). These factors affect the full REE biorecovery process either individually or simultaneously (Choi et al., 2004; Yaashikaa et al., 2022; Yang et al., 2009).

For any microorganism, the ideal temperature is a critical parameter as microbial growth, kinetics, and metabolic activity all depend on this. Temperatures between 25°C and 75°C are ideal for microorganisms to grow and metabolically perform (Arya & Kumar, 2020). Various microbial groups exhibit varying mineral oxidation properties based on their optimum growth temperature. Adsorption is enhanced at high temperatures due to the high kinetic energy and surface activity possible (Lhamo & Mahanty, 2022). Another crucial factor affecting bioleaching efficiency is pulp density. There is a direct correlation between pulp density and metal extraction. A higher pulp density increases the chances of metal recovery. There is a point at which pulp density inhibits microbial populations and can cause metal protein complexes to form that inactivate specific enzymes in the microbial population (Kumar et al., 2018). In general, the

optimal pulp density is within the range of 10 and 15 mg/l. The size, quantity, and concentration of metals in the waste also influence microbial growth and efficiency. Furthermore, a balance should be maintained between the liquid growth media and the source material to govern the bioleaching process. Microorganisms' ability to grow and extract metals from the source material is highly dependent on the pH. Optimum bacterial growth occurs at physiological pH range of 1.0 to 4.0 pH and thus shows high-efficiency metal solubilization (Arya & Kumar, 2020). Considering microorganisms have different optimum growth pHs, selecting the appropriate microorganisms should also consider the type of target metal. EPS production by microorganisms is also influenced by pH. Highly acidic pH results in the production of high EPS content, resulting in a stronger attachment of microorganisms to the mineral surface and an improved REE recovery (Dev et al., 2020). Even with all of the above factors in play, the nutrient for bacterial growth plays a crucial role in the overall population of microbes. The optimization of parametric conditions revealed that higher temperature, low pH, low pulp density, and small particle size are highly effective for extracting REE (Srichandan et al., 2019).

3. Data Collection

To analyze the factors that could affect bio-extraction efficiency, peer-reviewed articles were searched on the Web of Science and Google Scholar using the keywords “rare earth elements,” “rare earth metals,” “microbial extraction of REE,” “bioleaching,” and “bioleaching of rare earth metals.” A total of 102 research articles were identified as of 12/20/2021, of which 82 studies focusing on extraction efficiency were evaluated further based on their abstracts. Based on data availability, 61 articles were selected for analysis. The list and relevant information for each study are presented in the supplementary materials, and the aggregated data is made available via an online open-access reposit.

While inputting this data, some experiments yielded a range of extraction efficiencies. For this analysis, we took the average of each of these ranges and used each average value as a single data point in our analysis. The data used for the study is presented in table 1.

Table 1. List of studies that examined bioleaching of REE.

Microorganisms	Sources	pH	Pulp density (%)	Metals	Extraction efficiency (%)	Reference
1. <i>Gluconobacter oxydans</i> (DSMZ 46616)	Red mud	2.1-7.12	10	REE: Sc	94	(Abhilash et al., 2021)
2. <i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Aspergillus terreus</i> , <i>Aspergillus ficuum</i> , <i>Pseudomonas aeruginosa</i> , <i>Penicillium cyclopium</i> , <i>Penicillium diversum</i> , <i>Penicillium oxalicum</i>	Carbonaceous shales	4.24	1	Mixed REE	33-86	(Amin et al., 2014)
3. <i>Gluconobacter oxydans</i> NRRLB85	Synthetic phosphogypsum	2.1	2	REE: Y, Ce, Nd, Sm, Eu, Yb	36.7-91.2	(Antonick et al., 2019)
4. <i>Acidithiobacillus thiooxidans</i> , <i>Acidithiobacillus ferrooxidans</i> , <i>Leptospirillum ferrooxidans</i>	Magnets	3.2	10	REE: Pr, Nd	86.4-100	(Auerbach et al., 2019)
5. <i>Bacillus</i> sp.	Ion-adsorption clay	5.9	2	REE: La, Ce, Dy, Lu	14.0-64.8	(Barnett et al., 2016)
6. <i>Aspergillus</i> sp.	Ion-adsorption clay	2.6	2	REE: La, Ce, Dy, Lu	15.3-72.6	(Barnett et al., 2018)
7. <i>Aspergillus</i> sp. <i>Acidithiobacillus ferrooxidans</i>	Bauxite	2.8	Nd	REE: mixed/total REE	26.2-62.8	(Barnett et al., 2020)
8. <i>Leptospirillum ferrooxidans</i>	Low grade scrap TV circuit boards	1.7	Nd	Other: Cu	95	(Bas et al., 2013)
9. <i>Acidithiobacillus</i> sp.	Zircon	nd	Nd	REE: Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu	79.6	(Becker et al., 1986)

10. Acidophilic mixed culture, <i>Acidithiobacillus ferrooxidans</i>	CRT fluorescent powders, spent catalyst	2-7	10	REE: Y Others: Ni, V, Mo	40-90	(Beolchini et al., 2012)
11. <i>Penicillium simplicissimum</i> , <i>Aspergillus niger</i>	Dust from electronic scrap	nd	Nd	Other: Cu, Al, Ni, Zn, Au	65-95	(Brandl et al., 2001)
12. <i>Chromobacterium violaceum</i> , <i>Pseudomonas fluorescens</i> , <i>P. plecoglossicida</i>	Dust from electronic scrap	nd	Nd	Other: Au,	69	(Brandl et al., 2008)
13. <i>Aspergillus niger</i> , <i>Aspergillus terreus</i> , <i>Paecilomyces</i> spp.	Monazite	2-2.8	1	REE: Ce, La, Nd, Pr	0.15-5	(Brisson et al., 2016)
14. <i>Ferroplasma acidiphilum</i> , <i>Sulfobacillus benefaciens</i> , <i>At. caldus</i> , <i>L. ferriphilum</i>	Printed circuit boards	1.7	3	Other: Cu	99	(Bryan et al., 2015)
15. <i>Acidithiobacillus ferrooxidans</i>	Printed circuit boards	2	Nd	Other: Cu	95	(Chen et al., 2015)
16. <i>Sulfobacillus thermosulfidooxidans</i>	Zinc refinery residues	2	30	Other: Ge	98.5	(Chen et al., 2017)
17. <i>Penicillium</i> sp. CF1 + indigenous consortia	Monazite	2.41-6.77	0.5	REE: Ce, La, Nd, Pr	1.52-79	(Corbett et al., 2018)
18. <i>Aspergillus ficuum</i> , <i>Pseudomonas aeruginosa</i>	Th-U concentrate	3-5.3	0.75, 1.2	REE: La, Ce, Y	2.51-33	(Desouky et al., 2016)
19. <i>Enterobacter aerogenes</i> , <i>Acidithiobacillus ferrooxidans</i>	Monazite	3.3-6.5	1	REE: Ce, Nd, Pr, Y	1.28	(Fathollahzadeh et al., 2018)
20. Acidophilic mixed culture	Municipal solid waste incinerator fly ash	1.4-2	5	REE: Nd, Ce, Sm, La	9-76	(Funari et al., 2017)
21. <i>Pseudomonas chlororaphis</i>	Natural polysaccharides	nd	Nd	Other: Au, Ag, Cu	8-52	(Gao et al., 2017)
22. <i>Aspergillus ficuum</i> , <i>Pseudomonas aeruginosa</i> , <i>Acidithiobacillus ficuum</i>	Monazite, Th-U concentrate	3.0-7.8	0.6, 1.2	Nd	55.60-75.4	(Hassanien et al., 2014)
23. <i>Streptomyces fungicidicus</i>	Ore	7.2	nd	REE: U	37.3	(Hewedy et al.,

YH04							2013)
24. Acidithiobacillus thiooxidans	Ground electronic waste	0.5	Nd	Other: Cu	98	(Hong & Valix, 2014)	
25. Lactobacillus casei, Komagataeibacter xylinus, Yarrowia lipolytica	Fluorescent phosphors	2.8-3.9	2.85	REE: Y, La, Ce, Eu, Gd, Tb	6.1-12.6	(Hopfe et al., 2017)	
26. Aspergillus niger	Spent automobile catalyst	2.35-5	1	REE: Ce, La Other: Al, Pt, Pd, Cu, Al, Zn	25.9-100	(Hosseinzadeh et al., 2021)	
27. Acidophilic consortium		nd	Nd	Other: Cu, Al, Zn	88-97	(Hussein et al., 2004)	
28. Acidithiobacillus ferrooxidans	Gibbsite ore	3-3.5	Nd	REE: Mixed Other: Al, Zn	22.26-67.58	(Ibrahim & Sheikh, 2011)	
29. Sulfo bacillus thermosulfidooxidans	Printed circuit boards	2	10	Other: Cu, Al, Zn, Ni	91-96	(Ilyas & Lee, 2014)	
30. Thermoplasma acidophilum, Sb. Thermosulfidooxidans	Electronic waste	1.57-2.7	1	Other: Cu, Zn, Ni, Al	64-89	(Ilyas et al., 2007)	
31. Ps. putida	printed circuit boards	8.0 - 9.2	Nd	Other: Cu, Au	44-98	(İşildar et al., 2019)	
32. Alicyclobacillus tolerans ST, Aspergillus niger AsIA, Methyloversatilis thermotolerans MSO	Phosphorus-containing slags	3.5	2	REE: La, Ce, Nd	2.38-81.87	(Issayeva et al., 2020)	
33. Gluconobacter oxydans	spent fluid catalytic cracking (FCC) catalysts	3.1	Nd	REE: mixed/total REE	23.3-25.7	(Jin et al., 2019)	
34. At. ferrooxidans, At. thiooxidans, Thiobacillus denitrificans, Thiobacillus thio paras, Bacillus subtilis, Bacillus cereus	Printed circuit boards	5.0 - 7.0	Nd	Other: Cu, Ni, Zn	48-53	(Karwowska et al., 2014)	

35. <i>Aspergillus niger</i>	Monazite	6.41	2	REE: Ce	100	(Keekan et al., 2017)
36. <i>Acidithiobacillus thiooxidans</i>	Printed circuit boards	1.4-2.5	Nd	Other: Cu, Ni, Zn	89-94	(Liang et al., 2010)
37. <i>Acidithiobacillus thiooxidans</i>	WEEE Shredding dust	1-1.5	0.5, 1, 2	REE: La, Nd, Y, Ce, Eu	80-99	(Marra et al., 2018)
38. <i>Acidithiobacillus thiooxidans</i>	Lead slag, shaft furnace slag, granulated slag	4.57, 4.87, 6.18	1	REE: mixed/total REE	83-99	(Mikoda et al., 2019)
39. <i>Acidithiobacillus ferrooxidans</i>	Spent fluid catalytic cracking catalyst	2	1	REE: La, Ce	23	(Muddanna & Baral, 2021)
40. Acidophilic chemolithoautotrophs, <i>Acidithiobacillus ferrooxidans</i> , <i>Acidithiobacillus caldus</i> , <i>Sulfobacillus</i> sp.	Ash slag waste	1.15-2	10	REE: Y, Sc, La, Nd, Gd, Sm	22.5-59.5	(Muravyov et al., 2015)
41. <i>Acidithiobacillus ferrooxidans</i> , <i>Acidithiobacillus thiooxidans</i>	Monazite	1.8	Nd	REE: Ce, La	1-9	(Nancucheo et al., 2019)
42. <i>Gallionella</i> sp.	Waste electrical and electronic equipment	nd	Nd	Other: Cu	95	(Oguchi et al., 2012)
43. <i>Aspergillus niger</i>	Siderophores	5-9	Nd	REE: La, Ce, Sm, Tm	8-66.7	(Osman et al., 2019)
44. <i>Candida bombicola</i>	Coal fly ash	4-6.5	1	REE: Dy, Y, Sc, Er, Yb, Gd, Eu, Sm, La, Ce, Pr, Nd	27.3-67.7	(Park & Liang, 2019)
45. <i>Aspergillus niger</i> (grapeskin strain), <i>Aspergillus niger</i> (pistachio-husk strain)	Red mud	6	3	REE: Sc	29-38	(Pedram et al., 2020)
46. <i>Acidithiobacillus ferrooxidans</i> , <i>acidiphilium acidophilum</i> (mixed)	Waste printed circuit boards	2.0-3.7	Nd	REE: Sc, La, Ce, Nd	24-31	(Priya & Hait, 2017)

47. Penicillium tricolor	Red mud	7.8-12	2	REE: La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y, Sc	30-65	(Qu & Lian, 2013)
48. Penicillium tricolor (RM-10)	Red mud	9–10.4	Nd	REE: La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y, Sc	48-78	(Qu et al., 2013)
49. Aspergillus niger	Red mud	1.8-3.8	2	REE: Sc, La, Eu, Yb	27-62	(Qu et al., 2015)
50. Gluconobacter oxydans	Spent NiMH batteries	3, 6, 9	1	total REE	7.8-56.1	(Rasoulnia et al., 2021)
51. Gluconobacter oxydans	Fluid Catalytic Cracking (FCC) catalyst, phosphor powder	3.3	Nd	REE: Tb, Eu, Ce, La, Y	49	(Reed et al., 2016)
52. Sulfur- and iron-oxidizing bacteria	Ore	2	10	REE: U, Th	6-60	(Reynier et al., 2021)
53. Endogenous acidophilic chemolithoautotrophs, Endogenous acidophiles	Quartz-pebble conglomerate ores	3 (max)	2-5	REE: Y, Ce, Pr, La, Nd, Yb, Dy, Sm	16.3-76.1	(Sapsford et al., 2012)
54. Acetobacter aceti	Monazite	3-5.2	16.7	REE: Ce, La	0.11-0.13	(Shin et al., 2015)
55. Acidithiobacillus thiooxidans	Acid mine drainage	nd	Nd	REE: Nd, Ce, La, Y	52.5	(Tayar et al., 2022)
56. Gluconobacter oxydans	FCC catalyst	2.14-2.61	1.5	REE: Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Ho, Yb, Lu, Dy, Er, Tm	42-56	(Thompson et al., 2018)
57. Acidophilic	Ash slag waste	0.92-	3	REE: Sc, Y, La,	15-30	(Tsaplina et al.,

chemolithoautotrophs		2.6		Nd, Sm, Gd, Ce, Pr, Dy, Er, Eu		2015)
58. Environmental consortium isolated from Elliot Lake mine waters	Quartz-pebble conglomerate ores	nd	Nd	REE: La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu	6-54	(Williamson, 2014)
59. Streptomyces sp. FXJ1.172	Bastnaesite- bearing rock	5.2- 7.8	0.5	REE: Ce, La, Y, Pr, Nd, Sm, Eu, Gd, Tb	8-10	(Zhang et al., 2018)
60. Acidithiobacillus ferrooxidans, Acetobacter methanolicus	Zircon	nd	Nd	REE: Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu	67-80	Glombitza et al (1988)
61. Acetobacter methanolicus	Zircon	nd	Nd	REE: Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu	67	Iske U (1987)

4. Data Analysis

4.1. Effect of microorganism type on REE leaching

Analyzing 196 data points from 61 peer-reviewed articles, we determine fungi appear to have higher leaching efficiency than heterotrophic, and chemolithotrophic microorganisms. Fungi is one kind of heterotrophic microorganism but due to its significance for bioleaching, it is considered as a different group of microorganisms (Işıldar et al., 2019). A total of 17 studies used different fungal strains, of which the most used strains were: *Penicillium simplicissimum*, *Aspergillus niger*, *Aspergillus ficuum*, and *Penicillium tricolor*. A co-culture of different strains of fungi has been proven to be more effective than a single strain for bioleaching, indicating synergy or sharing of resources between fungal species (Alavi et al., 2021; Brandl et al., 2001). Fungi have advantages over bacteria, including a faster leaching rate, shorter lag phase, and the ability to tolerate toxic materials and grow under various pH (Muddanna & Baral, 2021). Fungi are suitable for alkaline waste leaching because they survive in an alkaline environment (Işıldar et al., 2019, Alavi et al., 2021). Few studies contradict the finding and show fungi strains are not always the most efficient ones (Sedlakova-Kadukova et al., 2020). Heterotrophic bacteria have better leaching efficiency than chemolithoautotrophs (Figure 3). Among 14 studies that used heterotrophic bacteria, the most common strains are found to be *Gluconobacter oxydans*, *Pseudomonas aeruginosa*, and *Acetobacter methanolicus*. Heterotroph bacteria require a continuous energy source of organic carbon for their metabolic activity. Despite this setback, heterotroph is more feasible than chemolithoautotrophs because of the high value of obtained products. In comparison to chemolithoautotrophs, heterotrophic bacteria can grow at high pH. (Srichandan et al., 2019). Chemolithoautotrophs show the lowest efficiency in bioleaching. Most chemolithoautotrophs have a high tolerance for heavy metals toxicity but can only perform

efficiently in low pH environments (Orell et al., 2010). The chemolithotrophs thrive at a higher acidic condition usually around pH 2.0 or below (Srichandan et al., 2019). Among 26 studies that used chemolithotrophs, the most common strains are found to be *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, *Acidophilic consortium*, *Sulfobacillus thermosulfidooxidans*, *Acidithiobacillus ferrooxidans*.

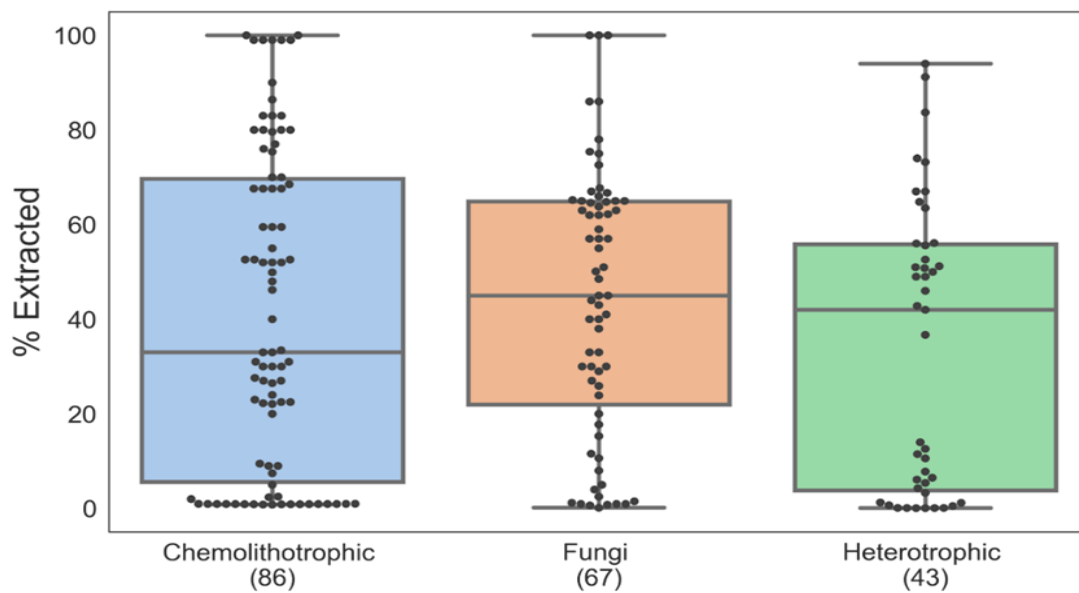


Figure 3: REE extraction efficiency varies with microorganism type: fungi, autotrophic, and chemolithotrophic. Fungi appear to have higher efficiency than heterotrophic, and chemolithotrophic microorganisms. There are 196 total data points on this graph gathered.

Six genera of microorganisms have been used most frequently as per the data we analyzed (Figure 4). Among chemolithotrophic bacteria group *Acidithiobacillus* and *Acidophilic*, from heterotrophic bacteria group *Acetobacter* and *Gluconobacter* species and from fungi group *Aspergillus* and *Penicillium* species show the greatest potential for REE mobilization *Acidithiobacillus* appears to be most effective, and *Acetobacter* is least effective. Though

Acidithiobacillus is a chemolithoautotrophic bacteria species, it showed better results than the fungi strains. Most of the bioleaching experimental work uses different strains of *Acidithiobacillus* because of the higher yield efficiency.

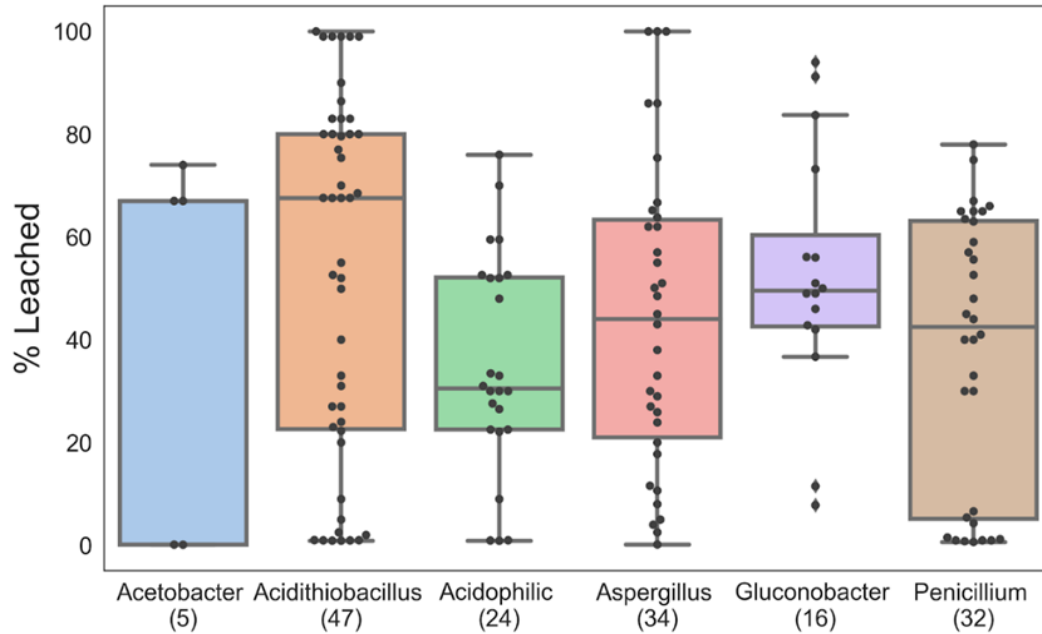


Figure 4: REE extraction efficiencies of 6 different microorganism genera. *Acidithiobacillus* appears to be most effective, and *Acetobacter* is least effective. There are 158 total data points on this graph gathered from 43 reference papers. Under each genus is written the number of data points that contribute to the corresponding box plot.

75% of our reviewed bioleaching studies resulted in 80-100% leaching efficiency, which signifies that bioleaching is indeed a very promising method for extracting REEs. The analysis also revealed that the REE removal efficiencies of all microorganisms could vary widely from the range of 0-100% (Figure 4). REE extraction efficiency could also vary with the composition or characteristics of the growth medium or leaching solution: pH, pulp density, and temperature

(Bryan et al., 2015). Results that had 80-100% yield had similar types of experimental conditions. However, the efficiency differed significantly with the change of source materials. Electronic waste products such as printed circuit boards spent automobile catalysts, equipment shredding dust, and NiMH batteries yielded more than leaching 80-100% efficiency. The elemental composition of the discarded devices is highly variable and complex, thus there is simply more to leach (Hadi et al., 2015). The second highest source material from where the leaching was significant was mineral ore like monazite.

4.2. Extraction efficiencies in a mixture of REEs with transition metals

Most REE-containing wastes also contain transition metals at concentrations greater than that of REE, which may be leached out by microorganisms often at greater concentrations than that of REE. Our analysis revealed that transitional metals have higher leaching efficiency than REEs (Figure 5). The bioleaching process and type of microorganism can selectively bind REEs or transitional metals from the solution and control the efficiency (Rasoulnia et al., 2021c). Cell-free culture media bioleaching is efficient in higher REE leaching, while the two-step bioleaching method is more efficient in leaching transitional metals. (Rasoulnia et al., 2021c). REEs get adsorbed on the microbial cells during the two-step bioleaching process which results in more transitional metal leaching. However for spent medium bioleaching, REE extraction is higher since the cells have been removed from the leaching solution (Qu et al., 2019; Rasoulnia et al., 2022). Our analysis indicated that 74% of data points used fungi to leach HREE from different sources. Fungi strains such as *Penicillium funiculosum*, *Aspergillus foetidus*, and *Penicillium simplicissimum* could adapt to high concentrations of heavy metals contributing to efficient leaching of HREE. LREE leaching was higher for chemolithoautotrophic bacteria. 44%

of data point used chemolithoautotrophic bacteria to leach LREE from different sources. Mix culture of chemolithotrophs was used to extract REE in most cases.

Biological ligands or organic acids can have different affinities to different REEs based on their atomic number and atomic weight (Mattocks & Cotruvo, 2020). The ionic radii of REEs decrease gradually with atomic number. Our analysis indicates that leaching increased with greater atomic numbers, except for yttrium and scandium. Yttrium has an atomic number of 39, it is generally classified as an HREE due to its similar chemical properties and ionic radius. With the increase in REE atomic number, organic compounds like citrate, oxalate, gluconate, and EDTA are more likely to form complexes (Barnett et al., 2018). The HREE-organic ligand complexes are more powerful and result in a greater percentage of leaching (Liu & Chen, 2021). Enhanced leaching of HREE has already been observed during the leaching of REE from red mud, with up to 70% Lu being leached compared to maximum of 27% for La (Qu & Lian, 2013). In contrast to leaching efficiency, the variability of LREE extraction efficiencies is much greater than that of HREE and transition metals (Figure 5). LREEs oxidize much faster than HREEs due to the variability of the resulting oxide product (Muravyov et al., 2015; Thompson et al., 2018). Estimating LREE and HREE in the same samples, we found that LREEs accounted for 90.9-99.6% of total leached REEs. This is reflected by the abundance of data points for LREE (Figure 5).

If there is more than one REE present in the source, the microorganism does not extract all the metals at the same rate. The leaching sequence depends on properties such as EPS layer, pH, redox potential, produced ligands, or siderophores. The mobilization of La, Gd and Y depends mainly upon pH value, whereas the mobilization of Ce depends both upon pH and redox

potential (Jalali & Lebeau, 2021). The siderophores produced by fungi can extract 68% uranium and 65% thorium, 4.3% lanthanum, 5.4% cerium, and 1.2% yttrium due to selective adsorption of Th^{4+} and UO_2^{2+} with secreted exogenous polysaccharides (Desouky et al., 2016).

Comparing the extraction efficiency of the four transition metals, two LREEs, and two HREEs that were reported most frequently (Figure 6), we found that the non-REE metals make up less of the entire data set than the REEs do. A majority of the 61 papers analyzed in this study primarily examined the extraction of REE, thus they did not report other elements even though they were present in the source material. The bioleaching extraction efficiency for REE decreased with the following order: Yttrium (Y) > Scandium (Sc) > Neodymium (Nd) > Cerium (Ce).

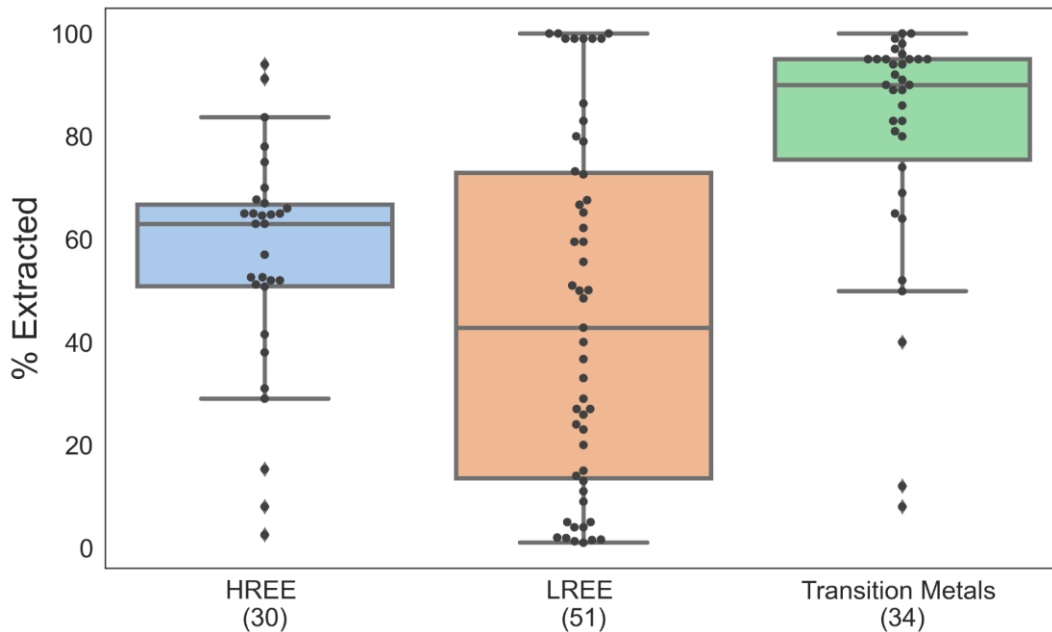


Figure 5: Extraction efficiencies of various metals, categorized by material group (HREE, LREE, transition metals). Transitional metals have higher leaching efficiency than REEs. There are 115

total data points on this graph, gathered from 35 reference papers. Under each type of metal is written the number of data points that contribute to the corresponding box plot.

The extraction efficiency varies largely due to the source material, which can often contain substantial quantities of non-REE metals. Electrical waste such as printed circuit boards (PCBs) was the major source of transitional metal leaching. HREE extraction was mainly from fly ash, industrial waste like fluorescent powder from CRT, phosphogypsum, phosphorus-containing slags, and red mud. The highest LREE was extracted from fly ash.

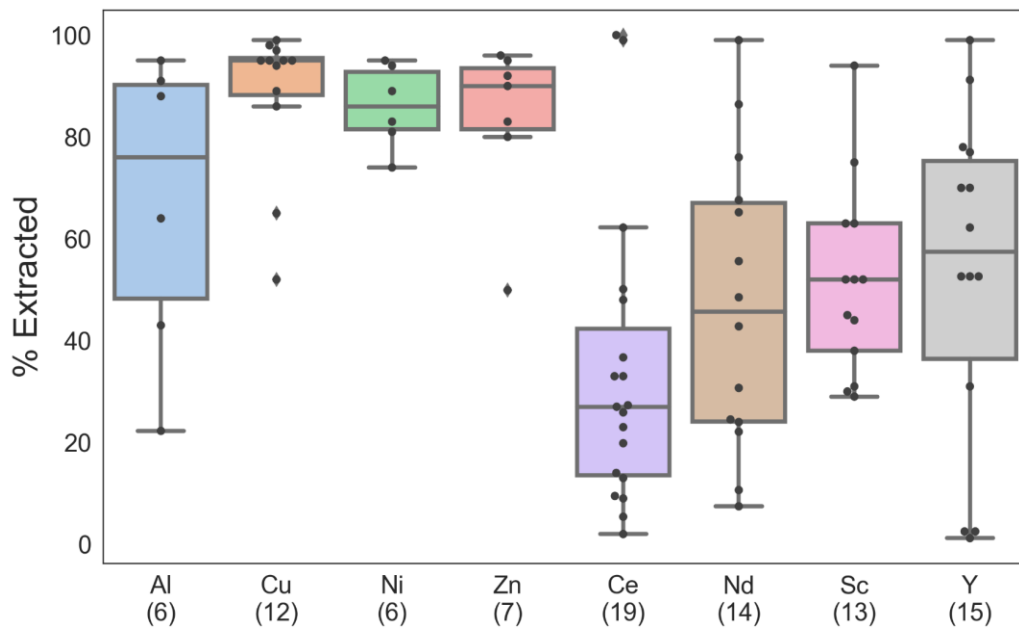


Figure 6: REE bioleaching levels are lower than their non-REE counterparts. There is a total of 92 data points, gathered from 28 reference papers. Under each metal is written the number of data points that contribute to the corresponding box plot.

4.3. Variation of efficiency with source type

Based on reported studies, we categorized REE sources into five major groups: (i) waste from electrical and electronic equipment (WEEE), (ii) industrial waste, (iii) multi-metallic solution, (iv) fly ash and (v) ore. WEEE includes magnets, NiMH batteries, dust from electronic scrap, printed circuit boards, and automobile catalyst—all major REE sources. Phosphogypsum, fluorescent powder, FCC catalyst, and red mud are all categorized as industrial waste for our analysis. Multi-metallic solution was collected from all the above sources where no individual metal percentage was mentioned. It contained mixture of REEs in an aqueous form. Municipal solid waste incinerator residue, shaft furnace slag, ash slag waste, coal fly ash is termed as fly ash for the analysis. Zircon, monazite, gibbsite, bastnasite, carbonaceous shales are categorized as ore.

Our analysis suggests that the leaching efficiency of REE varied with source types and was maximum for WEEE (Figure 7), indicating WEEE should be increasingly used as REE source. Extraction of REEs was found to be lowest in multi-metallic solutions as leaching of REE becomes less efficient in the presence of other transition metals. The leaching efficiency of fly ash and industrial waste was similar to ore.

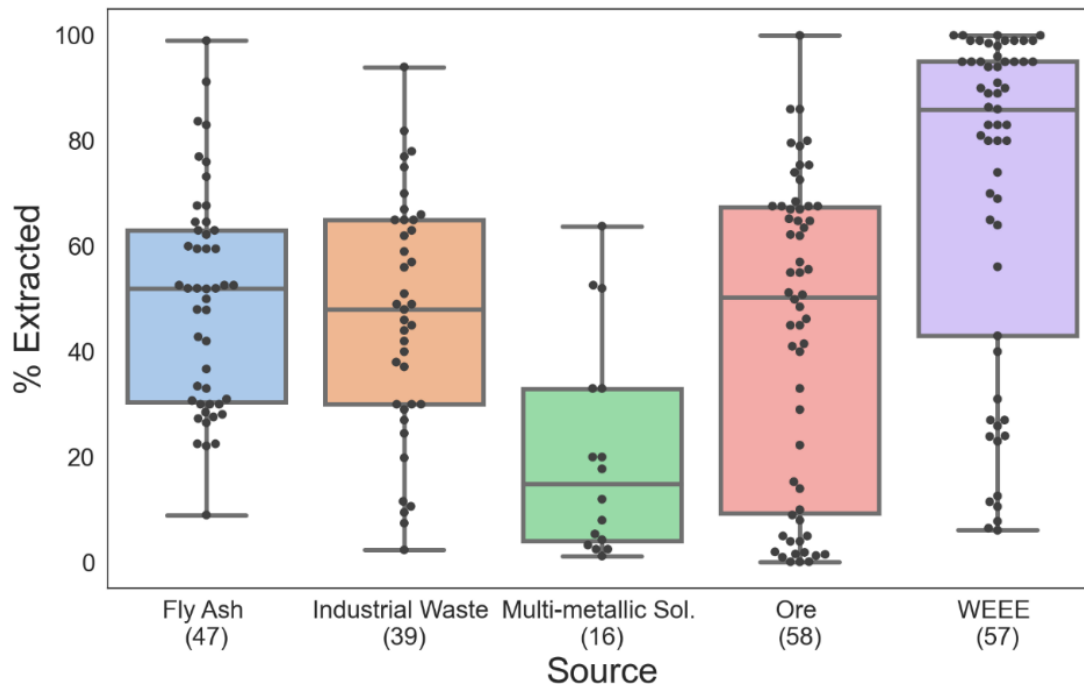


Figure 7: REE extraction efficiencies from 5 different groups of sources. Leaching of REE is maximum for WEEE and lowest in multi-metallic solutions. There are 218 total data points on this graph gathered from 61 reference papers.

To examine if the variability of REE extraction can be explained by the microorganism type used in those studies, we further divided each source group by microorganism types and found that fungi extracted the highest amount of REE from WEEE. WEEE increases the pH of the solution which is not favorable for other groups of microorganisms. This result differed from some studies where the chemolithotrophic group was most effective at leaching REEs from WEEE (S. Chen et al., 2015; Lee & Pandey, 2012). Heterotrophic microorganisms' extraction efficiency was lowest from electronic waste. Chemolithotrophic microorganisms extracted the highest percentage of REE from natural ore. Ore contains metal sulfides which help regeneration of oxidation agents for chemolithotrophic microorganisms (Figueroa-Estrada et al., 2020). Fungi extracted more REEs from ore compared to other heterotrophic bacteria. The extraction

efficiency of all the microorganism groups is lowest from the multimetal solution compared to other sources. The presence of several transitional metals is responsible for lower REE removal. Fungi extracted the highest amount of REE from industrial waste as well. Heterotrophic microorganisms resulted in a higher amount than chemolithotrophic groups. Fungi and heterotroph bacteria produce bio lixiviant which has proved promising for REE recovery from different industrial wastes (Antonick et al., 2019; Rasoulnia et al., 2021b). In the case of fly ash, fungi are the most effective group because fly ash is alkaline and fungi perform better in such conditions (Park & Liang, 2019).

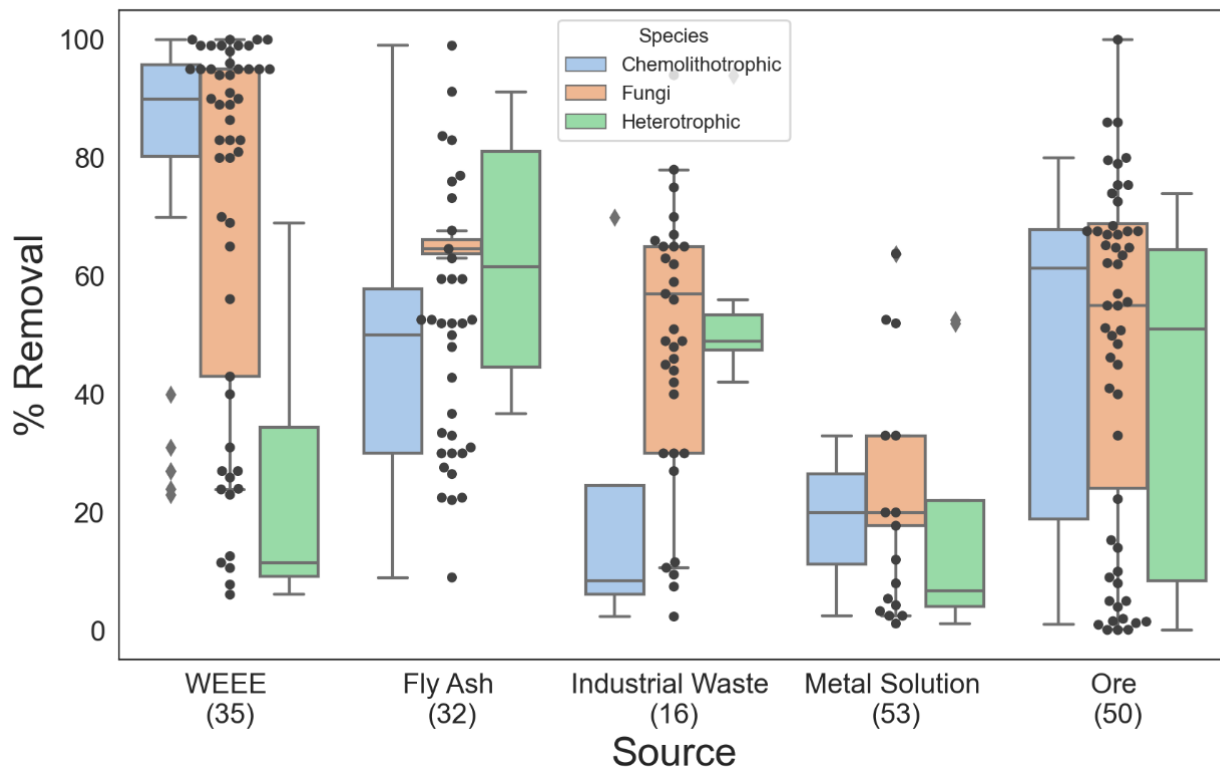


Figure 8: REE extraction efficiencies of secondary sources by different groups of microorganisms. There are 218 total data points on this graph gathered from 61 articles.

5. Challenges and Opportunities

This review highlighted the knowledge gap and the need for more detailed studies on the bioleaching process. The main challenge that we faced was the lack of explanation for experimental results. Most of the published article reports leaching percentages and measure different abiotic factors that may affect the process without proper interpretation. This could be due to a lack of understanding of leaching mechanisms. To maximize metal dissolution, microbial production of leaching agents and leaching conditions need to be optimized first. More research focused on the fundamental understanding of bioleaching needs to be done to maximize the process efficiency. Another major difficulty that we faced during the work is the classification of the microorganisms. Some well-known strains were easy to classify but there were a few strains mentioned in some papers which were not easily categorized. We could not find any database where this classification is well-listed.

The scarcity of rare earth elements makes finding other secondary sources even more important. Considering the low grade of REE present in ores, the future scope of the research lies in the application of microbial bioleaching technology for high-efficiency REE recovery from secondary sources like magnets, fluorescent lamps NiMH batteries, and industrial wastes. Coal ash and electronic waste are mainly considered alternative sources for REE in recent days. New source material needs to be established for the growing upcoming demand for The REE as critical raw materials. Bioleaching is likely to be the first step of leaching REE from the source. For better efficiency, a combination of two or three bio-recovery technologies might be more efficient. A combination of bioleaching with a biosorbent strategy can yield much higher efficiency More studies need to test which other techniques in combination with bioleaching

yield the best result. Process optimization and scale-up of current bioleaching methods should also be the focus of future studies to reach industrial-scale applications. Most of the studies included here are lab experiments. The major challenge with large-scale operations is the difficulty to maintain the microbial population and oxygen rate, which eventually affects bioleaching efficiency. Before advancing into large-scale operations, further investigations are required to determine the effectiveness of bioleaching, optimization of the overall process, and environmental sustainability. More focus should be given to the future development of novel microorganisms and highly efficient strategies for recovering critical metals. Recent publications point to synthesizing microorganisms and transplanting specific genes to improve their efficiencies. This might help meet the requirements to operate the bioleaching process on an industrial scale. It might also reduce operating costs. Furthermore, the co-culture of bacteria and fungi might be a potential way for a high recovery rate of REEs (Hopfe et al.2017).

6. Conclusions

By using bioleaching for REE extraction, many of the ongoing hazards connected with chemical extraction can be eliminated. As opposed to chemical leaching, which requires high temperatures and produces chemical wastes, bioleaching is believed to be a cleaner, more efficient, and less expensive method of mobilizing metals. Despite the fact that bioleaching of REEs cannot solve all the objectives, it is nevertheless an important tool for reducing market dependence and supply risks. It has been demonstrated that bioleaching is a significant means of extracting REEs from a variety of primary and secondary sources. Various types of microorganisms are capable of performing bioleaching to recover REE, and other processes have also been shown to be effective in recovering REE, such as biosorption, bioaccumulation, and bioprecipitation. There is generally a lack of selectivity associated with biological processes such as bioleaching, biosorption, biomineralization, bioaccumulation, and bioprecipitation. Various chemical methods of extraction by conventional methods have significant limitations as well, so bioleaching technology can offer an intelligent alternative. It is possible to significantly improve the overall bioleaching process by optimizing several factors.

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