IPEK TEZYAPAR KARA

ASSESSING RESOURCE RECOVERY POTENTIALS OF INDUSTRIAL METAL-BEARING BY-PRODUCTS USING BIOLEACHING

SCHOOL OF WATER, ENERGY AND ENVIRONMENT

PhD in Water

PhD

Academic Year: 2020 - 2023

Supervisor: Prof. Frédéric Coulon

Associate Supervisor: Dr. Stuart Wagland

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This thesis is submitted in partial fulfilment of the requirements for the degree of PhD

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ABSTRACT

The global transition to a circular economy calls for research and development on technologies facilitating sustainable resource recovery from wastes and by-products as some possessing comparable or superior quality to natural ores. Traditional methods e.g., pyrometallurgy and hydrometallurgy are considered as inefficient for processing secondary resources as they often cause harmful emissions and loss of metals, require high capital cost. Bioleaching, which emerges as an eco-friendly and cost-effective alternative to conventional methods, is well established for metal extraction from low-grade sulfidic ores, tailings, and metallurgical side streams, yet the method is at the research stage for other secondary resources such as metallurgical slag and dust, fly ash, e-waste. The aim of this PhD study was assessing the resource recovery potentials of industrial metal-bearing by-products using biomining. Firstly, this study critically reviewed the microbial diversity and specific mechanisms of bioleaching as well as the current operations and approaches of bioleaching at various scales and summarised the influence of a broad range of operational parameters. Then, suggested an optimisation route for bioleaching of metal-bearing materials for laboratory scale bioleaching operations to further inform pilot/commercial scale operations. Further to this, feasibility of extracting metals from two industrial metal-bearing by-products which were basic oxygen steelmaking dust (BOS-D) and goethite were investigated using *Acidithiobacillus ferrooxidans*. Taguchi orthogonal array design was used to evaluate the effect of four parameters which were pulp density, energy source concentration, inoculum concentration, and pH at three different levels. Then methods were explored for enhancing and scaling up the extraction of metals from BOS-D. Finally, techno-economic assessment conducted for two potential industrial scale bioleaching technologies including an aerated bioreactor and an aerated and stirred bioreactor, for different metal recovery scenarios from the industrial metal-bearing by-products.

Keywords: *Acidithiobacillus ferrooxidans*, biomining, bio-hydrometallurgy, secondary resources, Taguchi orthogonal array design, techno-economic assessment.

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LIST OF ABBREVIATIONS

ORP	Oxidation-reduction potential
BOS-D	Basic oxygen steelmaking dust
IRR	Internal rate of return
NPV	Net present value
PD	Pulp density
MBM	Metal-bearing materials
REEs	Rare earth elements

1 INTRODUCTION

Metals, metalloids, and rare earth elements, collectively termed ‘metals’, are in high demand as they are essential to develop modern cities and low carbon energy technologies (Lee et al., 2022; Gomes et al., 2020). Growing concerns about supply constraints due to geological scarcity and ever-increasing demand on metals has driven researchers investigating the feasibility of extracting metals from secondary resources (Watari et al., 2020; IEA, 2021). Secondary resources such as, e-waste, tailings, metallurgical by-products, spent catalyst and fly ash, may contain similar or even higher metal content than primary ore, makes them valuable for the circular economy (Kinnunen and Hedrich, 2023). For instance, electronic wastes can contain up to 26 times higher Cu and 50 times higher Au content compared to ores/concentrates (Akcil et al., 2015). Furthermore, tailings can have higher Co content (0.02%-1.38%) than a natural ore (0.05%-0.3%) (Gutiérrez-Gutiérrez et al., 2015; Sarker et al., 2022). Similarly, the iron content of some basic oxygen steelmaking dust, a metallurgical by-product, is close or better than virgin iron ores (Ma, 2016).

Metallurgical by-products including basic oxygen steelmaking dust (BOS-D), and goethite, potentially considered as a secondary iron resource for the steel making industry due to their high iron content (40-60%), yet their zinc content, >10 g/kg for BOS-D and 50-100 g/kg for goethite hinders their potential for reuse (Ma, 2016; Di Maria and Van Acker, 2018; Pelino et al., 1996). The reason is that when zinc charged into a blast furnace, due to its low boiling point (907 °C) it vaporizes at high temperatures (1600–1650 °C), condensing on blast furnace walls and forming zinc accumulations that affect the solid and gas flows through the furnace. This can decrease the productivity and damage the furnace linings (Stewart and Barron, 2020; Xue et al., 2022). Zinc also shortens the furnace operating life by attacking refractories in the upper stack of the furnace (Binnemans et al., 2020). Metallurgical by-products also contain wide range of different metals such as manganese and aluminium, critical metals such as lithium, cobalt, copper and rare earth elements (REEs) such as yttrium, cerium and neodymium. Recovering metals from by-products may be a profitable

business opportunity, also can provide better environmental management by reducing the environmental impact of waste (Di Maria and Van Acker, 2018; Kinnunen and Hedrich, 2023).

Mainly pyrometallurgy and hydrometallurgical processes are used for extracting metals from primary ores; however, they are considered inefficient for processing secondary resources due to the loss of metals during the process and the generation aqueous waste streams that require additional treatment (Roy et al., 2021a; 2021b; Binnemans et al., 2020). Pyrometallurgy also requires intensive energy, high temperature and pressure, high capital cost as well as cause harmful emissions (Arya and Kumar, 2020; Asghari et al., 2013). Over the last 10 years, bioleaching has attracted more attention for extracting metals from secondary resources as an environmentally friendly and economic alternative to conventional processes (Brown et al., 2023; Owusu-Fordjour and Yang, 2023; Al Sultan and Benli, 2023).

For decades bioleaching has been commercially available for extracting elements mainly copper (Cu), gold (Au) from low-grade sulphidic ores and tailings. However, the method is still under development for other waste streams such as metallurgical by-products, fly ash, e-waste to overcome the challenges with alkalinity, heterogeneity, toxic contents, and the lack of iron and sulphur source in metal-bearing materials (Kinnunen and Hedrich, 2023). Bioleaching is a method that uses naturally available microorganism and/or their metabolites to extract metals from solid matrixes. The efficiency of bioleaching relies on various operational parameters such as solid-liquid ratio, pH, energy source concentration, temperature, inoculum concentration, concentration of nutrient medium components, agitation speed, recirculation/irrigation (Srichandan et al., 2020). Simultaneous optimisation of these parameters rather than one-factor-at-a-time methodology can provide higher metal extraction yield and reveals the most influencing parameters and their optimum values (Naseri et al., 2023; Niu et al., 2016). Moreover, towards sustainable and commercially viable bioleaching operations, comprehensive techno-economic assessment studies considering

scalability of process, variations of leaching conditions, and recovery targets will support decision-making (Naseri et al., 2023).

1.1 Aim and Objectives

The aim of the PhD research is to assess the resource recovery potentials of metallurgical by-products by using biomining. To achieve the research aim, the following specific objectives have been addressed:

- **Objective 1:** To critically review relevant literature and identify gaps.
- **Objective 2:** To evaluate the key factors influencing bioleaching efficacy and flexibility to different materials.
- **Objective 3:** To explore the methods for enhancing and scaling up the extraction of metals.
- **Objective 4:** To perform a techno-economic assessment to inform pilot scale implementation.

1.2 Thesis structure and format

The PhD thesis is comprised of six chapters, of which four have been written as paper format (Figure 1.1). A brief description of each chapter is provided hereinafter:

Chapter 1: This chapter presents a general introduction and provide research context, background, aim and objectives of the research.

Chapter 2: This chapter provides a critical review of the microbial diversity and specific mechanisms of bioleaching as well as the current operations and approaches of bioleaching at various scales and summarises the influence of a broad range of operational parameters. Then, it offers a two-phase optimisation route for bioleaching of metal-bearing materials for laboratory scale bioleaching operations to further inform pilot/commercial scale operations. This chapter has been published in Environmental Chemistry Letters.

Chapter 3: This chapter investigates the feasibility of extracting metals from two industrial metal-bearing by-products, basic oxygen steelmaking dust (BOS-D) and goethite, using *A. ferrooxidans*. Various elements Mn, Zn, Pb, Al, Li, Co, Y,

and Ce, were selected to be investigated as some of them the most abundant metals, critical and rare earth elements. Four variables at three different levels including solid concentration (1, 5 and 10% w/v), energy source concentration (1, 2 and 3% w/v for BOF optimisation; 2, 3 and 4% w/v for goethite optimisation), inoculum concentration (1, 5 and 10% v/v) and pH (1.5, 1.75, and 2) were evaluated using L9 Taguchi orthogonal array design. The study provided the order of importance and optimum values of the parameters for individual metals. Optimum condition to leach selected metals form BOS-D and goethite was defined. This chapter has been published in Chemosphere.

Chapter 4: Methods for enhancing and scaling up the extraction of metals from BOS-D were explored in this Chapter. First, the effectiveness of three batch systems approaches: bioleaching using *A. ferrooxidans*, chemical leaching using choline chloride-ethylene glycol (ChCl-EG) and a combined approach were compared. Then, scaling up was evaluated through a semi-continuous bioleaching column system where leachate was recirculated at different regimes. Overall, this chapter emphasizes the considerable potential of bioleaching for metal recovery as well as challenges and need for further optimisation efforts. This chapter has been submitted in Hydrometallurgy.

Chapter 5: This chapter evaluated the economic feasibility of two potential industrial scale bioleaching technology including an aerated bioreactor and an aerated and stirred bioreactor for metal recovery from BOS-D and goethite. Techno-economic assessment (TEA) was conducted for different recovery scenarios. Further, project cash flow, net present value and internal rate of return for selected elements recovery were projected over 20 years.

Chapter 6: This chapter provides an overall discussion and summary of the key findings from each chapter. Further it provides overall implications and limitations of the study as well as recommendations for further studies.

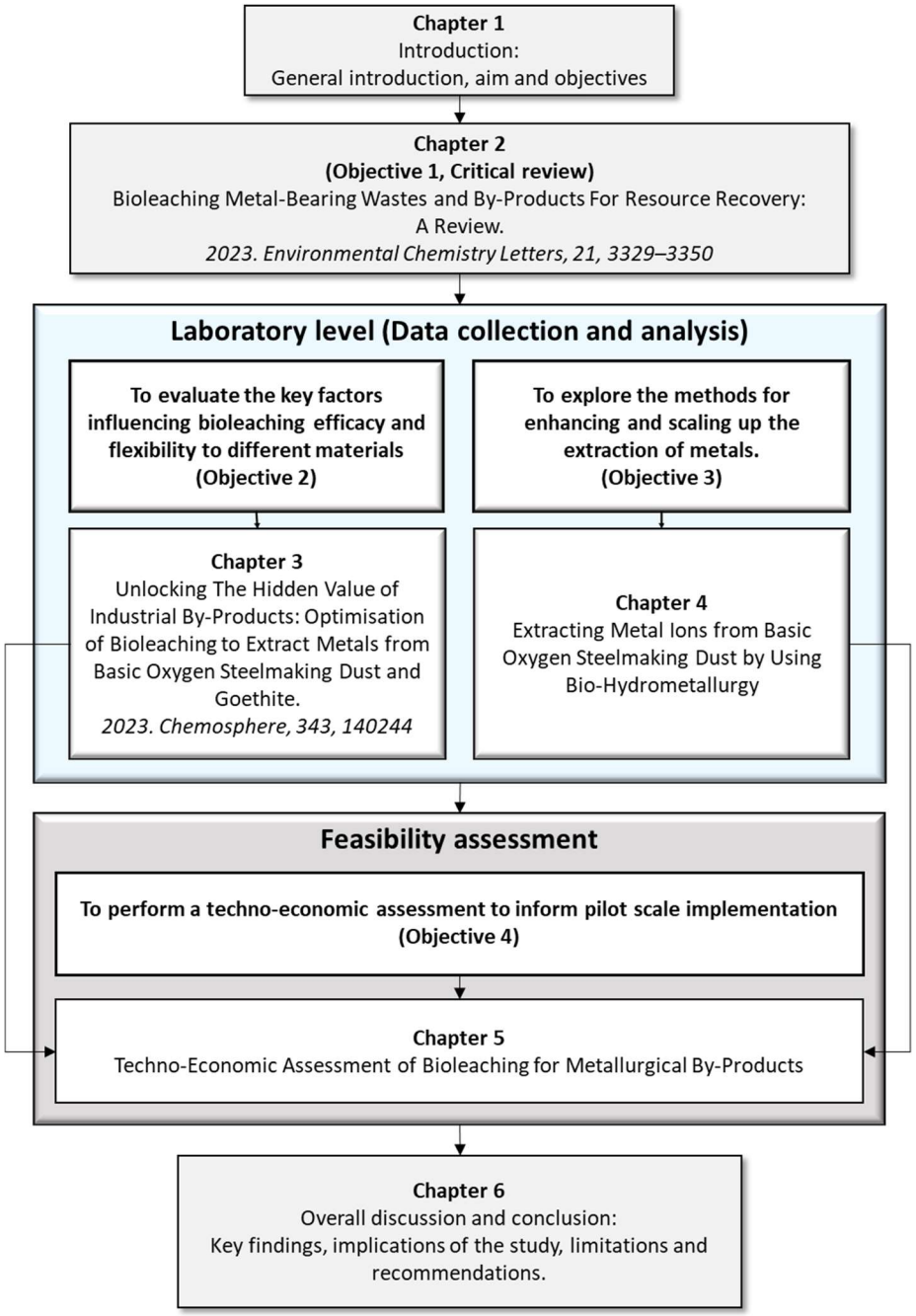

Figure 1-1. Thesis structure and chapters mapped against each objective

1.3 Publications

Publications included in the thesis

- **Tezyapar Kara, I.**, Kremser, K., Wagland, S.T., and Coulon, F. (2023) Bioleaching metalbearing wastes and by-products for resource recovery: a review, Environmental Chemistry Letters, 21: 3329–3350. **(Chapter 2)**

- **Tezyapar Kara, I.**, Simmons, N., Wagland, S.T. and Coulon, F. (2023) Unlocking the hidden value of industrial by-products: Optimisation of bioleaching to extract metals from basic oxygen steelmaking dust and goethite, Chemosphere, 343: 140244. **(Chapter 3)**

Other publications

- **Tezyapar Kara, I.**, Marsay, N., Huntington, V., Coulon, F., Alamar, M.C., Capstick, M., Higson, S., Buchanan, A., and Wagland, S. (2022) Assessing metal recovery opportunities through bioleaching from past metallurgical sites and waste deposits: UK case study, Detritus, 21: 62–71.

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- **Tezyapar Kara, I.**, Marsay, N., Huntington, V., Coulon, F., Alamar, M.C., Capstick, M., Higson, S., Buchanan, A., and Wagland, S. (2022) Assessing metal recovery opportunities from past metallurgical sites and waste deposits: UK case study, Sum2022, 6th Symposium on Circular Economy and Urban Mining, Capri, Italy, 18-20 May, (Platform presentation).
- **Tezyapar Kara, I.**, Wagland, S.T. and Coulon, F. (2023) Bioleaching of Metal Ions from Basic Oxygen Furnace (BOF) Sludge and Dust by *Acidithiobacillus Ferrooxidans*, Biomining '23, 11th International Symposium on Biomining, Falmouth, Cornwall, UK, 5-6 June (Platform presentation).
- **Tezyapar Kara, I.**, Wagland, S.T. and Coulon, F. (2023) Metals recovery from metallurgical wastes using biometallurgy, AquaConSoil, Prague, Czech Republic, 11-15 September (Platform presentation).

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2 BIOLEACHING METAL-BEARING WASTES AND BY-PRODUCTS FOR RESOURCE RECOVERY: A REVIEW

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Abstract: The global transition to a circular economy calls for research and development on technologies facilitating sustainable resource recovery from wastes and by-products. Metal-bearing materials, including electronic wastes, tailings, and metallurgical by-products, are increasingly viewed as valuable resources, with some possessing comparable or superior quality to natural ores. Bioleaching, an eco-friendly and cost-effective alternative to conventional hydrometallurgical and pyrometallurgical methods, uses microorganisms and their metabolites to extract metals from unwanted metal-bearing materials. The performance of bioleaching is influenced by pH, solid concentration, energy source, agitation rate, irrigation rate, aeration rate, and inoculum concentration. Optimising these parameters improves yields and encourages the wider application of bioleaching. Here, we review the microbial diversity and specific mechanisms of bioleaching for metal recovery. We describe the current operations and approaches of bioleaching at various scales and summarise the influence of a broad range of operational parameters. Finally, we address the primary challenges in scaling up bioleaching applications and propose an optimisation strategy for future bioleaching research.

Keywords: Bioleaching, multi variable optimisation, metal-bearing-materials, bioleaching approaches, biohydrometallurgy, biomining

2.1 Introduction

Metals, metalloids, and rare earth elements, collectively termed ‘metals’, are in high demand as they are essential to develop modern cities and technology products (Lee et al., 2022a). Catering the ever-increasing metal demand is become arduous due to the decrease in ore quality (IEA, 2021). Rare earth

elements and lithium (Li), cobalt (Co), antimony (Sb), copper (Cu) and indium (In) are labelled as critical metals due to their supply risk and economic importance (Gutiérrez-Gutiérrez et al., 2015; Muddanna and Baral, 2021). Although metals are recyclable, the end-of-life recovery rate is low, <50% for most of critical and base metals and <1% for Li and rare earth elements (Muddanna and Baral, 2021; IEA, 2021). This is because metals are often trapped in the complex metal-bearing materials, e.g., metallurgical by-products, catalysts, Li-ion batteries, and electric-electronic equipment (Binnemans et al., 2020; Işıldar et al., 2019; Moazzam et al., 2021). Last decade, the end-of-life metal-bearing materials, can be also called as metal bearing wastes, has been widely accepted as secondary resource of critical raw materials due to metal content of metal-bearing wastes is comparable with natural ores (Işıldar et al., 2016; Lee et al., 2022a; Sarker et al., 2022). For example, electronic wastes contain up to 26 times higher Cu and 50 times higher Au content compared to ores/concentrates (Akcil et al., 2015). Another example is that some tailings can have higher Co content (0.02%-1.38%) than a natural ore (0.05%-0.3%) (Gutiérrez-Gutiérrez et al., 2015; Sarker et al., 2022). Similarly, the quality of some basic oxygen furnace dust, a metallurgical by-product, is close or better than virgin iron ores (Ma, 2016).

Metal-bearing wastes, such as electronic waste, tailings, slag, and dusts, are typically stockpiled or landfilled due to the lack of efficient and sustainable recovery routes for the metals they contain (Binnemans et al., 2020). For instance, the UK alone has over 190 million tonnes of iron and steel waste stockpiled in current and former metallurgy sites (Riley et al., 2020). This underutilisation of metal-bearing material resources leads to economic losses and creates environmental challenges. To address this issue, enhanced landfill mining and urban mining have been proposed to recover secondary resources from stockpiled metal bearing wastes and integrate them into the circular economy (Arya and Kumar, 2020a; Jones et al., 2013; Lee et al., 2022b). Enhanced landfill mining and urban mining align with the goal of the bioeconomy to create more sustainable value chains and improve productivity and quality of products in economic sectors. Moreover, recovering metals from metal-bearing wastes not only provides economic benefits and waste reduction but also allows

for land reuse and treatment of heavy metal contamination. (BIT II, 2019; Gao et al., 2021; Potysz et al., 2018; Sur et al., 2018). However, metal-bearing wastes recovery remains a challenge due to the complex and refractory structure of the materials. Therefore, there is a need for the development of efficient, sustainable, and low-cost recovery routes for metals from metal-bearing wastes (Binnemans et al., 2020; Potysz et al., 2021; Yaashika et al., 2022).

The two main methods, pyrometallurgy and hydrometallurgy, are used to extract metals from primary (ore) and secondary sources e.g., tailings, e-waste, fly ash (Keshavarz et al., 2021; Potysz et al., 2018; Shahbaz, 2022). Hydrometallurgy is the process used to extract metals from solid matrices, which is achieved by recovering and dissolving the metals as salt in successive aqueous solutions-based steps, including leaching, purification, and recovery of the targeted metal by selective precipitation or electrowinning (Dutta et al., 2023). Hydrometallurgy also involves using water for the extraction of metals, but the extraction and purification are heat-based processes (from ambient to around 300°C). Extraction above 100°C is carried out under pressure to prevent boiling (Whitworth et al., 2022). In pyrometallurgy, there are typically three steps including: roasting, smelting in high temperatures (250-1000°C) and refining processes involving different kinds of furnaces and electrolytic processes (Arya and Kumar, 2020a; Mishra et al., 2022). The disadvantages of pyrometallurgy are the loss of metals during the process for example the loss of lithium form Li-ion batteries, and the production of hazardous gases (Roy et al., 2021). With pyrometallurgy, a vast amount of energy and high capital cost is required, compared to hydrometallurgy processes (Arya and Kumar, 2020a).

Bioleaching, which is also known as biomining, emerges as a promising eco-friendly and cost-effective biohydrometallurgical to traditional mining and metal recovery methods (Asghari et al., 2013; Gavrilescu, 2022; Moazzam et al., 2021). Bioleaching is mediated by wide range of microorganisms and their metabolites (Figure 2-1 and Table 2-1) (Gavrilescu, 2022). The efficiency of bioleaching relies on operational parameters such as solid-liquid ratio, pH, energy source and concentration, and aeration rate, which are crucial for the recovery of metals from

various solid matrices (see Appendix Table B-1-Table B-7). The process can be performed on different scales depending on the particle size of the metal-bearing material, with small to intermediate scale operations conducted in shake flasks, column reactors, and stirred bioreactors (Amiri et al., 2011a; Gomes et al., 2018; see Appendix Table B-8-Table B-11). Commercial scale operations are typically conducted ex-situ using vat, agitated tank reactors, heap, and dump methods (Natarajan, 2018; Zanbak, 2012). Although bioleaching has been industrialised for low-grade copper and refractory gold ores using acidophiles, it has not been widely adopted for other metal-bearing materials (Srichandan et al., 2019).

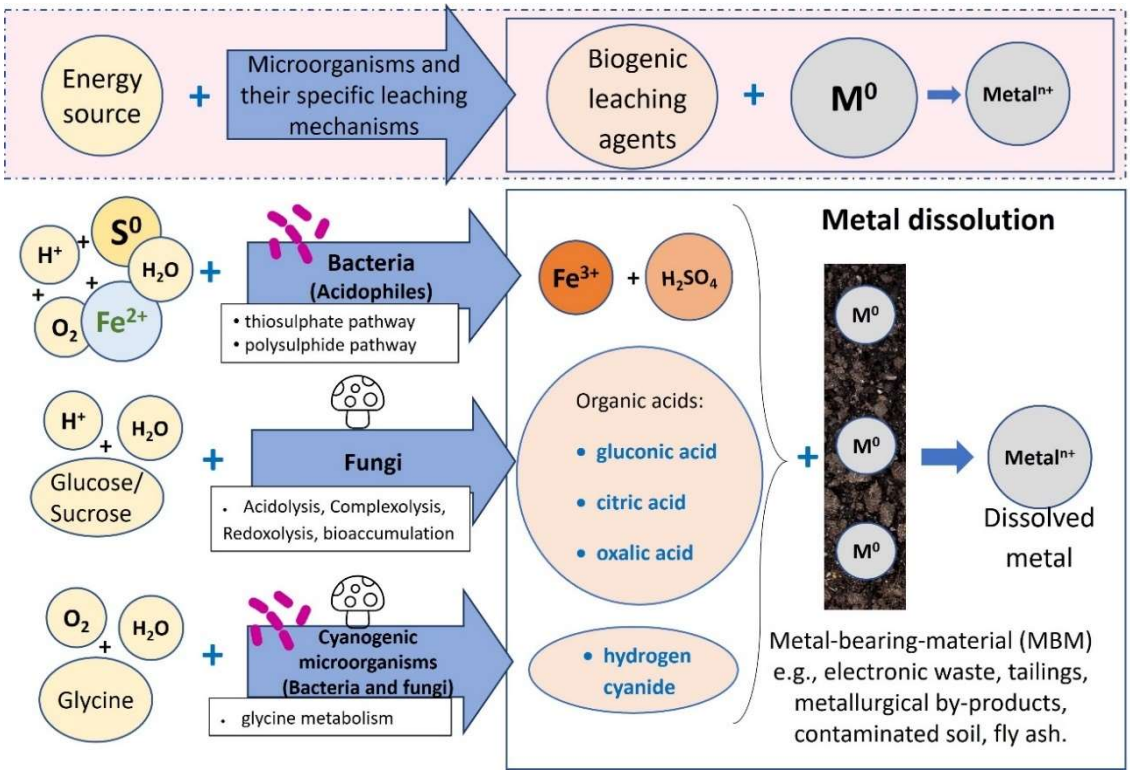


Figure 2-1. Illustration of bioleaching process. On the top, figure shows inputs for bioleaching process which are energy sources depending on microorganisms' type and microorganisms with their specific leaching mechanism. Microorganisms produce biogenic leaching agents which can be ferric iron, sulphuric acid, organic acids, and hydrogen cyanide. Then, biogenic leaching agents react with metal-bearing materials to dissolve metals. M^0 represents a metal in elemental state, Mn^+ represents a metal in ionic form is metal in elemental state.

This review provides a concise overview on the microbial diversity and associated bioleaching mechanisms used for solubilising metals. It then provides insights into their use through different bioleaching application strategies for various

metal-bearing-materials from lab-scale to pilot scale applications and summarises the key parameters influencing bioleaching process. The review further shed light on the remaining challenges for scale up alternatives and proposes an optimisation framework for future bioleaching research.

2.2 Microbial diversity and biological mechanisms

Based on their energy source preferences, microorganisms used for bioleaching are classified as chemolithotrophs, which oxidise inorganic compounds such as iron and sulphur to grow, and chemoorganotrophs, which oxidise reduced organic compounds (Srichandan et al., 2019, 2020). Chemolithotrophs are also commonly called acidophiles as they thrive under low pH values. Depending on their optimal growth temperatures, they can be further categorised as mesophiles, moderate thermophiles, and thermophiles (Natarajan, 2018). Chemolithotrophs can be classified as chemolithotrophic autotrophs, which utilises inorganic matter, and chemolithotrophic heterotrophs, which utilise organic carbon as an energy source. Acidophiles have been the most used group of microorganisms for bioleaching application, followed by fungi and cyanogenic microorganisms (see Appendix B for details).

2.2.1 Acidophilic bioleaching

Acidophilic microorganisms, mainly bacteria, are often used to extract metals from sulphidic ores e.g., pyrite. Most common bacteria are *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* (Table 2-1) (Mikoda et al., 2019; Yi et al., 2021). These microorganisms oxidize ferrous iron (Fe^{2+}) and/or sulphur compounds to produce ferric iron (Fe^{3+}) and sulphuric acid (H_2SO_4) which are effective oxidizing agents for metal solubilisation. For instance, *A. ferrooxidans* oxidizes ferrous iron and sulphur compounds, *A. thiooxidans* only oxidizes elemental sulphur and *Leptospirillum ferrooxidans* and *Leptospirillum ferriphilum* only use ferrous iron as energy source.

Table 2-1. Overview of the common microorganisms used for bioleaching application.

Microbial groups	Species	Microbial leaching mechanism	pH range and temperature	Energy source	Biogenic leaching agents	References
Acidophiles	*Acidianus brierleyi, A. infernus, Acidimicrobium species, Acidithiobacillus ferrooxidans, A. thiooxidans, A. caldus,* *Brevibacillus sp,* *Ferrimicrobium sp., Ferroplasma acidiphilum, F. acidarmanus, Leptospirillum ferrooxidans, L ferriphilum, Metallosphaera sedula, Sulfobacillus thermosulfidooxidans, S. thermotolerans, S. acidophilus, S. metallicus, S. acidocaldarius, S. solfataricus, S. brierleyi*	Thiosulphate pathway Polysulphide pathway	pH 0.8-2.5 Mesophiles: 28-37°C Moderate thermophiles: 40-60°C Thermophiles: 60-80°C	Fe^{2+} Reduced inorganic sulphur compounds e.g., S_8, $S_2O_3^{2-}$, H_2S	F^{3+} H_2SO_4	Figueroa-Estrada et al., (2020); Ma et al., (2021); Rodrigues et al., (2018); Tian et al., (2022)
Fungi	*Aspergillus niger, A. flavus, Penicillium simplicissimum,* *P. chrysogenum,*	Acidolysis Complexolysis Redoxolysis Bioaccumulation	pH 3.0-7.0 25-35°C	Glucose Sucrose	Gluconic acid Citric acid Oxalic acid Malic acid	Amiri et al., (2012); Qayyum et al., (2019); Dusengemungu et al., (2021)
Cyanogens	**Bacteria**: *Pseudomonas fluorescens, P. aeruginosa, P. Caldus, P. putida, P. aeruginosa, Chromobacterium violaceum* **Fungi:** *Clytocybe sp., Polysporus sp., Marasmius oreades*	Glycine metabolism	pH 7.0-11.0 25-35°C	Glycine	Hydrogen cyanide	Arab et al., (2020); Liu, Li and Ge, (2016); Srichandan et al., (2020)

Acidophiles typically use two pathways to solubilise metals from sulphidic ores: the thiosulphate and the polysulphide pathways. Ores such as pyrite, tungstenite, and molybdenite follow the thiosulphate pathway (Mishra et al., 2005; Srichandan et al., 2020). These ores do not soluble in acid; however, they are solubilised by Fe^{3+}. Thiosulfate pathway for pyrite ore oxidation with *A. ferrooxidans* can be expressed by the following equations (1-3):

$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (1)$$

$$FeS_2 + 6Fe^{3+} + 3H_2O \rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+ \quad (2)$$

$$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \rightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+ \quad (3)$$

On the other hand, ores such as chalcopyrite, arsenopyrite, sphalerite and galena are solubilised by collaboration of Fe^{3+} and H_2SO_4 or by H_2SO_4 only (Srichandan et al., 2020). Polysulfide oxidation pathway with *A. ferrooxidans* can be expressed by the following equations (4-6):

$$MS + Fe^{3+} + H^+ \rightarrow M^{2+} + 0.5H_2S_n + 2Fe^{2+} \qquad (n \geq 2) \quad (4)$$

$$0.5H_2S_n + Fe^{3+} \rightarrow 0.125S_8 + Fe^{2+} + H^+ \quad (5)$$

$$0.125S_8 + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ \quad (6)$$

Besides sulphidic form, metals are found as oxide and organically bound forms also present as hydroxides, carbonates, and silicates. Almost all acidophiles can manage to solubilise metals from these metallic forms at suitable conditions (Srichandan et al., 2020). Spent catalyst, fly ash and metallurgical slags can be given as examples of oxide form while sewage sludge is an organically bound metal form (Srichandan et al., 2020; Kremser et al., 2021). Thus, bioleaching has been one of the promising biotechnology applications used for recovering metal from wastes from these metal-bearing wastes (Ilyas and Lee, 2014; Villares et al., 2016).

A. ferrooxidans has the unique ability to oxidize ferrous iron and elemental sulphur to ferric iron and sulphuric acid, respectively (Asghari et al., 2013; Wang et al., 2016). Benzal et al. (2020) investigated copper bioleaching from printed

circuit boards from waste mobile phones by using *A. ferrooxidans*. 95-100% copper recovery was obtained in only 48 hours using two-step bioleaching process. Chen et al., (2015) examined the feasibility of copper recovery from waste printed circuit boards by using *A. ferrooxidans*. 95% copper recovery was achieved after 28 days. Both Benzal et al., (2020) and Chen et al., (2015) stated that copper bioleaching mechanism can be expressed by the following equations (7-9):

$$Cu^0 + 2Fe^{3+} \rightarrow Cu^{2+} + 2Fe^{2+} \quad (7)$$

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{A.f.} 4Fe^{3+} + H_2O \quad (8)$$

$$2Cu^0 + 4H^+ + O_2 \rightarrow 2Cu^{2+} + 2H_2O \quad (9)$$

Gholami et al. (2011) investigated aluminium (Al), Co, molybdenum (Mo), and nickel (Ni) recovery from a spent processing catalyst by using *A. ferrooxidans*. 63% Al, 96% Co, 84% Mo and 99% Ni dissolution were achieved after 30 days. Wang et al. (2009a) studied Copper, Lead and Zinc mobilization from printed wire boards by using *A. ferrooxidans*. 99% Cu recovery was achieved after 9 days, and more than 88.9% Zn and Pb become soluble after 5 days of leaching time. They indicated Zn and Pb solubilisation mechanisms by Fe^{3+} as following equations (10-11):

$$2Fe^{3+} + Zn^0 \rightarrow 2Fe^{2+} + Zn^{2+} \quad (10)$$

$$2Fe^{3+} + Pb^0 \rightarrow 2Fe^{2+} + Pb^{2+} \quad (11)$$

2.2.2 Fungal bioleaching

Aspergillus niger and *Penicillium simplicissimum* are the most common types of fungi found in bioleaching processes (Table 2-1). They produce various organic acids by using organic carbons such as glucose and sucrose as an energy source. The production of organic acids allows to dissolve metals from its ore or waste material. Among the organic acids produced, gluconic, citric, and oxalic acids have been shown as the most potent acids for bioleaching (Asghari et al., 2013; Srichandan et al., 2019). Fungi convert glucose or sucrose to organic acids

through several enzymatic reactions in cytosol and mitochondrion which are membrane-bound cellular compartments. By passing through cytoplasmic membrane to cytosol, glucose is converted into pyruvate via the glycolysis pathway. One of the produced two pyruvate molecules is decarboxylated to acetyl-CoA by mitochondrion via malate (Trivedi et al., 2022). The other pyruvate carboxylates to oxaloacetic acid in the cytosol. Oxaloacetic acid is transferred into the mitochondrion and produce citric acid by condensed with acetyl-CoA. In cytosol, oxaloacetic acid is converted into oxalic acid and acetic acid by oxaloacetase (Srichandan et al., 2019; Trivedi et al., 2022).

Dissolution of metals by using fungi generated organic acids follows acidolysis, complexolysis, redoxolysis and bioaccumulation pathways (Asghari et al., 2013). In the acidolysis process, fungi-generated organic acids provide protons, and these protons react with metal on the ore or waste surface. Metal ions generated from acidolysis step become stabilize in the complexolysis process. Complexation occurs between metal ion(s) and organic acid(s). Generalised acidolysis and complexolysis reactions for dissolution of Ni ion can be expressed with Eq. 12 and Eq. 13 and as follows (Asghari et al., 2013):

$$NiO + 2H^+ \rightarrow Ni^{2+} + H_2O \quad (12)$$

$$Ni^{2+} + C_6H_8O_7 \rightarrow Ni(C_6H_8O_7)^- + 3H^+ \quad (13)$$

In redoxolysis step, microorganisms dissolve metals through oxidation-reduction processes. Asgrahi et al. (2013) stated that redox processes have a minor role in fungal leaching; however, oxidation-reduction processes have a significant role in bioleaching employed chemolithoautotrophic microorganisms. Redoxolysis reaction for dissolution of Mn ion can be expressed as following equation (Asghari et al., 2013):

$$MnO_2 + 2e^- + 4H^+ \rightarrow Mn^{2+} + 2H_2O \quad (14)$$

Bioaccumulation mechanisms can be explained as actively transported metals through cell membrane accumulated into the cells and metal solubilisation continuously occur by disturbing equilibrium (Asghari et al., 2013; Srichandan et al., 2019).

2.2.3 Cyanogenic bioleaching

Cyanogenic microorganisms use organic carbon as energy source and mostly belong to Proteobacteria while there are few fungal species (Table 2-1). Their ability to produce cyanide, which is a good leaching agent, from glycine $(\mathrm{NH_2CH_2COOH})$ through several metabolic activities makes them promising microorganisms for precious metal recovery from ore and wastes (Arab et al., 2020; Brandl et al., 2008; Faramarzi et al., 2020; Liu et al., 2016).

Studies on *Chromobacterium violaceum*, *Pseudomonas putida* and *Pseudomonas florosence* showed that bioleaching mechanism is based on the neutral process in which produced cyanide (lixivant) derived from bio-generated hydrogen cyanide dissolves metals by forming a metal complex (Asghari et al., 2013; Faramarzi et al., 2020). Production of hydrogen cyanide from glycine is carried out by HCN synthase enzyme which is encoded by hcnABC operon. Hydrogen cyanide synthesis reaction equation can be expressed by following equation (Srichandan et al., 2019):

$$NH_2CH_2COOH + O_2 \rightarrow HCN + CO_2 + 2H_2O \quad (15)$$

According to Liu et al., (2016), bioleaching of gold by cyanogenic microorganisms typically involves an indirect process where biologically generated HCN forms soluble metallic complexes, such as $[Cu(CN_2)]^-$, $[Ag(CN_2)]^-$, $[Au(CN_2)]^-$ (Pourhossein et al., 2021). The equations proposed in this process are represented by equations (16-18) as follows:

$$4Au + 8CN^- \rightarrow 4Au(CN)_2^- + 4e^- \quad (16)$$

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad (17)$$

$$4Au + 8CN^- + O_2 + 2H_2O \rightarrow 4Au(CN)_2^- + 4OH^- \quad (18)$$

2.3 Bioleaching mechanisms, approaches, and processes parameters

2.3.1 Bioleaching mechanisms

There are two microbial leaching mechanisms that can be used to dissolve metals from ore or waste materials, which are classified based on their contact status: direct (Eq. 19 and Eq. 20) and indirect (Eq. 21-23) mechanisms. In the direct mechanism, microorganisms attach onto the ore/waste material surface via extracellular polymeric substances (Op de Beeck et al., 2021; Costa et al., 2018; Moazzam et al., 2021) and simultaneously dissolve metals with several reactions occurring in the region of the extracellular polymeric substances (He et al., 2014; Wang et al., 2018). In the indirect mechanism, the planktonic microorganism's mediate dissolution process by producing leaching agents such as Fe^{3+}, H_2SO_4, organic acids, HCN (Table 2-1) (Natarajan, 2018). These biogenic leaching agents dissolve metals in the ore or waste material without requiring a microbial attachment. As an example, dissolution of pyrite by *A. ferrooxidans* is shown in the equations (19-23) (Chen and Lin, 2010; Sand et al., 2001):

(a) Direct mechanism

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2H^+ + 2SO_4^{2-} \quad (19)$$

$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (20)$$

(b) Indirect mechanism

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 16H^+ + 2SO_4^{2-} \quad (21)$$

$$MS + 2Fe^{3+} \rightarrow M^{2+} + S^0 + 2Fe^{2+} \quad (22)$$

$$S^0 + 1.5O_2 + H_2O \rightarrow 2H^+ + SO_4^{2-} \quad (23)$$

Direct and indirect mechanisms are also called as contact and non-contact leaching, respectively. A third term, "cooperative leaching" is used to describe the combination of both contact and noncontact mechanism (Natarajan, 2018). In cooperative leaching, the microorganisms attach to the surface of metal-bearing-material (Eq. 19-20) and the free cells in the solution simultaneously mediate

dissolution (Eq. 21-23). The indirect/direct mechanism phenomena have led to development of different bioleaching engineering approaches in practice which are one-step, two-step, and spent-medium step bioleaching.

2.3.2 Bioleaching operations and approaches

2.3.2.1 Scale of bioleaching applications

Methods in bioleaching are mainly classified as agitation leaching for fine particles and percolation leaching process for coarse particles (Figure 2-2; Natarajan, 2018). At laboratory scale, most of the studies have been done in shake flask to reduce process complications and to obtain valuable information about the bioleaching process (Srichandan et al., 2020; Table B-8). Process parameters such as pH, energy source concentration, inoculum and solid concentration and agitation speed can be optimised in shake flask (Amiri et al., 2011a; Bayat et al., 2008). However, it is not sufficient to understand leaching kinetics to design commercial scale reactors.

Industrial scale applications using percolation methods are mostly on-site ex-situ methods which are heap, dump, vat reactor and the in-situ leaching method (Figure 2-2). Stirring method is used in commercial scale agitated tank reactor (ex-situ) (Maluckov, 2017; Zanbak, 2012; Table 2-2). Heap and dump have a similar construction, however; the difference is that to build heap metal-bearing-materials are subjected to size reduction, <25 mm, and placed on leach pads (Natarajan, 2018; Table 2-3). Microorganisms are maintained in a separate reservoir and irrigated on the top of the metal-bearing-materials. In-situ bioleaching is performed underground using natural porosity of rocks or creating porosity by blasting (Abhilash and Pandey, 2013; Vargas et al., 2020). Vat bioleaching is used as cost effective method to bioleach fine materials, 1-10 mm, in submerged condition, yet it is not prominent in commercial bioleaching operations (Natarajan, 2018; Watling, 2014). On commercial scale agitated tank reactors, the microorganisms and the metal-bearing-materials are maintained in the same place. Also, the complex process parameters are better controlled and observed in stirred condition for instance microbial growth kinetics, gas-liquid-solid mass transfer and temperature (Gericke et al., 2009; Natarajan, 2018).

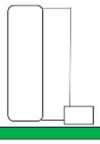
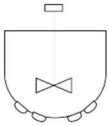
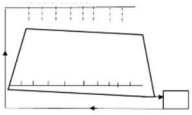
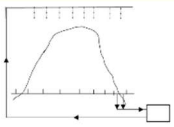
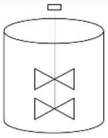
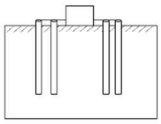
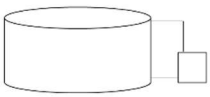
Small scale		
Shake flask		
	Particle size: 120 µm - 2mm Solid concentration: 0.1 - 20 %(w/v) Materials: low grade polymetallic ores, mine tailings, metallurgical waste (slag sludge and dust), fly ash, spent catalyst, waste electric electronic equipment (WEEE), contaminated soil, sewage sludge.	Table B-8
Intermediate scale		
Column reactor		
	Particle size: 0.1 - 30 mm Solid/liquid ratio: 3 - 1800 Material: low grade polymetallic ores, mine tailings, metallurgical by-products, spent catalyst, waste electric electronic equipment (WEEE), contaminated soil.	Table B-10
Stirred reactor		
	Particle size: < 1 mm Solid concentration: up to 20% (v/w) Material: spent catalyst, mine tailings, metallurgical by-products, contaminated soil, WEEE.	Table B-9
Large scale		
Heap		
	Particle size: 10-25 mm Solid amount and liquid volume: 1,000 - 180,000 tons. Up to 117,200 m^3 capacity solution pond. Material: low-grade ores	Table 3; Rawlings and Johnson (2007)
Dump		
	Particle size: 5mm – 2 m Solid amount and liquid volume: 120,000 – 500,000 or more. More or less than 194 m^3 capacity solution pond. Material: run-of-mines	Natarajan (2018); Groudeva et al., (2010)
Agitated tank		
	Particle size: < 1 mm Solid concentration: up to 20%, 900-1500 m^3 capacity Material: ground ores, flotation concentrates, refractory gold concentrates	Gericke et al., (2009); Natarajan (2018)
In-situ		
	Particle size: - Solid concentration:- Material: mineral deposits	Natarajan (2018); Vargas et al., (2020)
Vat		
	Particle size: 1-10 mm Solid concentration: - Material: copper oxide ores, refractory gold ores	Natarajan (2018); Rawlings and Johnson (2007); Kaksonen et al. (2018)

Figure 2-2. Bioleaching operations on different scales. Operations were divided by three categories including small scale, intermediate scale and large scale.

Table 2-2. Commercial scale stirred-tank reactor bioleaching operations for refractory gold concentrates using acidophiles

Owner/ operator	Mine/plant/ operation	Location	Concentrate treatment capacity t/d	Gold production, ounces	Year initiated	Reference
Anglo Gold Ashanti	Obuasi - BIOX®	Obuasi, Ghana	250	200,000	1994	Roberto and Schippers, (2022)
Kirkland Lake Gold	Fosterville - BIOX®	Victoria, Australia	211	150,000	2005	
Navoi Mining and Metallurgical Combinat	Kokpatas - BIOX®	Uzbekistan	2138	432,000	2009	
Polyus Gold	Olimpiada - BIONORD®	Krasnoyars, Russia	1500	965,000	2001	
Sino Gold Mining Limited & Guizhou	Jinfeng	China	790	-	2006	Gericke et al., (2009)
Golden Star Resources	Bogoso	Ghana	820	-	2006	
Centroserve	Suzdal	Kazakhstan	196	-	2005	Abhilash et al., (2015)

Table 2-3. Commercial scale heap bioleaching operations for secondary copper ores and/or mixed oxide/sulphide ores using acidophiles.

Owner/ operator	Mine/plant/ operation	Location	Cathode copper production, t/y	Year initiated	References
Iranian Babak Copper Company	Iranian Babak Copper Company	Iran	50,000	2020	Roberto and Schippers, (2022)
Zambia Consolidated Copper Mines	Chambishi	Zambia	10,000	2011	
BHP Billiton	-	Chile	115,000	1993	Gericke et al., (2009)
Freeport-McMoran	-	Arizona	380,000	2001	Abhilash et al., (2015)
Zijin Mining Group Ltd.	Jinchuan Copper	China	10,000	2006	
Straits Resources	Whim Creek and Mons Cupri	Australia	17,000	2006	
Barrick Gold Corporation	Zaldivar	Chile	150,000	1998	

However, compared to heap, it has drawback because it is only efficient up to 20% solid concentration (Natarajan, 2018).

From shake flask scale to production at industrial scale, column reactors and stirred bioreactors are used to perform intermediary scale operation. Lab scale stirred bioreactor is used to simulate commercial scale agitated tank bioleaching (often >1000 m^3) (Natarajan, 2018). Column bioleaching provides flexibility to optimise heap and dump specific parameters for example irrigation rate of the leachate. Microorganisms are mainly maintained in a separate reservoir. Column reactor can be operated in batch or recycling mode based on which one is desired to select to operate heap bioleaching (Srichandan et al., 2020). It can be operated both in fluidized bed, submerged, or drain mode, free flow, according to particle size (Natarajan, 2018; Pathak et al., 2019). Although aerated column is a good design to simulate a heap construction, there are studies have been tested column without aeration (Yang et al., 2013; Abhilash et al., 2010).

2.3.2.2 Bioleaching approaches

There are three approaches to operate microorganisms for bioleaching including: one-step (Figure 2-3a, b c), two-step (Figure 2-3d, e f) and spent-medium step (Figure 2-3g, h, i) bioleaching approaches (Moazzam et al., 2021). Each approach can yield different leaching efficiency mainly because of the metal toxicity and the difference of the associated bioleaching mechanism which are direct, indirect, or cooperative leaching mechanisms (Amiri et al., 2011b). In shake flask or stirred bioreactor, one-step bioleaching approach is applied by adding cells and the metal-bearing-material into a fresh growth medium at the same time (Figure 2-3b). Thus, microbial growth and metal dissolution begin simultaneously. In this approach, microorganisms are however often adversely affected by metal toxicity as well as alkaline nature of metal-bearing wastes before they have time to produce sufficient leaching agents resulting in insufficient metal dissolution (Srichandan et al., 2020; Kremser et al., 2022). In the two-step bioleaching (Figure 2-3e), first, microorganisms are added into a fresh growth medium. Then, cell cultures are incubated for 2-7 days or even

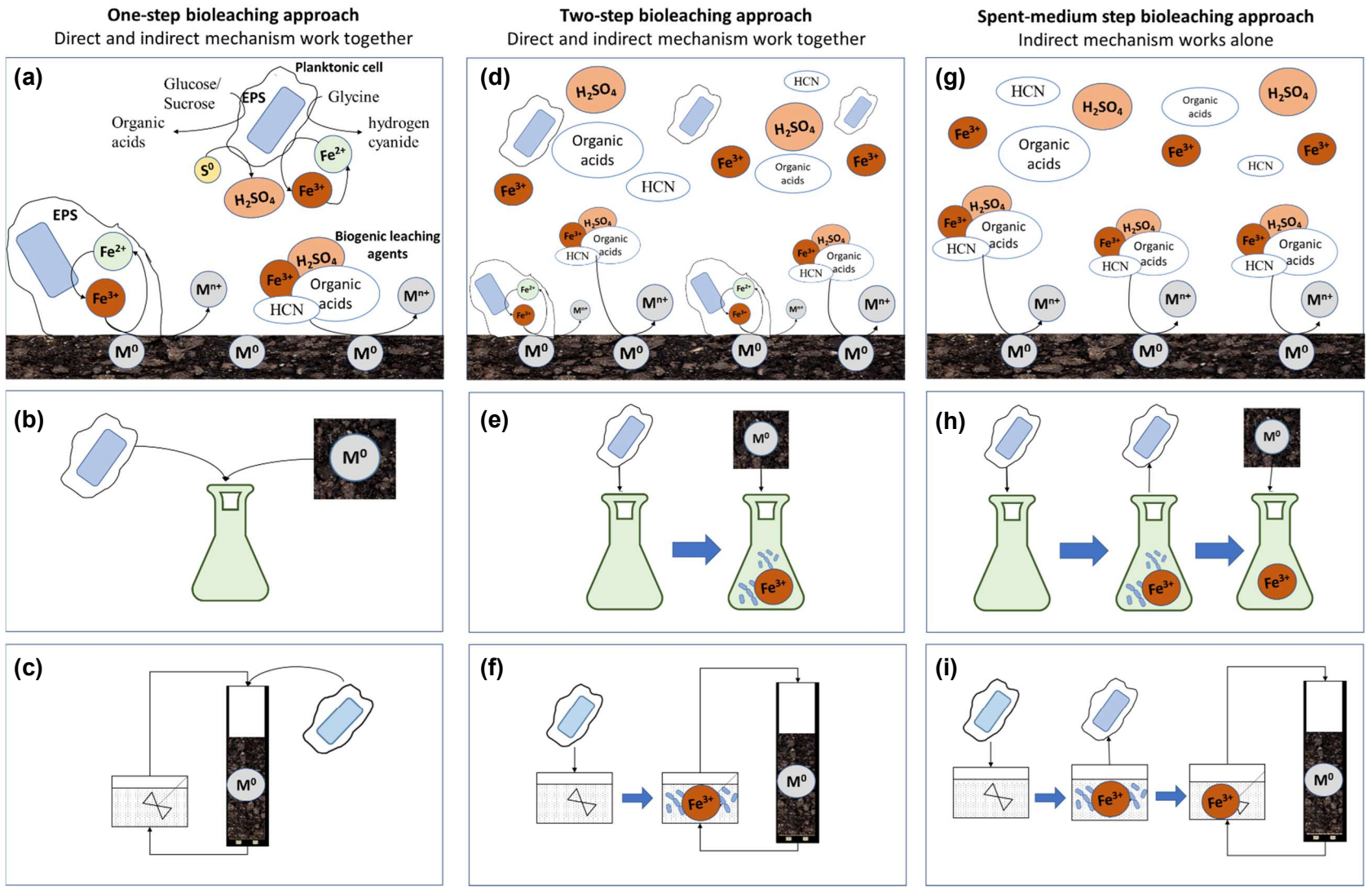

One-step bioleaching approach
Direct and indirect mechanism work together
Two-step bioleaching approach
Direct and indirect mechanism work together
Spent-medium step bioleaching approach
Indirect mechanism works alone
(a)
Planktonic cell
Glucose/
Sucrose
EPS
Glycine
Organic
acids
hydrogen
cyanide
S^0
Fe^{2+}
H_2SO_4
Fe^{3+}
EPS
Biogenic leaching
agents
Fe^{2+}
Fe^{3+}
M^{n+}
H_2SO_4
Fe^{3+}
Organic
acids
HCN
M^{n+}
M^0
M^0
M^0
(b)
M^0
(c)
M^0
(d)
H_2SO_4
Organic
acids
HCN
Fe^{3+}
M^{n+}
M^0
(e)
M^0
Fe^{3+}
(f)
Fe^{3+}
M^0
(g)
HCN
H_2SO_4
Organic
acids
Fe^{3+}
M^{n+}
M^0
(h)
M^0
Fe^{3+}
(i)
Fe^{3+}
M^0

Figure 2-3. Overview of bioleaching approaches used for metal solubilisation (a) mechanism of the one-step bioleaching approach; in this approach, both microorganisms and metal-bearing material are added to the medium at the same time, and the bioleaching process is carried out during the microorganisms culturing (b) one-step approach in shake flask, (c) one-step approach in column, (d) mechanism of the two-step bioleaching approach; in this approach, first, the microorganisms are cultivated in the absence of solid waste, then after microorganisms are reached the log phase, metal-bearing material is added into the culture to start the bioleaching, (e) two-step approach in shake flask, (f) two-step approach in column, (g) mechanism of the spent-medium step bioleaching approach; in this approach, the microorganisms are cultivated until stationary phase to produce maximum biogenic leaching agent. Then after microorganism are removed from lixiviant metal-bearing material is added into the cell free medium to start bioleaching, (h) spent-medium step approach in shake flask, (i) spent-medium step approach in column (Moazzam et al., 2021). EPS is the abbreviation of extracellular polymeric substances, HCN is hydrogen cyanide, M^0 represents a metal in elemental state, M^{n+} represents a metal in ionic form.

more, depending on the microbes used, in the absence of metal-bearing-material to allow microorganism to grow and produce leaching agents. Incubation time is 2 days or more for iron-oxidizing bacteria until the oxidation-reduction potential is ≥ 600 mV or until reduction is observed in pH in case of sulfur-oxidizing bacteria. Then the metal-bearing-material is added into the culture which still contains cells. In the spent-medium step (Figure 2-3h) bioleaching approach, can be also called cell-free bioleaching, cells are initially cultured until reaching the stationary phase of metabolites production. This follows removing cells to create cell-free, supernatant, medium. Then, metal-bearing-material is added in this cell-free medium (Moazzam et al., 2021). Both two-step and spent-medium bioleaching approaches usually provide better dissolution yield than one-step bioleaching (Amiri et al., 2011b). Spent-medium bioleaching can provide more flexible industrial application due to metal-bearing-material being not contaminated by microorganisms (Moazzam et al., 2021).

In bioleaching using the percolation method, the cells are typically kept separate from the metal-bearing material and maintained in a reservoir or external bioreactor (Figure 2-3c, f, i). The success of this approach depends on the location of inoculation, either in the column or reservoir. In laboratory-scale column operations, inoculating cells at the top of the column creates a one-step bioleaching process (Figure 2-3c), but this may result in lower yield (<20%) or

longer operation time (>100 days) due to metal toxicity (Ilyas et al., 2010b). Alternatively, a two-step approach involves first inoculating cells into a reservoir and allowing them to reach the log phase before feeding them into the column (Figure 2-3f; Mousavi et al., 2006). Another option is the spent-medium step (Figure 2-3i), where cells are first cultivated until the stationary phase of metabolite production and then removed to create cell-free medium that is fed into the column.

2.3.3 Conditions and parameters affecting bioleaching efficiency

Bioleaching efficiency is influenced by a wide range of parameters and conditions including pH, particle size, pre-treatment, solid concentration, inoculum concentration, energy source and concentration, agitation/irrigation rate, aeration rate, temperature, and catalysts.

2.3.3.1 Particle size

Particle size is a significant parameter for bioleaching in terms of both affecting leaching performance and guiding industrial application method (Natarajan, 2018). When the particle size decreases the available surface area increases. The rise in the available surface area provides high bioleaching yield and increase microorganisms interaction with ore/waste (Wang et al., 2018). Particles in bioleaching can be classified as either fine or coarse, with fine particles being less than 1 mm in size, and coarse particles ranging from 1 mm to over 25 mm depending on the type of leaching method used as follows: 1 mm - 10 mm (vat leaching); 10 mm - 25 mm (heap leaching); 25 mm < (dump leaching) (Natarajan, 2018). Fine particles have a higher potential for clogging, but this can be prevented by using acid agglomeration techniques (Srichandan et al., 2020). Additionally, fine particles can have a stronger buffering effect, while larger particle sizes can reduce buffering but can also lead to lower dissolution rates and longer operation periods due to limited mass transfer (Srichandan et al., 2020).

2.3.3.2 Pre-treatment

As a pre-treatment, acid washing, sterilisation, water washing and thermal treatment, are applied to increase the bioleaching performance in some cases (Chu et al., and2022; Wang et al., 2009b). When acidophiles are employed, acid washing is often performed for material which is alkaline in nature. To favour the microorganism pH stabilisation, ≤ pH 2, is carried out to by using dilute sulphuric acid (Chen et al., 2015; Ilyas et al., 2010b). Sterilisation by autoclaving, for 15–30 min at 121-135 °C, is often applied in lab scale bioleaching to remove indigenous microorganisms from metal-bearing-material to interpret the employed microorganisms' actual performance (Tezyapar Kara et al., 2022; Gomes et al., 2018; Wang et al., 2016). Water-washing are applied for removing water soluble inorganic salts from metal-bearing-materials. It has been found increased markedly the dissolution of Cd, Mn, and Zn from 76%, 45%, 53% to 96%, 91%, 68%, respectively, from municipal solid waste incinerator fly ash by A. Niger (Wang et al., 2009b).

Thermal pre-treatment is applied in some cases to remove organic matter from metal-bearing-material due to iron and sulphur-oxidising acidophiles are known to be sensitive to organic compounds (Torma and Itzkovitch, 1976). For example, Cu dissolution markedly increased from 47% to 100% after calcination of waste printed circuit boards in muffled furnace at 600°C for 60 minutes (Chu et al., 2022). Similarly, Mo and Co recovery from spent refinery catalysts increased from 18% to 100% and 79% to 94%, respectively, after thermal treatment at 400°C for 1-2 hours, in acidophilic bioleaching (Qian et al., 2020). On the other hand, more than 60% of Co, Ni, Cu, Zn, Fe release from polymetallic ore and 80–90% of U, Cu, Ni, Mn, Mo, Y and Zn dissolution from polymetallic black shale, with up to 10% organic matter content, have been reported by acidophiles (Bhatti, 2015; Watling et al., 2014). So, bioleaching can be performed for material contains up to 10% of organic content efficiently (>60% metal dissolution). In addition, Chu et al. (2022) stated that while thermal pre-treatment increased Cu dissolution from 47% to 100%, it decreased Ni dissolution from 100% to 35% due to the formation of acid insoluble NiO. Therefore, thermal pre-treatment can be considered for selective metal extraction.

2.3.3.3 pH and Oxidation-Reduction Potential

Oxidation-reduction potential and pH directly affect microbial activity, metal solubility, and generation of precipitation layers such as jarosite (Chen et al., 2015; Halinen et al., 2009; Zare Tavakoli et al., 2017c; Zhao et al., 2019). Ores and solid wastes contain acid consuming minerals such as sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg). When acidophilic microorganisms are employed, these high reactive minerals increase the pH and inhibit the acidophilic microorganisms. Thus, optimisation of pH is recommended (Ilyas et al., 2010a). Optimal pH range is guided by the types of microorganisms used for bioleaching (Table 2-1), and can be defined by conducting an optimisation study (Table 2-4). To avoid low dissolution yield, maintaining pH at optimum level by adding sulphuric acid is recommended (Chen et al., 2015; Roy et al., 2021). For the bioleaching employing fungi and cyanogenic bacteria pH of the culture medium can be adjusted my using HCl and NaOH (Naseri and Mousavi, 2022). During bioleaching an automatic control unit via connected pH sensor can be utilised to maintain optimum pH at the constant level (Kremser et al., 2022).

Oxidation-reduction potential indicates the activities of the species and the flow of ions from metal-bearing-material to leaching media (Muddanna and Baral, 2021; Roy et al., 2021). Muddanna and Baral, (2021) stated that oxidation-reduction potential ≥ 600 is good indicator for high Fe^{3+} for *A. ferrooxidans*. During acidophilic bioleaching oxidation-reduction potential above 400 mV indicates there is a high concentration of Fe^{3+} and high bacterial growth (Rawlings and Johnson, 2007; Roy et al., 2021). Zare Tavakoli et al., (2017c) achieved 100% uranium dissolution from low grade uranium ore when oxidation-reduction potential was 550 mV by using *A. ferrooxidans*.

2.3.3.4 Solid concentration

Solid concentration is expresses as the term pulp density (% w/v) for shake flask and stirred tank. Low solid concentration (<1%) provides efficient dissolution for both acidophilic and fungal bioleaching; however, this increases the cost of operation in large scale application (Srichandan et al., 2020). High solid

Table 2-4. Bioleaching yield (%) for different metals by pH optimisation

Material	Microorganisms	Element	pH	Bioleaching yield (%)	Operational conditions	Class	Reference
Dewatered electroplating sludge	Mixed acidophilic strains including *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*	Cu	1.5	97%	Temperature: 30 °C Period: 24 hours Particle size: ≤75 µm Inoculum rate: 10% (v/v) Agitation speed: 500 rpm Pulp density: 5% (w/v) Aeration rate: 20 L/h Bioleaching approach: Two-step	Bacteria	Zhang et al., (2020)
			2	100%			
			2.2	98%			
			2.5	94%			
		Ni	1.5	98%			
			2	100%			
			2.2	98%			
			2.5	81%			
		Zn	1.5	100%			
			2	100%			
			2.2	100%			
			2.5	52%			
		Cr	1.5	99%			
			2	100%			
			2.2	94%			
			2.5	65%			
Contaminated soil	*Aspergillus flavus*	Pb	5	8%	Temperature: 30 °C Period: 15 days Particle size: 2 mm Inoculum rate: 2% (v/v) Agitation speed: 130 rpm Pulp density: 5% (w/v) Bioleaching approach: -	Fungi	Qayyum et al., (2019)
			5.7	16%			
			7	18%			
			9	4%			
		Cd	5	25%			
			5.7	30%			
			7	40%			
			9	16%			
		Zn	5	27%			
			5.7	53%			
			7	53%			
			9	34%			
Waste Printed Circuit Boards	*Pseudomonas balearica*	Au	7	46%	Temperature: 30 °C Period: 7 days Particle size: 150 ≤ µm Inoculum rate: 5% (v/v) Agitation speed: 150 rpm Pulp density: 1% (w/v) Bioleaching approach: two-step	Cyanogenic bacteria	Kumar et al., (2018)
			8	55%			
			9	68%			
		Ag	7	16%			
			8	25%			
			9	34%			

concentration causes high buffering effect in acidophilic bioleaching, so acid addition into culture is needed to keep pH at favourable level during bioleaching (Chen and Lin, 2010). In some optimisation studies, solid concentration was observed as the most important parameter (Gu et al., 2017; Nkulu et al., 2013). Like the other parameters, different pulp density can provide variations on leaching efficiency of different metals. For instance, Muddanna and Baral, (2021) achieved the highest La dissolution (83%) when 1% pulp density of spent fluid catalytic cracking catalyst was used, while the highest Ce dissolution (23%) was achieved when 5% and 7% pulp density was used. Amiri et al. (2011b) investigated the effect of one-step, two-step and spent medium step approaches using 1-5% (w/v) pulp density of spent hydrocracking catalyst by P. simplicissimum. Highest recovery of W, Al and Mo was achieved in two-step approach when 3% pulp density was used. Dissolution of W and Al were decreased from 67% to 37% and from 17% to 12% in the one-step approach when pulp density was increased from 1% to 5%.

When the spent medium step approach was used, the dissolution of all investigated metals was decreased as the pulp density increased. In different approach, Fe and Ni dissolution remained unchanged or decreased when pulp density increased. Overall results suggested that different metals were affected at different rate from selected bioleaching approach and pulp densities, but the two-step approach can provide high metal dissolution (>90% W, Fe, Mo dissolution) at high pulp density (>1%). Pulp density can be optimised up to 20% (w/v) for agitated tank reactors (Natarajan, 2018) (Figure 2-2). Higher solid liquid ratio (>20% w/v) can be used for column, heap, and dump bioleaching. For instance, Mousavi et al., (2006) achieved 72% zinc dissolution by using 22 L of culture for 410 kg of low-grade sphalerite ore in column bioleaching after 120 days. BIOPROTM used 117,200 m3 capacity solution pond to bioleach 810,000 tons of refractory sulfidic ore in commercial heap process (Rawlings and Johnson, 2007).

2.3.3.5 Energy source choice and its concentration

The type of energy source is dependent on the selected microorganisms (Table 2-5). The choice of energy source is also important for the material that should be treated. Some oxidic materials such as waste incineration ashes and slags require the action of both Fe^{3+} and H_2SO_2 (Kremser et al., 2021). Wang et al., (2015) discussed that during bioleaching of Pb/Zn smelting slag Indium (In) dissolution was both acid dissolution by biogenic H_2SO_4 and oxidation/reduction by Fe^{2+}/Fe^{3+}, whereas Zn, Cd and Pb followed acid dissolution mechanism. Others for instance spent Li-Ion batteries or waste printed circuit boards require Fe^{2+}, Fe^{3+} or H_2SO_4 only (Chen et al., 2015; Ghassa et al., 2020; Roy et al., 2021).

Optimisation of energy source concentration ranging between 0.1 and 20% (w/v) can be recommended to achieve a high bioleaching yield and to make the process economical (Gu et al., 2017; Mousavi et al., 2006; Xu and Ting, 2004). Pathak et al. (2009) investigated the effects of ferric ion concentration on bioleaching of sewage sludge using two different energy sources, ammonium ferrous sulphate and ferrous sulphate, for iron-oxidising microorganisms. Using ferrous sulphate provided better metal bioleaching yield, 64% Cu, 58% Ni, 76% Zn, 52% Cr, than ammonium ferrous sulphate, 56% Cu, 48% Ni, 68% Zn, 42% Cr.

2.3.3.6 Microbial acclimatisation and inoculum concentration

Before starting the bioleaching process, it is recommended to acclimatise the selected microorganism(s) with the metal-bearing-material to enhance their metal toxicity tolerance (Amiri et al., 2011b). This is typically performed by culturing the microbes in liquid medium until the log phase is reached. Then small amounts (0.2% w/v) of solid material are added into the culture (Chen et al., 2015). Once the cells reach again the log phase, sub-culturing is performed fir increased material concentration. This step can be repeated as necessary to reach the desired solid:liquid ratio (Muddanna and Baral, 2021). Acclimatisation can also be performed on petri dish using a growth medium containing metal ions for fungi (Amiri et al., 2011b). Several bioleaching studies confirmed that acclimatised microorganisms provided higher metal dissolution than non-acclimatised

Table 2-5. Optimal energy source concentration for various microorganisms. Conc, stands for concentration, B stands for bacteria, F stands for fungi.

Material	Microorganisms	Energy source	Optimisation values % (w/v)	Optimal conc. or selected conc. % (w/v)	Leaching yield (%)	Type	Reference
Sewage sludge	Indigenous sulphur oxidising bacteria (*Acidithiobacillus ferrooxidans, A. thiooxidans and Thiobacillus thioparus)*	elemental sulphur	0.1, 0.2, 0.3, 0.55, 1.0	0.1-0.3%	>60% Cu, Zn, Pb, Ni	B	Chen and Lin (2010)
	Indigenous iron oxidizing bacteria	ammonium ferrous sulphate (source of Fe^{2+})	-	2	56% Cu, 48% Ni, 68% Zn, 42% Cr	B	Pathak et al. (2009)
		ferrous sulphate (source of Fe^{2+})	-	2	64% Cu, 58% Ni, 76% Zn, 52% Cr	B	
Low grade uranium ore	*A. ferrooxidans*	ferrous sulphate (source of Fe^{2+})	0.5, 1, 1.5, 2	1.45	90% U	B	Zare Tavakoli et al. (2017b)
Low grade uranium ore	*A. ferrooxidans*	ferrous sulphate (source of Fe^{2+})	1, 2, 4	2	100% U	B	Zare Tavakoli et al., (2017c)
Spent refinery catalyst	*Aspergillus niger*	sucrose (source of organic C)	9, 11, 13, 15, 17	9.40	100% Mo, 46% Ni, and 14% Al.	F	Amiri et al. (2012)
Power plant residual ash	*A. niger*	sucrose (Source of organic C)	5, 7.5, 10, 12.5	10.20	83% V	F	Rasoulnia and Mousavi (2016)
Soil from landfill of e-waste	indigenous cyanogenic bacteria	glycine	0.005, 0.2, 0.5, 1	0.20	96.7% Cu	B	Arab et al. (2020)
Printed circuit boards	*Pseudomonas aeruginosa*	glycine	0.06, 0.1, 0.2, 0.3, 0.34	0.10	90% Ag	B	Merli et al. (2022)

microorganisms. For example, Abhilash et al., (2013) acclimatised *A. ferrooxidans* and *L. ferrooxidans* up to 5% (w/v) sample concentration. Once both species were acclimatised, they were able to solubilise 57% and 66%, respectively of uranium from low-grade apatite rich uraninite ore. Muddanna and Baral, (2021) adapted *A. ferrooxidans* up to 20% (w/v) spent fluid catalytic cracking catalyst. They stated that acclimatized microorganisms ensured higher bioleaching yield than non-acclimatised culture. Chen et al., (2015) adapted *A. ferrooxidans* to waste printed circuit boards from 0.2% (w/v) to 3.5% (w/v) and achieved 94.8% Cu recovery after 28 days. Ilyas et al., (2010b) operated for 2 years a column bioleaching process by using adapted mixed culture of *Sulfobacilllus thermosulfidooxidans* and *Thermoplasma acidophilum*. They were able to recover 80% Zn, 64% Al, 86% Cu and 74% Ni from 10 kilos of electronic scrap.

Inoculum concentration is also an important parameter affecting leaching time and dissolution yield (Zare Tavakoli et al., 2017b). Several researchers tried to optimise inoculum concentration from 0.1% up to 30% (v/v) for different metal bearing material (Ilyas et al., 2012; Mo et al., 2019; Zare Tavakoli et al., 2017b; Table B-8; Table B-10). When the effect of inoculum concentration was evaluated with the one-factor-at-a-time methodology, it appeared that an increase in the inoculum concentration had positive effect on the bioleaching performance (Hosseinzadeh, Rastegar and Ashengroph, 2021; Jagannath, Vidya Shetty and Saidutta, 2017). When multivariable optimisation method was used (e.g., Plackett−Burman factorial design, central composite design), inoculum concentration was usually found to be the second, third or fourth important factor. So that, it mostly come after pH, solid concentration, and energy source concentration by the order of importance (Arshadi et al., 2019; Zare Tavakoli et al., 2017b; Xu and Ting, 2004). Overall, several studies revealed that increase in inoculation percent had positive effect on bioleaching yield; however, higher concentration than optimum value caused the precipitation of some metal ions in reactor during bioleaching of low-grade ore or e-waste (Jagannath et al., 2017; Zare Tavakoli et al., 2017b). Therefore, inoculum concentration is suggested as an optimisation parameter for future works due to it may have a positive effect on

bioleaching yield and the cost of operation. Since to reach a high inoculation percent high energy source needs to be used, it will also increase the operational cost.

2.3.3.7 Aeration rate and temperature

Aeration provides an efficient transfer of O_2 and CO_2 for microorganisms in both agitated and percolated systems (Srichandan et al., 2020). 1.5 – 4 mg/L dissolved oxygen concentration is recommended to be managed inside the reactor (Rawlings and Johnson, 2007). Ilyas and Lee, (2014) optimised aeration rate between 0.07 - 1 L/min for a stirred bioleaching tank inoculated with *Sulfobacillus thermosulfidooxidans*. They found that increasing aeration rate from 0.07 to 0.5 L/min accelerated the leaching rate for Al, Cu, Zn, Ni and reduced the lag time of the bacterial culture approximately 50%. However, increasing the aeration rate more than 0.5 L/min adversely affected the bacterial growth and resulted in decreased metal dissolution. Zare Tavakoli et al., (2017a) tested different air flows ranging between 50 and 250 L/h for 7.5 cm diameter column for U bioleaching. They reported that the optimum value was 100 L/h (1667 ml/min) to achieve 100% of U bioleaching. Higher air flow than 100 L/h caused decrease in bioleaching yield due to bacterial growth adversely affected by increasing excessive turbulence, shear stress and cellular attrition in column. Chen et al. (2015) used a 20 L/min of aeration rate for a column which has an internal diameter of 6 cm. They recovered 95% Cu form waste printed circuit boards. Natarajan (2018) reported the typical aeration rates for a heap operation are 0.1 - 0.5 m^3/m^2h.

Maintaining temperature at desired level based on employed microorganisms is important to achieve efficient metal dissolution (Table 2-1) (Mousavi et al., 2006; Srichandan et al., 2020). Irrigation and aeration rate influence the temperature in percolation systems (Mousavi et al., 2006). Due to bioleaching is an exothermic process and ores have diverse indigenous organisms, the dominant microbial community can change with time in heap bioleaching (Natarajan, 2018). Stirred tanks are designed to maintain the reactor temperatures at desired levels to cope with exothermic heat generation, thus they provide more controlled environment

than heap (Natarajan, 2018). To avoid significant temperature changes in column bioleaching, using water bath for reservoir and water jacket around the column can be preferred (Ilyas et al., 2010b).

2.3.3.8 Agitation speed and irrigation rate

While agitation rate is a specific parameter for shake flask and stirred bioreactor, irrigation rate is a specific parameter of the percolated systems e.g., column, heap, dump (Natarajan, 2018). Good mixing condition is needed to ensure gas-liquid-solid transfer and heat distribution (Natarajan, 2018). Optimum agitation speed will be depended on the type and design of the reactor (Amiri et al., 2011b; Eisapour et al., 2013) and the target metal in the metal-bearing-material (Nkulu et al., 2013). High metal dissolution (>90%) can be achieved when agitation speed is set up between 120-240 rpm for shake flask (Amiri et al., 2011b; Işıldar et al., 2016), and 150-600 rpm for stirred tank bioleaching (Eisapour et al., 2013; Kremser et al., 2020; Pathak et al., 2015). For example, Nkulu et al., (2013) optimised agitation speed between 200 and 400 rpm along with temperature, pH, leaching duration and pulp density for bioleaching of a polymetallic flotation concentrate by using acidophiles. As a result, agitation speed appeared as the second most important parameter for the Co leaching and optimum value was 250 rpm. On the other hand, for Ni and Cu, it was the least important factor, and the optimum values were 300 and 350 rpm, respectively. Ilyas et al., (2010a) investigated the best operational conditions for bioleaching of metal ions from low grade sulphide ore by optimising agitation speed along with four other parameters. The agitation speed was the third significant parameter for *S. thermosulfidooxidans*, and the optimum value was 180 rpm to achieve 72 Zn%, 68% Co, 78% Cu, 81% Ni and 70% Fe dissolution.

In terms of irrigation rate, Mousavi et al., (2006) reported that liquid irrigation rate is an important operational parameter to increase the bioleaching kinetic, and it must be optimised. Like agitation speed there is no clear trend for irrigation rate as it will be depended on the type and design of the reactor and the metal-bearing-material (Chen et al., 2015; Zare Tavakoli et al., 2017a, b). Typical irrigation rates are 5-20 L/m2h for heap and 5-40 L/m2h for column (Natarajan,

2018). Mousavi et al., (2006) tested three irrigation rates (5, 10, 20 L/m2h), for A. ferrooxidans and Sulfobacillus. The lowest irrigation rate provided higher Zn dissolution, >70%, from the low-grade sphalerite ore while the highest irrigation rate provided only <50% Zn dissolution for both microorganisms. In another study, Chen et al., (2015) used an irrigation rate of 40 ml/min by recirculating the leaching solution. They suggested that increasing the cycling velocity (> 40 ml/min) of the leached solution can accelerate the kinetics of bioleaching yield in the 6 cm diameter column reactor. In contrast, Zare Tavakoli et al., (2017) evaluated two irrigation rates (0.25 and 2.25 ml/min) along with seven independent parameters, aeration rate, the concentration of initial ferrous, pH, temperature, inoculation percent and particle size, in column (no circulation) bioleaching for uranium recovery. They found that that irrigation rate was not a statistically important parameter.

2.3.3.9 Catalyst

Bioleaching performance can be enhanced by the addition of catalysts into the bioleaching medium, such as silver ions (Ag^+), graphene, biochar. For example, Zeng et al., (2013) investigated the effect of silver ions (Ag^+) on the solubilisation of cobalt (Co) from spent lithium batteries by using *A. ferrooxidans*. 98% Co recovery was achieved after 7 days of bioleaching, whereas Co solubilisation was only 43% in the absence of Ag^+. Wang et al., (2016) showed that the addition of biochar to e-waste contribute to the complete solubilisation of copper (Cu) in 2 days, while only 80% was achieved without it. Gu et al., (2017) explored the catalytic effect of graphene on the dissolution of copper from waste printed circuit board by using *A. ferrooxidans*. Copper dissolution was enhanced by 10% compared to the control. Based on the conducted studies it can be assumed that utilisation of catalyst can provide higher bioleaching yields. However, understanding the additional materials effect on bioleaching kinetic can be difficult. Thus, it can be suggested that once the main operational parameters, which are pH, solid concentration, energy source concentration, irrigation rate/agitation speed, are optimised, the catalyst addition can be evaluated to increase further the bioleaching yield.

2.4 Industrial application needs and future perspectives

Bioleaching is already performed on industrial scale for various types of ores to extract copper and gold by using acidophiles (Demergasso et al., 2010; Galleguillos et al., 2008; Gericke et al., 2009; Halinen et al., 2009; Soto et al., 2013). Heap and dump as well as agitated tank are the most preferred operation types for industrial scale applications. However, in large scale changes in pH and microbial community structure due to poor management of operational parameters e.g., air flow and CO_2 availability lead to a decreased leaching rate (Gao et al., 2021; Marín et al., 2021). Recent advances in biomining such as gene libraries, Polymerase Chain Reaction (PCR)-based techniques are promising for a better understanding of the microbial dynamics (Marín et al., 2021; Natarajan, 2018). Besides, optimisation of new influencing parameters such as micro-cracks (Chen et al., 2020) and characterisation of novel microorganisms (Natarajan, 2018) may help to overcome industrial scale limitations. In-situ bioleaching is successfully applied for Cu currently. Yin et al., (2018) reported some underground in-situ bioleaching operations for copper in China, achieving over 95% recovery in Tongguanshan Copper Mine in 1980 and >500 t/a in Zhongtiaoshan Copper Mine in 2000. In terms of uranium, a few in-situ bioleaching studies have been reported with 50-70% efficiency (Abhilash et al., 2015). Lots of research are promising to be in-situ bioleaching widely applicable in future (Götze et al., 2022; Huang et al., 2018; Laurent et al., 2019) and to reduce its possible environmental impacts (Ballerstedt et al., 2017).

To the best of the authors knowledge there is no commercial bioleaching applications for other metal-bearing-wastes than ores (Gao et al., 2021; Kaksonen et al., 2020; Natarajan, 2018). Several promising intermediate scale, which are column and stirred tank, bioleaching studies have been published for various types of metal-bearing-material (Table 2-2 -Table 2-4), yet they are not commercialised (Gao et al., 2021; Srichandan et al., 2019). Although lab bioleaching experiments revealed high bioleaching yields for some metals (>80% Al, Cu, V, Zn, Ni) at low pulp densities (≤5% w/v), research needs to be intensified to understand and accelerate the bioleaching kinetics towards industrial scale (Chen et al., 2015; Nkulu et al., 2013; Pathak et al., 2019). One limitation about

acidophiles that during bioleaching leached metal ions in the solution can precipitate due to jarosite formation (Baniasadi et al., 2019). This adversely affects the recovery and inhibits the bioleaching. To maintain jarosite formation optimisation of temperature, pH and ferrous iron concentration as well as continuous pH adjustment is recommended (Opara et al., 2022). In many bioleaching studies one-factor-at-a-time methodology was used to identify the optimum value for these parameters. However, rather than using one-factor-at-a-time methodology, multiparameter optimisation is recommended to understand the interactions between parameters and to identify optimal conditions in the process (Niu et al., 2016). According to this, optimisation can be performed as two-phases process. First, analysing of process parameters using a screening method, such as Plackett-Burman, this will allow researcher to understand the most influencing factors as a first insight (Amiri et al., 2011a; Zare Tavakoli et al., 2017b). Then, further optimisation can be performed with response surface methods, such as a central composite design or Box–Behnken, or Taguchi orthogonal array design to find optimum values for the identified most influencing parameters (Amiri et al., 2011a; Jalali et al., 2019; Mo et al., 2019; Nkulu et al., 2013). Here, we propose a two-phase optimisation route to achieve a high metal recovery yield from small to large scale bioleaching operation (Figure 2-4) (see Figure B-1 for the detailed flow chart). Our procedure modified from Potysz et al., (2018) the “flowsheet to develop optimised bioleaching protocol for slags” and improved and generalized for all types of metal-bearing-materials. Besides, analysing metals leaching kinetics provide broader understanding of the leaching behaviours of different metals during bioleaching (Amiri et al., 2012; Chen et al., 2015; Pathak, Srichandan and Kim, 2019). Therefore, selective metal solubilisation can be possible (Nkulu et al., 2013). In addition, there are limited studies about selective recovery of target metals from the biolixiviant after bioleaching (Kremser et al., 2022). In conclusion, more collaborative research with chemist, biologist and metallurgist is needed to make bioleaching of metal-bearing-wastes commercially feasible (Holmes, 2008; Roy et al., 2021).

To recover metals from some metal-bearing-materials, such as e-waste, biohydrometallurgy requires low installation and operation costs and provides

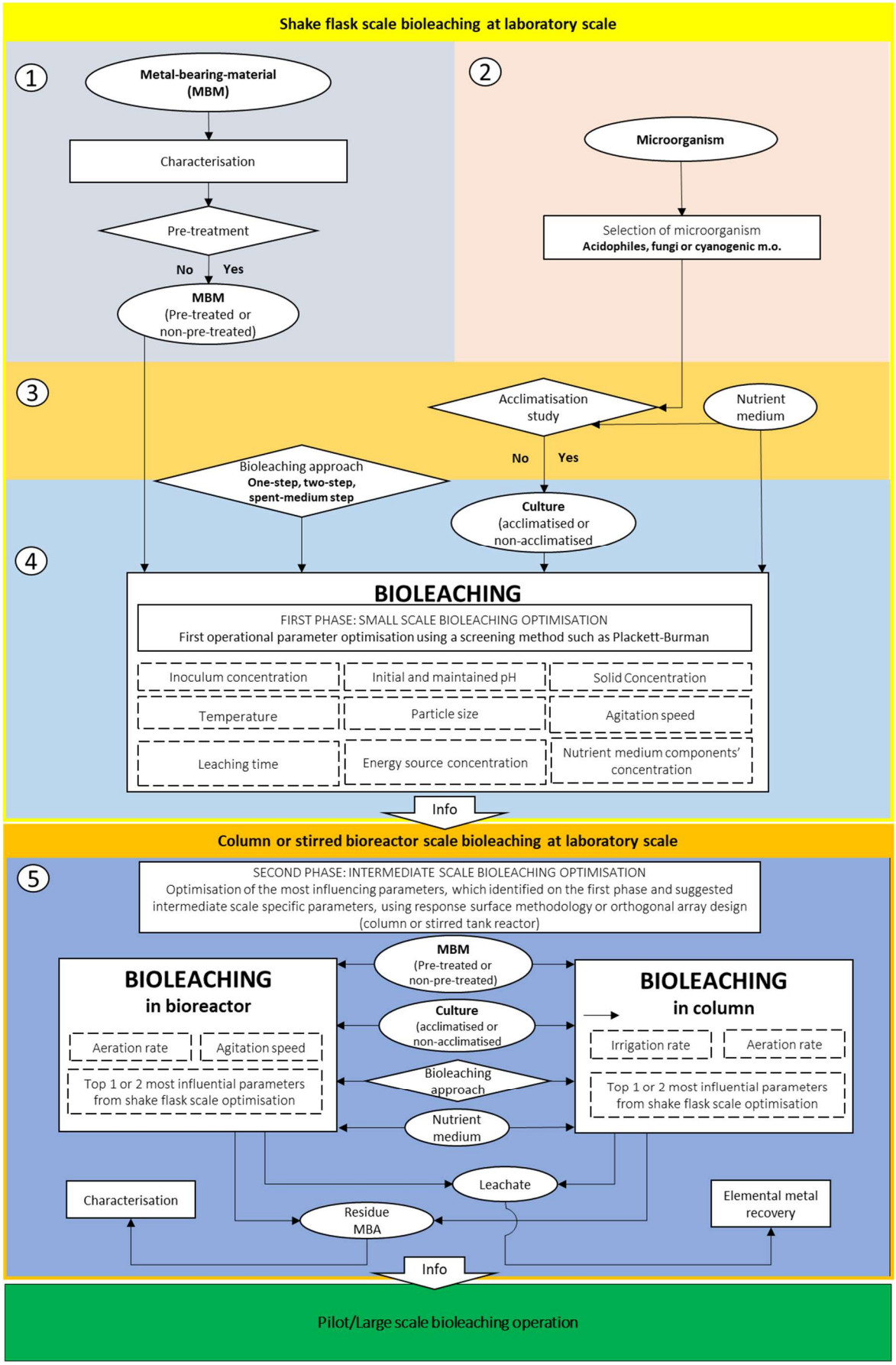

Shake flask scale bioleaching at laboratory scale
1
Metal-bearing-material (MBM)
Characterisation
Pre-treatment
No
Yes
MBM (Pre-treated or non-pre-treated)
2
Microorganism
Selection of microorganism
Acidophiles, fungi or cyanogenic m.o.
3
Acclimatisation study
Nutrient medium
No
Yes
Bioleaching approach
One-step, two-step, spent-medium step
Culture (acclimatised or non-acclimatised
4
BIOLEACHING
FIRST PHASE: SMALL SCALE BIOLEACHING OPTIMISATION
First operational parameter optimisation using a screening method such as Plackett-Burman
Inoculum concentration
Initial and maintained pH
Solid Concentration
Temperature
Particle size
Agitation speed
Leaching time
Energy source concentration
Nutrient medium components' concentration
Info
Column or stirred bioreactor scale bioleaching at laboratory scale
5
SECOND PHASE: INTERMEDIATE SCALE BIOLEACHING OPTIMISATION
Optimisation of the most influencing parameters, which identified on the first phase and suggested intermediate scale specific parameters, using response surface methodology or orthogonal array design (column or stirred tank reactor)
MBM (Pre-treated or non-pre-treated)
Culture (acclimatised or non-acclimatised
Bioleaching approach
Nutrient medium
BIOLEACHING in bioreactor
Aeration rate
Agitation speed
Top 1 or 2 most influential parameters from shake flask scale optimisation
BIOLEACHING in column
Irrigation rate
Aeration rate
Top 1 or 2 most influential parameters from shake flask scale optimisation
Leachate
Characterisation
Residue MBA
Elemental metal recovery
Info
Pilot/Large scale bioleaching operation

Figure 2-4. Two-phase optimisation routes for bioleaching of metal-bearing materials. Flow chart covers characterisation and pre-treatment of metal-bearing materials, microorganisms selection and acclimatisation followed by first phase of optimisation which is on shake flask scale. In the second phase of optimisation, intermediate scale bioleaching is suggested using column or bioreactor. MBM stands for metal-bearing material. Modified and improved from Potysz et al., (2018). See Figure B-1 for the detailed flow chart.

more environmentally friendly process when compared to pyrometallurgy and hydrometallurgy (Arya and Kumar, 2020b; Baniasadi et al., 2019). Applying life cycle assessment and techno economic analysis are recommended by several researchers as it would be very helpful to quantifying the environmental impact of the process (Baniasadi et al., 2021; Sadhukhan, Ng and Hernandez, 2014; Villares et al., 2016). Some small-scale life cycle assessment provided valuable insights about the impacts of the bioleaching process as well as guide to researchers to identify potential hotspots to reduce the process overall impact. For instance, Sun et al., (2016) found that during bioleaching of Zn-Mn batteries, cutting and crushing the batteries have the highest environmental impact on the human toxicity and marine ecotoxicity. In another techno-economic analysis life cycle assessment showed that during bioleaching of fluidized catalytic cracking catalysts 44% of the cost was due to the using of raw energy source for microorganisms, and raw energy source and electricity requirement was responsible for the largest proportion of the environmental impact (Thompson et al., 2018). Researchers suggested that utilisation of alternative energy sources, for example agricultural waste or real organic wastewater for heterotrophs, and optimisation of energy source can reduce the environmental impact of the process (Baniasadi et al., 2021; Gavrilescu, 2022; Jin et al., 2019). Moreover, an ex-ante life cycle assessment study, based on small scale laboratory and pilot bioleaching study results, can help to predict the environmental impact of a commercial scale bioleaching application on the early development stage (Villares et al., 2016).

2.5 Conclusion

Bioleaching has emerged as a promising cost-effective and environmentally friendly alternative for recovering metals from complex metal-bearing materials.

Acidophilic bacteria and fungi are key microorganisms that play an important role in metal dissolution through specific mechanisms, and recent advances in gene libraries and PCR-based techniques offer effective on-site monitoring and better understanding of microbe-mineral/waste interactions. Although bioleaching has been successfully applied at a commercial scale for various types of low-grade ores and tailings, slow dissolution kinetics remain a challenge. To address this, multivariable optimisation methods such as orthogonal array design, Plackett-Burman, and response surface analysis should be used to achieve higher bioleaching yields. Solid concentration, pH, energy source concentration, and particle size are the most influential parameters, and analysing reaction kinetics is essential to enable selective metal extraction. Furthermore, we recommend that more pilot studies and collaborative research involving chemists, biologists, and metallurgists working together should be conducted to scale up bioleaching for industrial use, especially for other metal-bearing materials such as e-waste, metallurgical sludge, and dust, spent catalysts, and fly ash. Finally, applying life cycle assessment and techno-economic assessment can help evaluate and reduce the environmental impact and cost of the bioleaching process. Our proposed optimisation flow chart can help achieve high bioleaching yield from general metal-bearing materials from laboratory to pilot/large scale operations. Implementing these recommendations can lead to the continued advancement of the field, providing a sustainable solution for metal recovery that minimises environmental impact.

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3 UNLOCKING THE HIDDEN VALUE OF INDUSTRIAL BY-PRODUCTS: OPTIMISATION OF BIOLEACHING TO EXTRACT METALS FROM BASIC OXYGEN STEELMAKING DUST AND GOETHITE

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Abstract: In this study, the potential of bioleaching to extract valuable metals from industrial by-products, specifically basic oxygen steelmaking dust (BOS-D) and goethite was investigated. These materials are typically discarded due to their high zinc content and lack of efficient regeneration processes. By using *Acidithiobacillus ferrooxidans*, successful bioleaching of various metals, including heavy metals, critical metals, and rare earth elements was achieved. The Taguchi orthogonal array design was used to optimise the bioleaching process, considering four variables at three different levels. After 14 days, the highest metal extraction for the BOS-D (11.2 mg Zn /g, 3.2 mg Mn /g, 1.6 mg Al /g, 0.0013 mg Y /g, and 0.0026 mg Ce /g) was achieved at 1% solid concentration, 1% energy source concentration, 1% inoculum concentration, and pH 1.5. For goethite, the optimal conditions were 1% solid concentration, 4% energy source concentration, 10% inoculum concentration, and pH 2 resulting in an extraction of 26.6 mg Zn /g, 2.1 mg/g Mn, 1.8 mg Al /g, 0.01 mg Co /g, 0.0022 mg Y /g. These findings are significant, as they demonstrate the potential to extract valuable metals from previously discarded industrial by-products. The extraction of such metals can have substantial economic and environmental implications, while simultaneously reducing waste in the metallurgical industry. Furthermore, the preservation of initial concentration of iron in both BOS-D and goethite residues represents a significant step towards implementing more sustainable industrial practices.

Keywords: *Acidithiobacillus ferrooxidans*, bioleaching, metallurgical by-products, metal dissolution, biohydrometallurgy

3.1 Introduction

Metallurgical by-products including basic oxygen steelmaking dust (BOS-D), and goethite are often considered as waste and stockpiled on former and current metallurgical sites, despite containing valuable metals such as manganese (Mn), lead (Pb), aluminum (Al), critical metals like lithium (Li) and cobalt (Co), and rare earth elements (REE) such as yttrium (Y) and cerium (Ce) (Binnemans et al., 2020; Hoeber and Steinlechner, 2021; Ma, 2008). Approximately, 20 kg of BOS-D is produced for per ton of liquid steel (Stewart and Barron, 2020; Binnemans et al., 2020), and 250 kg goethite is produced from per ton of ore (Rodriguez Rodriguez et al., 2020). Unfortunately, the high zinc content in metallurgical by-products, such as BOS-D (>10 g/kg) and goethite (ranging between 50-100 g/kg), hinders their potential for reuse (Binnemans et al., 2020; Ma, 2016; Di Maria and Van Acker, 2018). Recovering metals from these secondary resources is not only critical for sustainable development but also for reducing environmental risks posed by historic contaminated sites (Di Maria and Van Acker, 2018; Riley et al., 2020). However, the current pyro- and hydro-metallurgical methods for extracting metals from these by-products are not economically feasible due to low extraction yields, metal loss, and the generation of aqueous waste streams that require additional treatment (Binnemans et al., 2020; Di Maria and Van Acker, 2018).

Bioleaching, also known as biomining, is a promising eco-friendly and cost-effective alternative to traditional hydro- and pyro- metallurgical process. It can be operated at low temperature (25-35°C) and atmospheric pressure, producing no harmful gas emissions. The process involves the use of bacteria and fungi and their metabolites, to solubilise metals from metal-bearing materials (MBM). Bioleaching can occur directly, where microorganisms attach to the MBM surface to solubilise metals; indirectly where the metabolites react with the MBM, or cooperatively, where the two phenomena occur simultaneously. Acidophiles are the most commonly used microbial group, utilising ferrous iron and/or reduced sulphur compounds (S_8, $S_2O_3^{2-}$, H_2S) to generate leaching agents such as ferric iron (Fe^{3+}) and sulphuric acid (H_2SO_4). Metal dissolution from acidophiles form sulphide ores (Eq. 1-3) (Mishra et al., 2005; Srichandan et al., 2019), oxidic ores

(Eq. 4) (Abhilash and Pandey, 2013; Zare Tavakoli et al., 2017) and electronic wastes (Eq. 5-6) (Işıldar et al., 2016; Pourhossein and Mousavi, 2019).

$$2Fe^{2+} + 0.5O_2 + 2H^+ \xrightarrow{A.f.} 2Fe^{3+} + H_2O \quad (1)$$

$$FeS_2 + 6Fe^{3+} + 3H_2O \rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+ \quad (2)$$

$$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \rightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+ \quad (3)$$

$$UO_2 + Fe_2(SO_4)_3 \rightarrow UO_2SO_4 + 2FeSO_4 \quad (4)$$

$$Cu^0 + 2Fe^{3+} \rightarrow 2Fe^{2+} + Cu^{2+} \quad (5)$$

$$Ni^0 + 2Fe^{3+} \rightarrow 2Fe^{2+} + Ni^{2+} \quad (6)$$

The bioleaching process performance is influenced by multiple parameters including pH, solid concentration (also termed as pulp density), energy source concentration, temperature, leaching time. Simultaneously optimising these parameters can enhance bioleaching efficiency (Niu et al., 2016). Several multivariable optimisation methods such as a central composite design (CCD), Box–Behnken, and Taguchi orthogonal array design have been used for bioleaching studies (Amiri et al., 2011a; Jalali et al., 2019; Mo et al., 2019; Nkulu et al., 2013). Compared to other experimental designs, the Taguchi method is advantageous because it requires fewer experiments to achieve optimal conditions. It uses orthogonal arrays to efficiently explore a large parameter space with a relatively small number of experiments, making it a cost-effective and time-saving method. In addition, the Taguchi method allows to evaluate simultaneously the relative importance of different parameters and to identify the optimal combination of parameter values that will produce the highest yield with the least variation. This is particularly useful in bioleaching studies where it is crucial to optimise the yield of metal extraction and minimise the variability of the process. In this study, leachability of metals from BOS-D and goethite using *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) was investigated. The influence of four factors including solid concentration, energy source concentration

($FeSO_4.7H_2O$ as a source of Fe^{2+}), inoculum concentration and pH was evaluated at three levels using Taguchi orthogonal array design. This study sheds light on the potential of bioleaching as a viable and sustainable alternative to conventional hydrometallurgical and pyrometallurgical processes. It highlights the positive impact of bioleaching on the leachability of certain metals, thus underscoring its potential as a promising technology for efficient and eco-friendly metal extraction.

3.2 Materials and Methods

3.2.1 Characterisation of materials

A bulk mixture of BOS-D from a historical iron and steelwork plant stockpiled in Teesside, UK, was received in a large, sealed drum and stored at ambient temperature. Additionally, goethite, a mineral residue from zinc ore refining throughout a hydro-metallurgical process, was obtained from the Nyrstar plant in Auby, France and stored in a bucket at ambient temperature. Both samples were air-dried, ground, and sieved through a 2 mm mesh. The physical-chemical analysis results for BOS-D and goethite can be found in Appendix C (Figure C-1). Characterisation methods used have been previously described in Tezyapar Kara et al., (2022). To prepare the materials for bioleaching experiments, both materials were autoclaved for 15 minutes at 121°C. The elemental composition of the autoclaved materials was determined using aqua regia digestion, with 1:3 ratio of nitric acid (HNO_3) and hydrochloric acid (HCl), followed by microwave digestion as described by Gutiérrez-Gutiérrez et al., (2015). Extracts were analysed with a PerkinElmer's NexION® Inductively Coupled Plasma Mass spectrometer (ICP-MS) using uranium (U) as internal standard as described Gutiérrez-Gutiérrez et al., (2015). Several standard solutions sourced from Perkin Elmer for calibration were used: mercury ICP-MS standard, single ICP-MS standard for precious metals, single ICP-MS standard for As, Mo, Re, Sb, Si and single ICP-MS standards for the rest of the metals with the analyte concentration of 10 µg/ml. The mineral composition of both BOS-D and goethite was analysed using X-ray diffraction (XRD) using a Bruker D5005 diffractometer with a copper tube ($\lambda K\alpha$ = 0.154nm) (Kremser et al., 2022). The step size was 0.02° 2θ, the time per step was 1 s, the angular range was 10-90° 2θ, and the total scan

duration was 67 min. The data acquisition software used was Bruker XRD Commander, and the data processing software used was Bruker Diffrac.EVA. The surface morphology and elemental distribution of both BOS-D and goethite were examined through scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) analysis (TESCAN VEGA4) prior to and post-bioleaching.

3.2.2 Taguchi orthogonal array design

To assess the impact of various parameters on the dissolution of metals from BOS-D and goethite materials the Taguchi orthogonal array design L9 (3^4) was chosen. Details of the computational method of Taguchi array design are provided in Tang et al., (2016), Gu et al., (2017), Nkulu et al., (2013). This design involved optimising four parameters, including solid concentration (1, 5 and 10% w/v), energy source concentration (1%, 2% and 3% w/v for BOS-D optimisation and 2%, 3% and 4% w/v for goethite optimisation), inoculum concentration (1, 5 and 10% v/v) and pH (1.50, 1.75, and 2.00), in three different levels under 9 different conditions (Table 3-1). The range of parameters for optimisation was selected based on Tezyapar Kara et al., (2023) and Mo et al., (2019). For the bioleaching of oxidic BOS-D, an intermediate energy source concentration of 2.22% was chosen, in accordance with the findings of Chen et al. (2015) and Gomes et al. (2018). Additionally, for the bioleaching of goethite, we considered an energy source concentration of 4.44%, as supported by the research of Jensen and Webb (1995), Roy et al. (2021), and Zeng et al. (2013). The optimisation results were analysed using Minitab® 19.2020.1 (Eisapour et al., 2013). Based on these responses signal-to-noise ratios (S/N) were calculated using larger is better function (Eq. 7) and optimum responses (Y_{opt}) (Eq. 8) were predicted using Minitab (Awolusi et al., 2022; Nkulu et al., 2013).

$$SN_l = -10log_{10}\left(\frac{1}{m}\sum_{j=1}^{m}\frac{1}{y_{ij}^2}\right) \quad (7)$$

$$Y_{opt} = \frac{T}{n} + \left(A_i - \frac{T}{n}\left(B_j - \frac{T}{n}\right)\right) + \cdots \quad (8)$$

where y_{ij} is the response y_j at the level I, T/n is the mean of all the responses; and A_i, B_j, ... are the means of the responses corresponding to the optimal conditions (Nkulu et al., 2013). Then, S/N values were used to define the order of importance of the parameters and the optimum level of the parameters. The difference of maximum and minimum S/N values for each parameter is called relative score (∆) and larger relative score represents the most influencing parameter (Gu et al., 2017; Tang et al., 2016).

Table 3-1. L9 (3^4) experimental design for both BOS-D and goethite materials.

Factor (parameter)	**Variation level/real values**					
Material	**BOS-D**			**Goethite**		
Level	**1**	**2**	**3**	**1**	**2**	**3**
A - Solid conc. % (w/v)	$A_1 = 1$	$A_2 = 5$	$A_3 = 10$	$A_1 = 1$	$A_2 = 5$	$A_3 = 10$
B - Energy source conc.% (w/v)	$B_1 = 1.11$	$B_2 = 2.22$	$B_3 = 3.33$	$B_1 = 2.22$	$B_2 = 3.33$	$B_3 = 4.44$
C - Inoculum conc. % (v/v)	$C_1 = 1$	$C_2 = 5$	$C_3 = 10$	$C_1 = 1$	$C_2 = 5$	$C_3 = 10$
D - pH	$D_1 = 1.50$	$D_2 = 1.75$	$D_3 = 2.00$	$D_1 = 1.50$	$D_2 = 1.75$	$D_3 = 2.00$

Conditions	**Experimental scheme**	**Factors and their variation levels**							
		BOS-D				**Goethite**			
		A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
C1	$A_1B_1C_1D_1$	1	1.11	1	1.50	1	2.22	1	1.50
C2	$A_1B_2C_2D_2$	1	2.22	5	1.75	1	3.33	5	1.75
C3	$A_1B_3C_3D_3$	1	3.33	10	2.00	1	4.44	10	2.00
C4	$A_2B_1C_2D_3$	5	1.11	5	2.00	5	2.22	5	2.00
C5	$A_2B_2C_3$D1	5	2.22	10	1.50	5	3.33	10	1.50
C6	$A_2B_3C_1D_2$	5	3.33	1	1.75	5	4.44	1	1.75
C7	$A_3B_1C_3D_2$	10	1.11	10	1.75	10	2.22	10	1.75
C8	$A_3B_2C_1D_3$	10	2.22	1	2.00	10	3.33	1	2.00
C9	$A_3B_3C_2D_1$	10	3.33	5	1.50	10	4.44	5	1.50

3.2.3 Batch bioleaching optimisation experiments

Actively growing culture of *A. ferrooxidans* (DSM 583) was sourced from the Leibniz Institute (DSMZ), Braunschweig (Germany). The culture was adapted to

5% (w/v) of BOS-D as detailed in Tezyapar Kara et al., (2022). To adapt the culture to goethite material, the same procedure was used (results are not shown). As ORP did not reach ≥ 600 mV within seven days for 2.5% (w/v) solid concentration, acclimatised culture for 1% (w/v) was used for bioleaching of goethite (Muddanna and Baral, 2021). Adapted cultures were preserved in Erlenmeyer flasks with a concentration of 10% (v/v). These cultures were maintained in 90 ml of optimised 4.5 K salt medium at pH 1.75 at 30°C at 150 rpm as described by Chen et al., (2015). Prior to their use in the batch optimisation experiment, the adapted cultures were sequentially cultivated for 2 days on two separate occasions. Following the 2 days of cultivation, the pH of the cultures was 2.04 for both BOS-D and goethite, while the ORP values were 637 mV and 628 mV for BOS-D and goethite, respectively. The adapted cultures were then utilised in 250 ml batch optimisation experiments (Table 3-1). To begin with, three different growth media were prepared based on the desired ferrous iron concentration, as energy source concentration is an optimisation parameter. The optimised growth media consist of modified basal salt medium (MBSM) and a ferrous iron solution. MBSM was prepared by adding 2 g of $(NH_4)_2SO_4$, 0.25 g of K_2HPO_4, 0.25 g of $MgSO_4 \cdot 7H2O$, 0.10 g of KCl and 0.01 g of $Ca(NO_3)_2$ to 700 ml of deionised water (Chen et al., 2015). The ferrous iron solution was prepared by adding varying amounts of $FeSO_4.7H_2O$, based on the optimisation level, to 300 ml deionised water. For BOS-D, 11.1 g, 22.2 g and 33.33 g $FeSO_4.7H_2O$ was used to obtain 2.22, 4.44 and 6.66 g/L Fe^{2+} in the medium, respectively. Both solutions were adjusted at pH 2.00 using 5 M H_2SO_4. After pH adjustment, MBSM was autoclaved at 121°C for 15 minutes and the ferrous iron solution was filtered using 0.2 µm Millipore filter. The two solutions were then mixed to get the growth medium with the desired ferrous iron concentration (Table 3-1, parameter B). A two-step bioleaching approach was employed for this study (Amiri et al., 2011b). In the first step, the growth media were placed into 250 ml flasks, and the pH was adjusted to the different levels (Table 3-1, parameter D). Before inoculation, an equal amount of growth medium volume was discarded from the flask to create volume for inoculum. The flasks were inoculated with different concentrations (Table 3-1, parameter C) and incubated at 30°C on an orbital shaker at 150 rpm.

Bioleaching experiments were performed in triplicate for BOS-D and in duplicate for goethite. Abiotic control experiment was performed for 1% pulp density using 1.11% energy source for BOS-D and 2.22% energy source for goethite at pH 1.75. Acid control experiment was performed under same condition using only 5M H_2SO_4 without $FeSO_4.7H_2O$. Control experiments were performed in duplicate. Throughout the experiment, the oxidation-reduction potential (ORP) and pH of the culture and control were monitored on a daily basis, with pH being adjusted daily to the initial concentration, except for the goethite material where pH and ORP were only monitored and adjusted on the designated sampling days (days 0, 1, 3, 5, 8, 11, 16). In the second step, after 2 days of incubation, different amounts of materials were added into the flasks (Table 3-1, parameter A).

3.2.4 Analytical methods

The pH of the shake flask medium was measured using a Jenway 3540 pH meter by dipping the probe into flask and mixing the slurry after cleaning the probe with alcohol (70% v/v isopropanol) or biocleanse (5% v/v Teknon™ Biocleanse Concentrate) and rinsing it with autoclaved distilled water. The oxidation of Fe^{2+} to Fe^{3+} was used as an indicator of microbial growth and was monitored using an Ag/AgCl ORP probe (Roy et al., 2021). Leachate samples (2 ml) were collected and filtered through a 0.2 µm Millipore filter for further analysis on day 1, 3, 5, 8, 11, and 16. The concentration of Fe^{2+} was determined through titration with $K_2Cr_2O_7$ (Third et al., 2000), while metal concentration was determined by ICP-MS. The metal extraction yield was calculated using the following equation (Jalali et al., 2019):

$$R = m/m_0\, x100 \qquad (9)$$

where R is % metal extraction, *m* is soluble metal amount in the leachate (g) and m_0 is initial metal amount in autoclaved BOS-D and goethite.

3.3 Results and Discussion

3.3.1 Characterisation of materials

In this study, the elemental composition of BOS dust and goethite, both subjected to autoclaving (Table 3-2) as well as their non-autoclaved counterparts (Table C-1) were compared. The results showed that autoclaving goethite led to a loss of certain elements compared to BOS dust. While the focus of this study is not the effect of autoclaving, it is important to discuss how this process could potentially impact subsequent bioleaching. Autoclaving has been reported to cause weight loss in minerals due to the removal of volatile components or the breakdown of certain mineral phases (Hubau et al., 2020). In the case of goethite, autoclaving may lead to the loss of elements that are more susceptible to thermal degradation or volatilisation (Roberts et al., 2023). For elements with an initial concentration exceeding 6 mg/kg, the reduction in weight was minimal, remaining below 20% for both BOS-D and goethite. Conversely, for other elements, the weight loss could reach up to 50% when associated with goethite, whereas it was limited to 40% when interacting with BOS-D. (Table C-1). This weight loss could affect the subsequent bioleaching process, as the availability and accessibility of metals for microbial activity may be altered. Furthermore, autoclaving can induce changes in the mineral structure, such as phase transformations or surface modifications (Yazici et al., 2009). These alterations can affect the reactivity of the mineral and its interaction with the microbial consortium responsible for bioleaching. For example, changes in surface area, mineral crystallinity, or the formation of new mineral phases may influence the attachment of microorganisms and their ability to access the mineral surface for bioleaching (Jenneman et al., 1986). It is worth noting that the specific effects of autoclaving on subsequent bioleaching can vary depending on the mineral composition, temperature, pressure, and duration of autoclaving. The impact may also differ for different microorganisms involved in the bioleaching process. Therefore, further investigations are necessary to comprehensively understand the consequences of autoclaving on bioleaching efficiency and metal extraction.

Table 3-2. Elemental composition of the autoclaved BOS-D (n=2) and goethite (n=3)

Element	BOS-D		Goethite	
REE	mg/kg	RSD (%)	mg/kg	RSD (%)
Y	1.3	12	2.3	7
La	0	-	1.8	9
Ce	3.8	1	5.6	7
Pr	0.4	25	0.6	9
Nd	1.8	7	2.4	4
Sm	0.3	23	0.4	7
Eu	<LOD	-	0.08	28
Gd	0.3	22	0.4	8
Tb	<LOD	-	<LOD	-
Dy	0.2	54	0.4	5
Ho	<LOD	-	0.04	38
Er	0.1	52	0.2	8
Tm	<LOD	-	<LOD	-
Yb	0.1	74	0.2	11
Lu	<LOD	-	<LOD	-
Sc	<LOD	-	<LOD	-
Critical metals	mg/kg	RSD (%)	mg/kg	RSD (%)
Li	4	4	3.2	10
Co	10	3	9	1
Sb	12	5	697	6
Cu	127	1	**203667**	6
Heavy metals	mg/kg	RSD (%)	mg/kg	RSD (%)
Cr	157	4	223	7
Ni	59	2	34.1	6
Zn	**18300**	**8**	**61615**	**7**
As	16	12	**5157**	7
Cd	62	10	423	5
Hg	1.0	139	75	6
Pb	**1738**	**9**	**24800**	**7**
Others	mg/kg	RSD (%)	mg/kg	RSD (%)
Be	0.38	22	0.5357	52
B	24	2	<LOD	-
Mg	**5062**	**9**	513.7	7
Al	**2660**	**3**	**11164**	**7**
Si	<LOD	-	<LOD	-
V	51	1	41.1	6
Mn	**5870**	**4**	**1457**	**7**
Fe	**390500**	**6**	**162333**	**7**
Ga	13	2	224	7
Mo	7.3	6	71.3	6
Ba	54	7	30.6	35
Re	0.03	141	-	-

Bold ones represent most abundant elements. RSD: relative standard deviation; <LOD: below limit of detection.

For this bioleaching study, autoclaved materials were used to ensuring precise evaluation of the targeted microbial activity and metal leaching without interference from the native microorganisms (Faraji et al., 2018; Gomes et al., 2018). Both BOS-D and goethite were dominated by iron, zinc, lead, manganese, aluminium, and magnesium. The XRD analysis results (Figure C-2) further reveal that magnetite (Fe_3O_4) is the dominant mineral in both materials, which is consistent with previous studies (Hoeber and Steinlechner, 2021).

BOS-D also contained calcite, graphite, kamacite, and wustite, while goethite contained lautite, zinc titanium oxide, and corkite. The exact differences in composition and concentration of elements between the two materials are shown in Table 3-2. The pH values of BOS-D and goethite were 9.40 and 4.30, respectively. This revealed that to perform acidophilic bioleaching using *A. ferrooxidans* pH should be adjusted during bioleaching at 1.50-2.25.

The SEM-EDS analysis results of BOS-D and goethite reveal the presence of globular iron particles in BOS-D and fine aggregated particles in goethite (Figure 3-1) which is in agreement with previous studies (Kelebek et al., 2004; Stewart et al., 2022); after bioleaching, morphological changes were observed, with increased visibility of spheroids in BOS-D and a reduction in the size of large agglomerates in both materials, as observed in Figure 3-1c and Figure 3-1f, along with the detection of Fe, Al, Mn, Pb, and Zn elements through EDS mapping (Tian et al., 2022).

3.3.2 Bioleaching experiment

ORP values and ferrous iron consumption during bioleaching of BOS-D are shown in Figure 3-2. To facilitate the interpretation, results were grouped based on the tested solid concentration which are 1%, 5% and 10. In addition, the series names represent the values of “energy source concentration | inoculum concentration | pH”. Before inoculation (Day 0), ORP values in all the flasks were around 300 (mV). On the Day 0 ferrous iron concentration were 2.22, 4.44 and 6.66 g/L in the flask that contain 1.11%, 2.22% and 3.33% (w/v) FeSO4.7H2O, respectively. First, flasks were inoculated with adopted cultures and cultivated for two days without BOS-D material. After two days of cultivation (Day 2), a linear

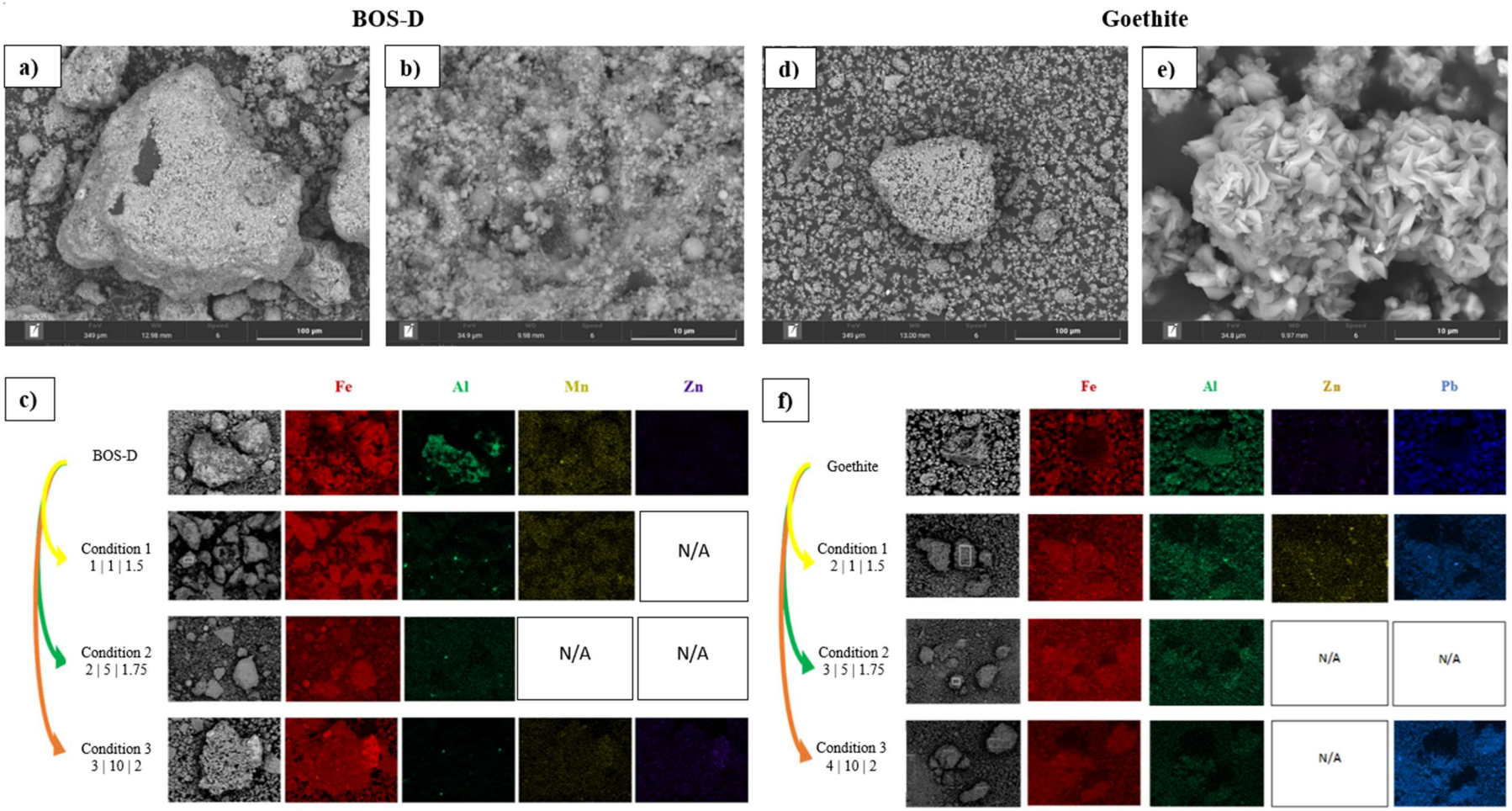

Figure 3-1. SEM-EDS images of bioleaching residues of BOS-D and goethite under 1% (w/v) solid concentration. SEM result for BOS-D at 100 µm (a) and 10 µm (b); EDS result for BOS-D (c); SEM result for goethite at 100 µm (d) and 10 µm (e); EDS result for goethite (f). Series names represent the values of "energy source concentration | inoculum concentration | pH".

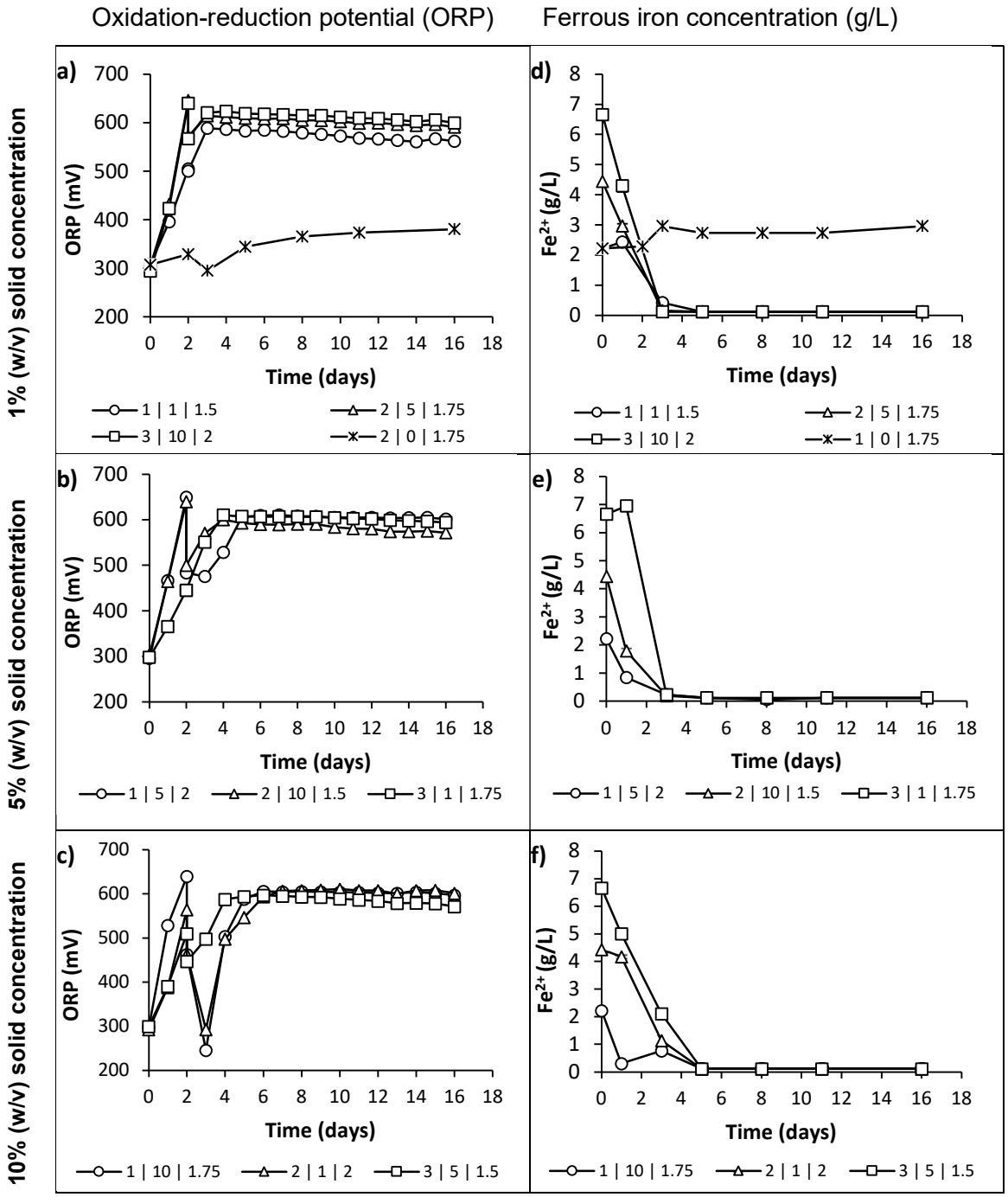

Figure 3-2. Evolution of the ORP values (mV) and ferrous iron concentration (g/L) of BOS-D overtime (series names represent the values of "energy source concentration | inoculum concentration | pH". n=3; RSD for all elements ≤ 20%).

increase in the ORP values (Figure 3-2a, b, c) and a decrease in Fe^{2+} concentrations (Figure 3-2d, e, f) were observed in all the flasks expect abiotic control. During acidophilic bioleaching ORP above 400mV indicates there is a

high concentration of Fe^{3+} and high bacterial growth (Rawlings and Johnson, 2007; Roy et al., 2021). According to Muddanna and Baral (2021), ORP ≥ 600

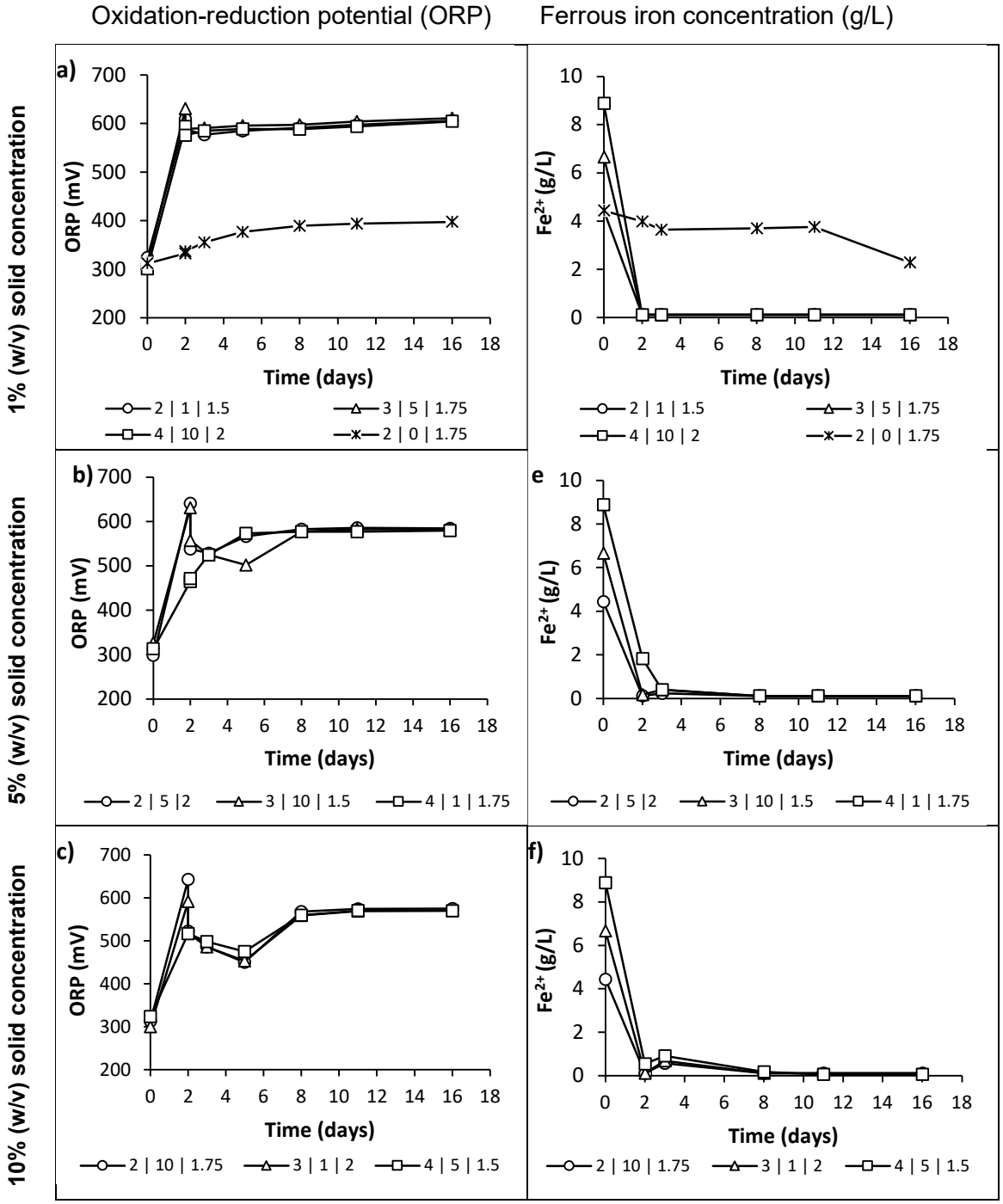

Figure 3-3. Evolution of the ORP values (mV) and ferrous iron concentration (g/L) of goethite over time (series names represent the values of "energy source concentration | inoculum concentration | pH". n=2; RSD for all elements ≤ 20%).

mV represents maximum ORP. ORP values in all the flasks were exceed the 400 mV in two days indicated that logarithmic phase of growth occurred (Roy et al.,

2021). All flasks, except the flasks inoculated with 1% (v/v) culture and the flask with condition 3 | 5 | 1.5, reached maximum ORP (≥ 600 mV). After ORP measurement, BOS-D with different concentrations were added into the flasks. Sharp decrease in ORP (below 600 mV) had occurred instantly (Figure 3-2a, b, c) and pH were increased (results not shown) due to alkali nature of the BOS-D (pH = 9.40). The pH was adjusted to the predefined values for each flask (Table 3-1) after BOS-D addition. On the next day (Day 3), ORP started to increase all the flasks that contain 1% (w/v) solid concentration and two of them reached out 600 mV again (Figure 3-2a). ORP values of the rest of the flasks were below 570 mV. Following days all flasks were shown increasing trend until different times. Supportively, ferrous iron concentration followed a decreasing trend showing ferrous iron consumption by *A. ferrooxidans* (Figure 3-2d, e, f). After Day 10, ORP values were in a downward trend for all the flasks until the end of the experiment (Day 16). Similarly, the ORP values and ferrous iron consumption during bioleaching of goethite are shown in Figure 3-3. Results were grouped based on the tested solid concentration which are 1%, 5% and 10 to facilitate the interpretation, and the series names represent the values of “energy source concentration | inoculum concentration | pH”. Before inoculation (Day 0), ORP values in all flasks were around 300 (mV). Besides, ferrous iron concentrations were 4.44, 6.66 and 8.88 g/L in the flask that contain 2.22%, 3.33% and 4.44% (w/v) $FeSO_4.7H_2O$, respectively. First, flasks were inoculated with adopted cultures and cultivated for two days without goethite. After two days of cultivation (Day 2), a linear increase in the ORP values (Figure 3-3a, b, c) and a decrease in Fe^{2+} concentrations (Figure 3-3d, e, f) were observed in all the flasks expect abiotic control. All flasks, except the flasks have 4.44% (w/v) energy source and the flask with condition 3 | 1 | 2, reached maximum ORP (≥ 600 mV) (Day 2). After the ORP measurement, goethite with different concentrations were added into the flasks. Sharp decrease in ORP had occurred instantly (Figure 3-3a, b, c). As a following step, the pH was adjusted to the predefined values for each flask (Table 3-1) after the goethite addition. An increase in the ORP values were observed following days for the flasks that contain 1% (w/v) goethite and reached the maximum ORP (≥ 600 mV) by the end of the experiment (Figure 3-3a). Flasks

that contain 5% and 10% goethite also showed increase in ORP indicating that bacterial activity was continuing. The cumulative acid consumption during the experiment is illustrated in Figure C-3, and it is evident that BOS-D required 2 to 6 times more acid compared to goethite to maintain the desired pH, primarily due to its higher initial pH of 9.40 in contrast to goethite's pH of 4.30.

This discrepancy can be attributed to BOS-D significantly containing higher Mg element content (5062 mg/kg) compared to goethite (514 mg/kg), which contributes to an increase in pH during bioleaching (Chen et al., 2015). Furthermore, it was observed that acid consumption increased with an increase in solid concentration, as expected. The acid consumption also increased when the maintained pH value decreased from 2.00 to 1.50 for the same solid concentration.

3.3.3 Taguchi statistical analysis

A comprehensive overview of the experimental setups and the average bioleaching yields (%) for each parameter studied, namely solid concentration, energy source concentration, inoculum concentration, and pH, individually evaluated for Mn, Zn, Pb, Al, Li, Co, Y, and Ce are summarised for BOS-D and goethite in Table C-2 and Table C-3, respectively. The metals were categorised into three groups: the most abundant metals, critical metals, and rare earth elements (REEs).

3.3.3.1 Most abundant metals

For the bioleaching of Zn, Mn, and Al the calculated means of S/N ratios, relative scores (Δ), optimal level for each factor are presented in Table 3-3. Dissolution of Zn from BO-P was most affected by the energy source concentration (Δ = 1.12), followed by solid concentration (Δ = 0.82), pH (Δ = 0.69), and inoculum concentration (Δ = 0.58). On the other hand, for dissolution of Zn from goethite solid concentration appeared as the most influenced factor (Δ = 2.19) followed by pH (Δ = 1.08), inoculum rate (Δ = 0.57) and energy source concentration (Δ = 0.41).

Optimum experimental scheme for maximum dissolution of Zn from BOS-D was corresponded to $A_1B_1C_1D_3$ which is 1% solid concentration, 1% energy source concentration, 1% inoculum concentration and pH 2.00. For goethite, optimum experimental scheme was defined as $A_1B_3C_3D_2$ which indicated as follows 1% solid concentration, 4% energy source concentration, 10% inoculum concentration and pH 1.75. Taguchi analyses predicted 64.3% and 44.4% of Zn dissolution from BOS-D and goethite, respectively, by using optimum experimental schemes.

For the manganese bioleaching solid concentration was the most influenced parameter for both BOS-D (Δ = 1.43) and goethite (Δ = 9.59). This factor followed by different parameters, yet inoculum concentration appeared as the less effective parameter for both. Taguchi analysis predicted that Mn extraction will increase 3% if optimum condition applies for Mn extraction from BOS-D. For Al dissolution, pH was the most affected parameter for both BOS-D (Δ = 1.64) and goethite (Δ = 1.56). Optimum experimental schemes were showed up as $A_2B_1C_1D_1$ for BOS-D and $A_3B_1C_1D_1$ for goethite. Pb did not involve Taguchi analysis due to Pb dissolution (13%) was only observed in $A_1B_1C_1D_1$, corresponding to: 1% solid concentration, 1% energy source concentration, 1% inoculum concentration and pH 1.50.

3.3.3.2 Critical Metals

Lithium and cobalt were selected as critical metals to be investigated using Taguchi statistical analysis (Table 3-3). Overall, solid concentration was appeared as most influencing factor with 1% (w/v) optimum value for both BOS-D and goethite. This followed by different parameters and optimum values. For example, for Li dissolution from BOS-D, the second important factor was energy source concentration (Δ = 3.74) followed by inoculum concentration (Δ = 2.04). On the other hand, pH was the second important factor, followed by energy source concentration for bioleaching of Li from goethite. However, even the order of importance of the pH was different for dissolution of Li, optimum pH value was appeared as 2.00 for both bioleached materials.

Table 3-3. Mean of S/N ratios for each factorial level; the left column belongs to BOS-D and right column belongs to goethite

	Zn_{obs} = 61% (C1,C4), Zn_{pre} = 64%				Zn_{obs} = 43% (C3), Zn_{pre} = 44%			
	Levels	Factors						
		SC	ESC	IC	pH	SC	ESC	IC	pH
Most abundant metals	1	1% = 35.01	1% = 35.2	1% = 34.9	1.5 = 34.51	1% = 32.1	2% = 30.65	1% = 30.61	1.5 = 30.28
	2	5% = 34.7	2% = 34.62	5% = 34.67	1.75 = 34.35	5% = 30.67	3% = 30.97	5% = 30.61	1.75 = 31.36
	3	10% = 34.19	3% = 34.08	10% = 34.32	2 = 35.04	10% = 29.91	4% = 31.06	10% = 31.18	2 = 31.04
	Optimal level	**A_1 = 1%**	**B_1 = 1%**	**C_1 = 1%**	**D_3 = 2**	**A_1 = 1%**	**B_3 = 4%**	**C_3 = 10%**	**D_2 = 1.75**
	Δ	0.82	1.12	0.58	0.69	2.19	0.41	0.57	1.08
	Rank	2	1	4	3	1	4	3	2
	Mn_{obs} = 57% (C1), Mn_{pre} = 60%					Mn_{obs} = 100% (C2, C3), Mn_{pre} = 100%			
	1	1% = 34.73	1% = 34.01	1% = 34.06	1.5 = 33.49	1% = 39.55	2% = 32.88	1% = 34.28	1.5 = 33.7
	2	5% = 33.61	2% = 34.05	5% = 33.71	1.75 = 33.65	5% = 32.26	3% = 34.13	5% = 33.7	1.75 = 34.39
	3	10% = 33.3	3% = 33.58	10% = 33.87	2 = 34.51	10% = 29.96	4% = 34.75	10% = 33.78	2 = 33.68
	Optimal level	**A_1 = 1%**	**B_2 = 2%**	**C_1 =1%**	**D_3 = 2**	**A_1 = 1%**	**B_3 = 4%**	**C_1 =1%**	**D_2 = 1.75**
	Δ	1.43	0.48	0.34	1.02	9.59	1.87	0.57	0.71
	Rank	1	3	4	2	1	2	4	3
	Al_{obs} = 59% (C1), Al_{pre} = 61%					Al_{obs} = 24% (C9), Al_{pre} = 26%			
	1	1% = 33.23	1% = 34.04	1% = 33.99	1.5 = 34.32	1% = 25.7	2% = 26.63	1% = 26.69	1.5 = 27.19
	2	5% = 33.88	2% = 33.26	5% = 33.07	1.75 = 33.23	5% = 26.44	3% = 26.54	5% = 26.4	1.75 = 26.35
	3	10% = 33.12	3% = 32.93	10% = 33.18	2 = 32.68	10% = 27.03	4% = 26.01	10% = 26.08	2 = 25.63
	Optimal level	**A_2 = 5%**	**B_1 = 1%**	**C_1 = 1%**	**D_1 = 1.5**	**A_3 = 10%**	**B_1 = 1%**	**C_1 = 1%**	**D_1 = 1.5**
	Δ	0.77	1.1	0.92	1.64	1.33	0.62	0.61	1.56
	Rank	4	2	3	1	2	3	4	1
Critical metals	Li_{obs} = 92% (C1), Li_{pre} = 94%					Li_{obs} = 64% (C3), Li_{pre} = 64%			
	1	1% = 36.3	1% = 35.82	1% = 34.78	1.5 = 33.35	1% = 35.65	2% = 34.7	1% = 34.66	1.5 = 34.06
	2	5% = 32.91	2% = 33.07	5% = 33.46	1.75 = 32.94	5% = 34.63	3% = 34.55	5% = 34.6	1.75 = 34.9
	3	10% = 31.76	3% = 32.08	10% = 32.73	2 = 34.68	10% = 33.72	4% = 34.74	10% = 34.74	2 = 35.03
	Optimal level	**A_1 = 1%**	**B_1 = 1%**	**C_1 = 1%**	**D_3 = 2**	**A_1 = 1%**	**B_3 = 4%**	**C_3 = 10%**	**D_3 = 2**
	Δ	4.54	3.74	2.04	1.74	1.93	0.2	0.14	0.98
	Rank	1	2	3	4	1	3	4	2
	Co_{obs} = 54% (C2), Co_{pre} = 55%					Co_{obs} = 100% (C2, C3), Co_{pre} = 100%			
	1	1% = 33.68	1% = 32.02	1% = 31.93	1.5 = 31.24	1% = 38.72	2% = 30.22	1% = 31.9	1.5 = 31.17
	2	5% = 31.88	2% = 32.44	5% = 32.67	1.75 = 32.68	5% = 29.93	3% = 32.48	5% = 31.88	1.75 = 32.76
	3	10% = 31.24	3% = 32.34	10% = 32.2	2 = 32.88	10% = 27.19	4% = 33.14	10% = 32.06	2 = 31.92
	Optimal level	**A_1 = 1%**	**B_2 = 2%**	**C_2 = 5%**	**D_3 = 2**	**A_1 = 1%**	**B_3 = 4%**	**C_3 = 10%**	**D_2 = 1.75**
	Δ	2.43	0.42	0.73	1.65	11.53	2.92	0.18	1.6
	Rank	1	4	3	2	1	2	4	3
REEs	Y_{obs} = 100% (C5, C6), Y_{pre} = 100%					Y_{obs} = 100% (C1, C4, C7, C8), Y_{pre} = 100%			
	1	1% = 36.56	1% = 38.68	1% = 38.89	1.5 = 39.32	1% = 39.75	2% = 40	1% = 39.98	1.5 = 39.71
	2	5% = 39.61	2% = 38.78	5% = 38.83	1.75 = 38.97	5% = 39.91	3% = 39.8	5% = 39.65	1.75 = 39.86
	3	10% = 37.36	3% = 36.06	10% = 35.81	2 = 35.24	10% = 39.78	4% = 39.63	10% = 39.8	2 = 39.87
	Optimal level	**A_2 = 5%**	**B_2 = 2%**	**C_1 = 1%**	**D_1 = 1.5**	**A_2 = 5%**	**B_1 = 2%**	**C_1 = 1%**	**D_3 = 2**
	Δ	3.05	2.72	3.08	4.08	0.17	0.37	0.33	0.16
	Rank	3	4	2	1	3	1	2	4
	Ce_{obs} = 75.6% (C2), Ce_{pre} = 75%					Ce_{obs} = 14.8% (C6), Ce_{pre} = 15%			
	1	1% = 35.7	1% = 34.21	1% = 33.89	1.5 = 34.9	1% = 23.18	2% = 23.27	1% = 23.11	1.5 = 23.19
	2	5% = 34.4	2% = 34.5	5% = 34.71	1.75 = 34.87	5% = 23.36	3% = 23.06	5% = 23.15	1.75 = 23.24
	3	10% = 32.12	3% = 33.49	10% = 33.6	2 = 32.45	10% = 22.9	4% = 23.12	10% = 23.18	2 = 23.01
	Optimal level	**A_1 = 1%**	**B_2 = 2%**	**C_2 = 5%**	**D_1 = 1.5**	**A_2 = 5%**	**B_1 = 2%**	**C_3 = 10%**	**D_2 = 1.75**
	Δ	3.58	1.01	1.11	2.45	0.46	0.21	0.07	0.22
	Rank	1	4	3	2	1	3	4	2

3.3.3.3 REEs

Bioleaching of yttrium and cerium was further analysed as rare earth elements (Table 3-3). Results showed that the factor with the highest significant is solid concentration for dissolution of Ce followed by pH for both BOS-D and goethite. Optimum experimental scheme for dissolution of Ce from BOS-D was $A_1B_2C_2D_1$ respectively to 1% solid concentration, 2% energy source concentration, 5% inoculum concentration and pH 1.50. For maximum Ce extraction from goethite experimental scheme defined as $A_2B_1C_3D_2$ which is 5% solid concentration, 2% energy source concentration, 10% inoculum concentration and pH 1.75.

Optimum energy source level which is 2% (w/v) was the same for Ce dissolution from both BOS-D and goethite. In terms of yttrium, complete dissolution was achieved both by bioleaching and acid leaching from both materials. Overall, based on the predicted results for individual metal extraction, an increase of more than 3% is not anticipated. However, the pH parameter emerged as the most crucial factor after solid concentration. Therefore, implementing automated monitoring and adjustment systems for pH during the bioleaching process could potentially enhance metal extraction rates.

3.3.4 Metal extraction potential of BOS-D and goethite

To compare bioleaching efficiency on shake flask Mn, Zn, Pb, Al, Li, Co, Y, Ce were selected due to some of them the most abundant metals, critical and rare earth elements. The extraction yields for metals that have the initial concentrations are lower than 1 mg/kg was not reported. After 14 days of bioleaching, both materials, highest metal dissolution was achieved using 1% (w/v) solid concentration. From BOS-D, highest metal extraction from majority of selected elements, Zn, Pb, Al, Li and Y, was achieved under the condition of 1% solid concentration, 1% energy source concentration, 1% inoculum concentration, and pH 1.50 ($A_1B_1C_1D_1$) (Figure C-4a and Table 3-4). Under this condition, 54% Mn, 61% Zn, 13% Pb, 59% Al, 92% Li, 40% Co, 99% Y and 67% Ce were dissolved from BOS-D. This percentages correspond to 3.2 mg Mn, 11.2 mg Zn, 0.2 mg Pb,1.6 mg Al, 0.004 mg Li, 0.004 mg Co, 0.0013 mg Y and 0.0026

mg Ce extraction. Overall, 0.005 mg of REE, 0.09 mg of critical metals, 11.5 mg of heavy metals and 4.8 mg other metals were extracted from 1 g of BOS-D (Table 3-4). Although bioleaching yield of Zn (61%) was lower than acid leaching (73%), more additional elements such as Li, Y and Ce were also extracted from BOS-D. This result indicates the benefit of bioleaching. Bioleaching provided higher metal dissolution than acid leaching for Pb, Al, Li, Co, Y and Ce. The reason is that Fe^{3+} contributed leaching of these metals additionally when compared to control that contains only sulphuric acid (Kremser et al., 2021). For example, Li and Co dissolution under the influence of the energy sources and the products of bacterial activity can be explained by Equation 10 (Moazzam et al., 2021).

$$2FeSO_4 + 2LiCoO_2 + 4H_2SO_4 \rightarrow Fe_2(SO_4)_3 + 2CoSO_4^{2+} + Li_2SO_4 + 4H_2O \qquad (10)$$

Table 3-4. Amounts of extracted elements of elements after bioleaching from 1 gram of BOS-D and goethite. n=3 for BOS-D and n=2 for goethite; SD for all elements ≤ 20%.

	BOS-D		Goethite	
Element	Bioleaching yield (%)	Amounts of extracted element (mg)	Bioleaching yield (%)	Amounts of extracted element (mg)
REE				
Y	99	0.0013	96	0.0022
La	-	-	3	0.0001
Ce	67	0.0026	14	0.0008
Nd	80	0.0014	21	0.0005
Total REE		**0.005**		**0.004**
Critical metals				
Li	92	0.004	64	0.002
Co	40	0.004	100	0.013
Sb	7	0.001	0	0.000
Cu	61	0.077	77	15.7
Total critical metals		**0.09**		**15.71**
Heavy metals				
Cr	15	0.0	17	0.0
Ni	23	0.0	48	0.0
Zn	61	11.2	43	26.6
Cd	100	0.1	49	0.2
Pb	13	0.2	0	0.0
Total heavy metals		**11.5**		**26.8**
Others				
Al	59	1.6	16	1.8
V	21	0.0	0	0.0
Mn	54	3.2	100	2.1
Ga	6	0.0	1	0.0
Ba	26	0.0	0	0.0
Total other metals		**4.8**		**3.9**
Total elements		**16.4**		**46.4**

For goethite, highest metal extraction from majority of selected elements, Mn, Zn, Li, and Co, was achieved under the condition of 1% solid concentration 1% solid concentration, 4% energy source concentration, 10% inoculum concentration, and pH 2.00 ($A_1B_3C_3D_3$) (Figure C-4b and Table 3-4). Under this condition 100% Mn, 43% Zn, 16% Al, 64% Li, 100% Co, 96% Y and 14 % Ce were dissolved from goethite. This percentages correspond to 2.1 mg Mn, 26.6 mg Zn, 1.8 mg Al, 0.002 mg Li, 0.013 mg Co, 0.0022 mg Y and 0.0008 mg Ce extraction. Overall, 0.004 mg of REE, 15.71 mg of critical metals, 26.8 mg of heavy metals and 3.9 mg other metals were extracted from 1 g of goethite (Table 3-4).

Bioleaching demonstrated superior metal dissolution compared to acid leaching for Mn, Zn, Li, and Co. Additionally, all the iron was preserved in the residues of both BOS-D and goethite (results not presented). Further research is required to reduce the Zn content to less than 1% in order to utilise goethite and BOS-D as secondary iron resources in the steel-making industry. Further to this, the SEM-EDS analysis results (Figure 3-1c and Figure 3-1f) demonstrate notable morphological changes in both BOS-D and goethite after bioleaching. In the case of BOS-D, increased visibility of spheroids is observed, while both materials exhibit a reduction in the size of large agglomerates post-bioleaching. EDS mapping reveals the presence of Fe, Al, Mn, Pb, and Zn elements. Following the bioleaching process, the distribution of Al, Zn, and Mn decreased in both BOS-D and goethite. The findings align with the leaching results, confirming that bioleaching, under optimised conditions, facilitates enhanced Zn extraction (Tian et al., 2022).

3.3.5 Kinetics of batch bioleaching

For a liquid/solid reaction system, the reaction rate is usually controlled by three main process which are diffusion through the liquid film, diffusion through the product layer, the chemical-controlled reaction at the surface of the solid particles (Asghari et al., 2013; Mishra et al., 2008; Naseri et al., 2019). The shrinking core model (SCM) theory considers that the leaching process is controlled by one of these steps (Gharabaghi et al., 2013; Naseri, et al., 2019; Pedram et al., 2020). In this study, the bioleaching kinetics were compared with the product layer

diffusion theory and chemical reaction theory (Amiri et al., 2012; Gomes et al., 2018; Pathak et al, 2019; Wang et al., 2019). As the bioleaching solution was non-viscous, film diffusion as the rate-controlling step was disregarded (Pathak et al, 2019; Srichandan et al., 2020). The bioleaching yields of metals under the best overall conditions were used to calculate the kinetics: 1% energy source concentration, 1% inoculum concentration, and pH 1.50 for BOS-D, and 1% solid concentration, 4% energy source concentration, 10% inoculum concentration, and pH 2.00 for goethite (Figure C-5). The results obtained under the optimised conditions were used to fit two kinetic models: chemical reaction and product layer diffusion (Table C-4). The correlations indicated that the dissolution of Zn, Li, Co, and Y from BOS-D aligned well with the chemical reaction model, suggesting the absence of a passivation layer on the surface of BOS-D or the presence of a porous layer that allowed the movement of leaching agents to reach the surface (Pathak et al., 2019). However, it's important to highlight that the pH range of 1.00 to 3.00 is conducive to the formation of jarosite, as indicated by Hou et al. in 2015. Consequently, in the context of this research, it is highly probable that jarosite formation will take place. This occurrence could potentially account for the reduction in the extraction of Li and Y, hinting at the possibility of co-precipitation of dissolved elements with jarosite, in addition to any experimental errors (Figure C-5) (Opara et al., 2022; Pirsaheb et al., 2022). In the case of Al and Ce, the results suggested that the overall dissolution kinetics were likely controlled by the diffusion step (Rastegar et al., 2015). The bioleaching of Mn from both BOS-D and goethite, as well as Co from goethite, were influenced by both the chemical reaction and product layer diffusion. However, for goethite, most metals showed the opposite outcome. This discrepancy may be attributed to the daily acid adjustment performed during BOS-D bioleaching, while goethite bioleaching only involved sampling on specific days, leading to the formation of production or passivation layers such as jarosite, which slows down the leaching kinetics (Pathak et al., 2019). Regular acid addition can help prevent the formation of jarosite (Chen et al., 2015).

3.4 Conclusion

The study employed a L9 Taguchi orthogonal array design to evaluate the effects of solid concentration, energy source concentration, inoculum concentration, and pH on bioleaching. After a 14-day period, the highest metal extraction from BOS-D was achieved at 1% solid concentration, 1% energy source concentration, 1% inoculum concentration, and pH 1.50, while for goethite, the optimal condition was 1% solid concentration, 4% energy source concentration, 10% inoculum concentration, and pH 2.00. Solid concentration emerged as the most influential parameter, followed by pH. Regular monitoring and adjustment of pH can effectively prevent the formation of jarosite and enhance the bioleaching yield. The study successfully extracted metals such as Zn, Mn, Al, Li, Co, Y, and Ce from the materials. However, further research is necessary to reduce the Zn content to less than 1% in order to utilise goethite and BOS-D as secondary iron resources in the steel-making industry.

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4 EXTRACTING METAL IONS FROM BASIC OXYGEN STEELMAKING DUST BY USING BIO-HYDROMETALLURGY

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Abstract: This study aimed to explore methods for enhancing and scaling up the extraction of metals from secondary sources, such as basic oxygen steelmaking dust (BOS-D). Initially, the study compared the effectiveness of three batch systems approaches: bioleaching using *Acidithiobacillus ferrooxidans*, chemical leaching using choline chloride-ethylene glycol (ChCl-EG) and a combined approach. Then, scaling up was evaluated through a semi-continuous bioleaching column system where leachate was recirculated at different regimes over 21 days. The metals of interest included Y (1.3 mg/kg), Ce (3.8 mg/kg), Nd (1.8 mg/kg), Li (4.5 mg/kg), Co (10.2 mg/kg), Cu (126.5 mg/kg), Zn (18,300 mg/kg), Mn (5,870 mg/kg), Al (2660 mg/kg). Bioleaching outperformed the control experiments achieving metal extraction recovery between 38% and 92% within 3 days, demonstrating the key role of *A. ferrooxidans.* On the other hand, ChCl-EG leaching, conducted with a solid concentration of 12.5% (w/v), successfully dissolved over half amount of all metals (58 - 63%) within two hours. When examining the extraction of rare earth elements (REE) at the ppm level, both bioleaching (98% Y, 64% Ce, 66% Nd) and hybrid leaching (89% Y, 82% Ce, 83% Nd) outperformed chemical leaching (63% Y, 59% Ce, 58% Nd). However, considering factors such as process duration, overall efficiency, and ease of extraction, chemical leaching was the most effective, followed by bioleaching and then hybrid leaching. After day 11 of leachate recirculation, a plateau was reached in the extraction of most elements, resulting in an overall extraction efficiency of 39% (10,590 mg/kg extracted from an initial concentration of 26,978 mg/kg). This suggests that concluding the column bioleaching process at day 11 can save time, energy, and resources, yielding higher overall metal extraction than extending it to day 21. Interestingly, variations in recirculation rates did not

influence the extraction efficiency. Overall, this study emphasizes the considerable potential of bioleaching for metal recovery, but it also highlights the need for further optimisation, particularly in parameters such as aeration rate, when transitioning to larger scale systems.

Keywords: Bioleaching, deep eutectic solvent, metallurgical waste, metal extraction, biomining

4.1 Introduction

Steel industry by-products are various including slags, dusts, and sludges from a blast furnace, basic oxygen furnace, electric arc furnace, and sinter plant, as well as oily mill scale and pickling sludge (Binnemans et al., 2020). Majority of the slag are internally recycled to the plant, or valorised using different application such as road construction, fertilizer, and cement components (Naidu et al., 2020). Yet, it is known that more than 190 million tonnes of legacy iron and steel slag are present across current and former iron and steel working regions of the UK (Riley et al., 2020). On the other hand, while basic oxygen steelmaking dust (BOS-D) is potentially considered as a secondary iron resource due to their high iron content (40-60%), they usually stockpiled on the marginal areas due to internal use of them are limited by its high zinc content (>1%) (Xue et al., 2022; Binnemans et al., 2020). When zinc loaded into a blast furnace, it vaporizes due to high temperatures (1600–1650°C) and its low boiling point resulting zinc accumulations on the blast furnace walls (Ma 2016; Stewart and Barron, 2020). This adversely affect the solid and gas flows through the furnace, damage the furnace linings and decrease the productivity (Stewart and Barron, 2020; Xue et al., 2022). Dust also contains wide range of different metals such as manganese and aluminium, critical metals e.g., lithium, cobalt, copper and rare earth elements such as yttrium, cerium and neodymium. It is estimated that globally 20 million tonnes of BOS-D were generated only in 2013. The zero-waste valorisation concept and the call for closing the loop within a circular economy emphasis the resource recovery from industrial process wastes using low cost, sustainable technologies, such as bioleaching (Gomes et al., 2020; Binnemans et al., 2015). This drives researchers to investigate the possible technologies that

turns BOS-D as a resource rather than a waste (Kinnunen and Hedrich, 2023; Binnemans et al., 2015; Ma, 2016).

To extract metals from complex metal-bearing materials hydrometallurgical methods can provide some advantages over pyrometallurgy due to pyrometallurgical methods require a high capital cost and cause harmful emissions and metal loss during the process (Arya and Kumar, 2020). In hydrometallurgy the extraction can be performed ambient to 300°C and above 100°C reactions carried out under pressure (Whitworth et al., 2022). Bioleaching, one of the biohydrometallurgical methods, emerges as more environmentally friendly extraction method due to it is mediated by naturally available microorganisms, required low capital cost, and performed under atmospheric pressure (Arya and Kumar, 2020; Srichandan et al., 2020). Process is influenced by various process parameters such as solid concentration (also termed as pulp density), pH, energy source concentration, temperature, inoculum concentration, agitation speed and recirculation/irrigation. Simultaneous optimisation of these parameters can provide higher metal extraction yield (Tezyapar Kara et al., 2023a; Niu et al., 2016). In addition, although scaling up bioleaching brings challenges, amplifying the process will widen the possibilities of implementing the extraction of values on commercial scale as well as will unlock the difficulties and possible solutions by providing technical know-how (Gao et al., 2021; Kinnunen and Hedrich, 2023; Srichandan et al., 2020).

Moreover, the use of green solvents, which derived from agricultural processing, can provide an environmentally friendly alternative to traditional chemicals e.g., EDTA (Abbott et al., 2009; Huntington et al., 2023; Tran et al., 2019). Deep eutectic solvents are promising discoveries in green chemistry that can be infinitely reused, biodegradable, and non-toxic. The use of green solvents has been proven to recover high yields of critical metals from contaminated waste. Abbott et al., (2009) used choline chloride – urea and ChCl-EG as a deep eutectic solvent and achieved >70% zinc recovery from electric arc furnace dust. Tran et al., (2019) used ChCl-EG as a deep eutectic solvent on spent lithium-ion batteries and achieved >90% of lithium and cobalt recovery. Therefore, hybrid leaching

processes could potentially enhance metal extractions from metallurgical wastes (Pourhossein et al., 2021; Hao et al., 2020; Priya and Hait, 2018).

For this study, leachability of metal ions of Y, Ce, Nd, Li, Co, Cu, Zn, Mn, Al from basic oxygen steelmaking dust (BOS-D) was investigated using bio-hydrometallurgy. Bioleaching was evaluated on a semi-continuous column set-up by recirculating the leachate to understand the effect of cycling rate. Also, the efficiency of a hybrid leaching process using ChCl-EG followed by bioleaching was evaluated. This research is important as it provides insights into wider bioleaching applications for secondary resources and sheds light on the limitations and opportunities for scaling up.

4.2 Materials and Methods

4.2.1 Sample Characterisation and Pre-treatment

Bulk basic oxygen steelmaking dust (BOS-D) which excavated from a stockpile of a historical iron and steelwork plant, Teesside UK, was received in a large, sealed drum and stored at ambient temperature. It then was air-dried, grounded, and sieved using a 2 mm mesh. The physical-chemical analysis results for BOS-D can be found in Tezyapar Kara et al., (2022; 2023b). For bioleaching study, autoclaved BOS-D, for 15 minutes at 121°C, was used to ensure precise evaluation of the targeted microbial activity and metal leaching without interference from the native microorganisms (Faraji et al., 2018; Gomes et al., 2018). The elemental composition of the autoclaved and non-autoclaved materials was determined using aqua regia digestion, with 1:3 ratio of nitric acid (HNO3) and hydrochloric acid (HCl), followed by microwave digestion as described by Gutiérrez-Gutiérrez et al., (2015). Extracts were analysed with a PerkinElmer's NexION® Inductively Coupled Plasma Mass spectrometer (ICP-MS) using uranium (U) as internal standard. Single ICP-MS standard solutions sourced from Perkin Elmer for calibration were used with the analyte concentration of 10 µg/ml.

4.2.2 Microorganisms and culture conditions

For this study, actively growing culture of *Acidithiobacillus ferrooxidans* (DSM 583), was received from Leibniz Institute (DSMZ), Braunschweig (Germany). Culture was then adapted to 5% (w/v) of BOS-D as described in Tezyapar Kara et al. (2022). Adapted culture was preserved in Erlenmeyer flasks with a concentration of 10% (v/v). The culture was maintained in 90 ml of optimised 4.5 K salt medium at pH 1.75 at 30°C at 150 rpm as described by Chen et al., (2015) and Tezyapar Kara et al., (2023b). Prior to their use in the bioleaching experiment, the adapted cultures were sequentially cultivated for 2 days on two separate occasions. Following the 2 days of cultivation, the pH of the cultures above 2.00, while the oxidation-reduction (ORP) values were ≥ 600 mV as a high ORP is a good indicator of a bacterial growth (Muddanna and Baral 2021). The adapted cultures were then utilised in 250 ml batch optimisation experiments. To avoid inaccurate cell density measurement, we have not applied a microbiological method, e.g., microscopy as the many bacterial cells were deposited on the precipitate that formed during the *A. ferrooxidans* growth as previously reported by Roy et al., (2021). Instead, we accepted that the oxidation of ferrous iron to ferric iron by *A. ferrooxidans*, the formation of ferric iron that creates red colour compared to abiotic control which is light green and the fast increase in ORP compared to abiotic control are good indicator of the bacterial growth (Muddanna and Baral 2021; Roy et al., 2021) A platinum electrode with an Ag/AgCl reference electrode was used for measurements of the redox potential (Srichandan et al., 2014). To prepare the growth medium, a modified salt medium (Chen et al., 2015) was used. The optimised growth medium consists of two solutions: solution A (modified basal salt medium) and solution B (ferrous iron). The solution A was prepared by adding 2.00 g of $(NH_4)2SO_4$, 0.25 g of K_2HPO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.10 g of KCl and 0.01 g of $Ca(NO_3)_2$ into a 700 ml of deionised water. The solution B was prepared by adding 11.1 g of $FeSO_4.7H_2O$, in 300 ml deionised water get 2.22 g/L Fe^{2+} in the medium, respectively. Both solutions were adjusted at pH 2 using 5 M H_2SO_4. After pH adjustment, solution A was autoclaved at 121°C for 15 minutes. The ferrous iron solution was filtered using

0.2 µm Millipore filter. The solutions A and B were then mixed (7:3). The pH is then adjusted to desired level for bioleaching study using 5 M H_2SO_4.

4.2.3 Bioleaching vs chemical leaching vs hybrid leaching

Bioleaching batches were conducted with 1% solid concentration, 1% energy source concentration, 1% inoculum concentration and pH 1.75 as described by Tezyapar Kara et al., (2023b). Bioleaching efficiency was evaluated for nine elements, including Y, Ce, Nd, Li, Co, Cu, Zn, Mn and Al, being the most abundant metals, critical and rare earth elements contained within BOS-D. A two-step bioleaching approach, following the methodology described by Amiri et al., (2011) and Muddanna and Baral (2021) was used. Briefly, BOS-D was introduced to the leaching media after 2 days of *A. ferrooxidans* incubation in the media and this was referred to as a two-step bioleaching process. While Muddanna and Barral (2021) introduce the metal-bearing material in the leaching media after 24 hours of *A. ferrooxidans* incubation. ORP was monitored, and ferrous iron concentration was measured as an indicator of bacterial growth on days 0, 2, 3, 5, 8, 11, 16. The pH was adjusted to 1.75 on the same days if needed. The abiotic controls were performed as the inoculated bioleaching batches with 1% solid concentration using 1% energy source at pH 1.75. The acid control batches were conducted under identical conditions, albeit with the sole use of 5M H_2SO_4 instead of $FeSO_4.7H_2O$.

The hybrid leaching was performed as follows: initially, a 2-hour chemical leaching was conducted using a 1:1 ChCl-EG solution with 1.25% solid concentration and pH 4. The 100 ml batches were placed in an agitated incubator at room temperature and operating at 150 rpm. The resulting BOS-D residue was then air dried and autoclaved at 121°C for 15 minutes before to be used for the subsequent bioleaching phase, which followed the conditions described above. All leaching conditions were performed in duplicate.

4.2.4 Scale-up bioleaching

Column bioleaching was performed to evaluate how the leachate recirculation rate, set at 40 mL/min and 80 mL/min, influenced the metal extraction yield. The

dimensions of the PVC columns are 5 cm diameter × 30 cm height. A total of 10 g, equivalent to 1% (w/v) of air-dried and autoclaved BOS-D was carefully inserted between two layers of glass wool in the column that featured a PVC support plate with multiple holes at its base. The glass wool layers also served as a barrier to prevent suspended solids from entering the reservoir. (Pathak et al., 2019; Abhilash et al., 2013). Air flow was supplied to the columns and reservoirs from the bottom at a rate of 1.33 L/min using an air pump (Pawfly®). Continuous leachate circulation of the system (40 or 80 ml/min) were supplied using and a peristaltic pump (Watson Marlow 505s). As per the batch bioleaching approach, a two-step approach was used for the bioleaching column system (Amiri et al., 2011; Muddanna and Barral, 2021). Similar to batch bioleaching, initially, a growth medium (ORP = 293 mV) of 1 L, containing 1.11% $FeSO_4 \cdot 7H_2O$ and adjusted to a pH of 2 was inoculated with 1% (v/v) of a *A. ferrooxidans* culture (ORP ≥ 600 mV, day 0). Culture was cultivated in the aerated reservoir in a water bath at 30°C. After 2 days if incubation, when ferrous iron was rapidly consumed compared to abiotic control, red colour occurred in the leaching medium and the ORP increased the first step was considered complete. For the second step, to initiate the bioleaching, the aerated columns were supplied with the culture from the bottom via the reservoir, at flow rates of 40 mL/min or 80 mL/min (Chen et al., 2015). The samples remained submerged by collecting leachate from the top of the column and recirculating the leachate between column and reservoir at the constant flow rate using the peristaltic pump throughout the experiment (Pathak et al., 2019). All conditions were performed in duplicate. Ferrous iron concentration and ORP were monitored on day 0, 2, 3, 5, 7, 13, 18, and 23. Day 2 onwards, the pH was adjusted to 1.75 on the same days as ferrous iron and ORP were measured. An attempt was made to conduct the experiment with a 5% (w/v) solid concentration under the same conditions. However, the columns experienced clogging as they started to be fed with the culture on day 2.

4.2.5 Analysis methods

The pH of the cultures was determined using a pH meter (Jenway 3540). The oxidation of Fe^{2+} to Fe^{3+} is an indication of microbial growth (Tezyapar Kara et al.,

2023b; Muddanna and Baral, 2021; Roy et al., 2021), and therefore ORP was monitored using a platinum electrode with an Ag/AgCl reference electrode on days 0, 2, 3, 5, 7, 13, 18, 23 (Srichandan et al., 2014). Leachate (2 ml) was collected and filtered using a 0.2 µm Millipore filter for further analysis. Fe^{2+} concentration was measured by titration against $K_2Cr_2O_7$ (Third et al., 2000). Metal concentration in leachate was determined by inductively coupled plasma mass spectrometry (ICP-MS) as described in Tezyapar Kara et al., (2023). Metal extraction yield was calculated using the following equation (Jalali et al., 2019):

$$R = m/m_0\, x100 \qquad (1)$$

Where R is % metal extraction, m is soluble metal amount in the leachate (g) and m_0 is initial metal amount in BOS-D.

4.2.6 Statistical analyses

The descriptive statistics was performed to evaluate the differences between corresponding groups. Data were subjected to one-way ANOVA statistical method with the significance level at $P < 0.01$ (Feng et al., 2023; Kremser et al., 2021).

4.3 Results and Discussion

4.3.1 Bioleaching vs chemical leaching vs hybrid leaching

The elemental composition results showed that BOS-D was dominated by iron, zinc, lead, manganese, aluminium (Table 4-1). For the bioleaching study, autoclaved BOS-D was used to ensuring precise evaluation of the targeted microbial activity and metal leaching without interference from the native microorganisms (Faraji et al., 2018; Gomes et al., 2018). The results showed that autoclaving BOS-D caused loss of most of the element. While weight loss was limited below 10% for most of the element, it reached 38% for Li. The focus of this study is not the effect of autoclaving, yet it is worth noting that autoclaving can change the mineral structure such as surface area, mineral crystallinity, or it may cause the formation of new mineral phases (Yazici et al., 2009). These alterations can influence the bioleaching efficiency as the they potentially affect

the attachment of microorganisms as well as the availability and accessibility of metals (Jenneman et al., 1986). Therefore, further study is necessary to understand the consequences of autoclaving on bioleaching efficiency and metal extraction. The dry matter, water content and loss on ignition were found as 99%, 1%, and -0.19, respectively. Negative loss of ignition value associated with the high iron content in the materials as Vandenberghe et al., (2010) suggested that iron oxidation can cause the weight gain and it overcame the weight loss of carbon.

Table 4-1. Elemental composition of the autoclaved and non-autoclaved BOS-D (n = 2). RDS stands for relative standard deviation.

	autoclaved		non-autoclaved	
Element	mg/kg	RSD	mg/kg	RSD
Y	1.3	12	1.26	2
Ce	3.8	1	4.09	8
Nd	1.8	7	1.89	12
Li	4.5	4	5.52	6
Co	10.2	3	9.73	2
Cu	126.5	1	126.5	1
Zn	18,000	8	18,950	0
Al	2,660	3	2,865	8
Mn	5,870	4	6,060	0
Fe	390,500	6	399,500	1

ORP values and ferrous iron consumption during bioleaching of BOS-D are shown in Figure 4-1. Before inoculation (day 0), ORP value of the growth medium was around 300 mV and the ferrous iron concentration was 2.22, g/L. First, the fresh medium (ORP = 293 mV) was inoculated with adapted culture and cultivated for 2 days without BOS-D material. The ORP values of bioleaching flasks significantly increased (>575 mV compared to abiotic control (ORP =318 mV) indicating effective bio-oxidation of Fe^{2+} to Fe^{3+} by *A. ferrooxidans* and a good bacterial growth (Roy et al., 2021; Kremser et al., 2021). When 1% (w/v) autoclaved BOS- D was added in the flask (day 2), a sharp decrease in ORP observed (from 622 mV to 597 mV) due to alkali nature of the BOS-D (pH = 9.40). Following day (day 3) the ORP exceeded 600 mV by reaching 606 mV. This

indicated that bacterial activity was continuing (Tezyapar Kara et al., 2023b) (Figure 4-1a). This is supported by the decrease of Fe^{2+} concentration to 0.1 g/L Fe^{2+} indicating biooxidation of ferrous iron to ferric iron by *A. ferrooxidans* (Figure 4-1b). On the other hand, throughout the experiment, ORP value of the abiotic control increased slightly yet it did not exceed the 400 mV. The reason of this increase is probably due to the chemical oxidation of the Fe^{2+} in the medium by atmospheric oxygen (Kremser et al., 2021).

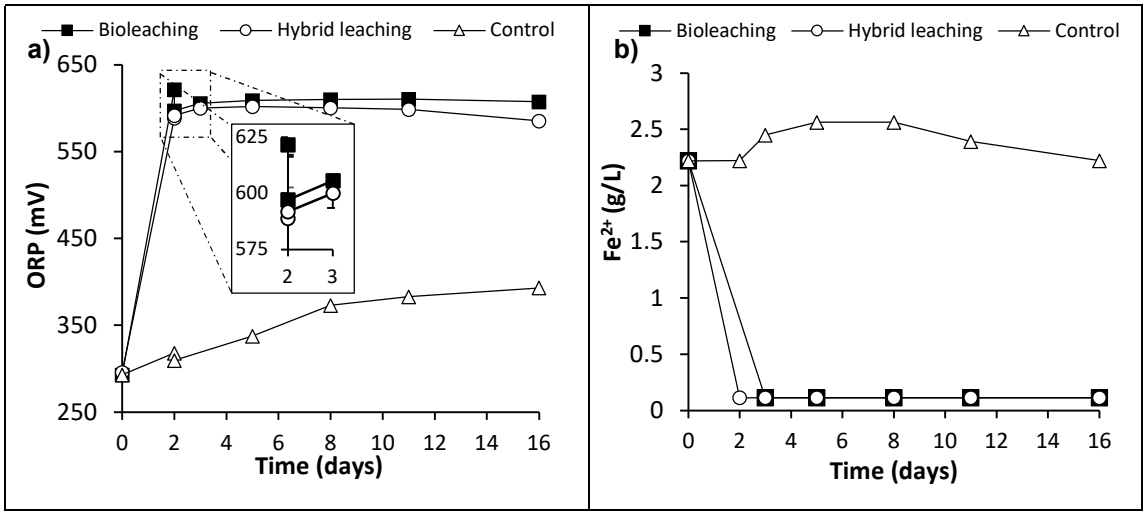

Figure 4-1. Evolution of the ORP values (mV) (a) and ferrous iron concentration (g/L) (b) of bioleaching batches overtime (SD for all series ≤ 20%). As series, "bioleaching" represents the BOS-D bioleaching (n=2); "hybrid bioleaching" represents the bioleaching (n=2) of residue of BOS-D from choline chloride-ethylene glycol leaching.

Comparison of the metal extraction yields from bioleaching, abiotic leaching and acid leaching for the 9 selected elements, including Y, Ce, Nd, Li, Co, Cu, Zn, Mn and Al is shown in Figure 4-2. After one day of bioleaching, 73% Y, 50% Ce, 51% Nd, 46% Li, 53% Co, 41% Cu, 39% Zn, 37% Mn, and 30% Al were dissolved (Figure 4-2). Until the end of the experiment, metal extraction yields increased between 10% to 20% for Y, Ce, Nd and Cu. In terms of other elements, extraction yields were slightly increased or remained stable. On the other hand, lower extraction was observed in both acid leaching and abiotic leaching compared to bioleaching on Day 3. The result indicates that biogenic Fe^{3+} contributed leaching additionally when compared to controls which can be explained by the Eq. (2-4) (Benzal et al., 2020; Srichandan et al., 2020; Wang et al., 2009).

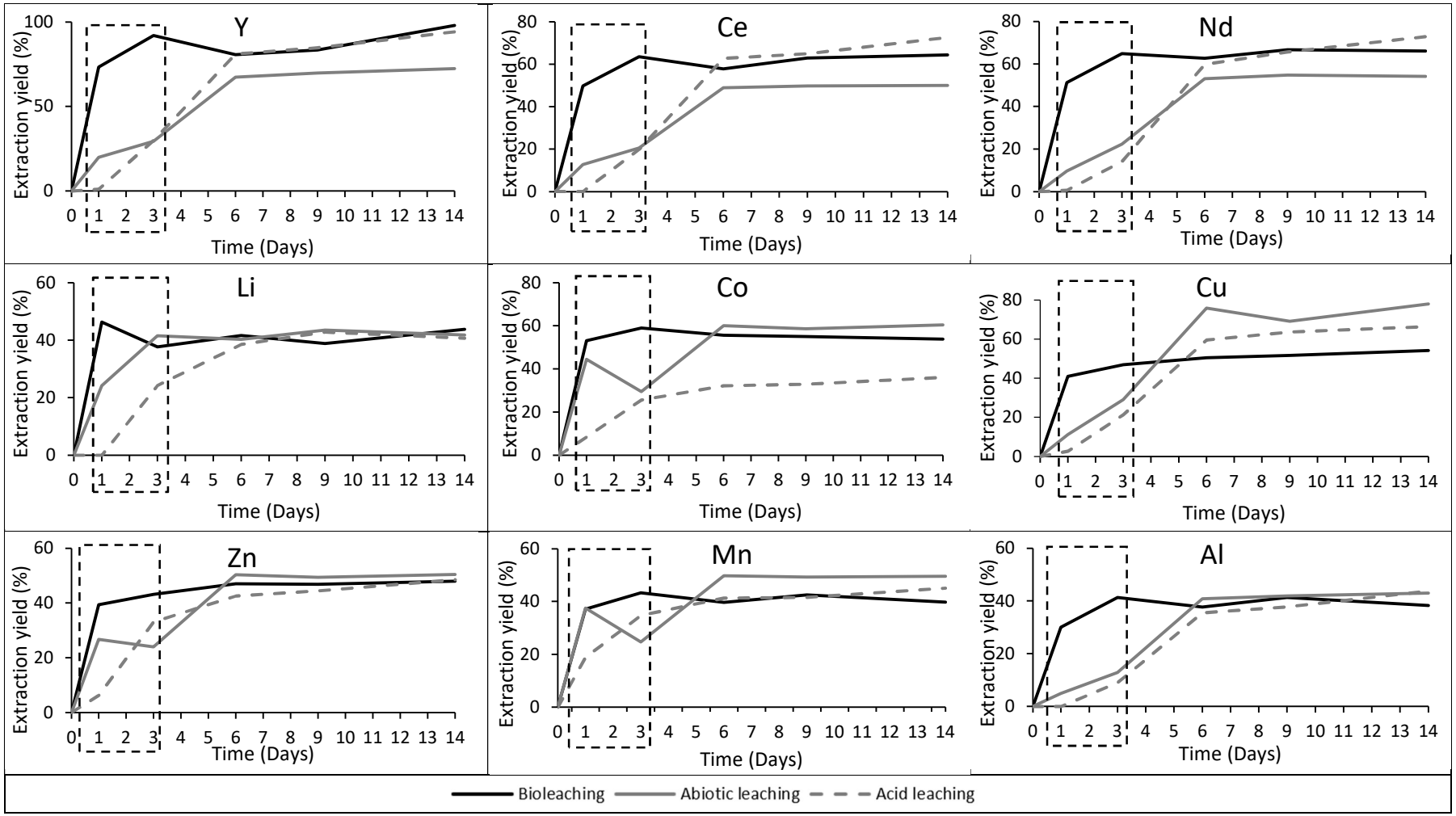

Figure 4-2. Metal extraction yields of selected elements over 14 days of leaching period (t = 0 equals to day 2 on Figure 1, which is BOS-D addition day) (n=2; SD for all elements ≤ 20%). The dashed box encompassing data for the first three days indicated the initial leaching stage that bioleaching provided higher dissolution than controls.

$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (2)$$

$$Cu^0 + 2Fe^{3+} \rightarrow Cu^{2+} + 2Fe^{2+} \quad (3)$$

$$Zn^0 + 2Fe^{3+} \rightarrow Zn^{2+} + 2Fe^{2+} \quad (4)$$

Abiotic leaching results were higher than acid leaching suggesting that ferrous iron acted as a reducing agent resulting effective metal extraction than acid leaching (Kremser et al., 2021; Moazzam et al., 2021). Day 3 onwards, metal extractions in controls followed an increasing trend until day 8. After day 8, metal extractions plateaued. Overall, the metal extraction was always superior when bioleaching was used compared to the controls (day 1 - day 3) indicating that *A. ferrooxidans* played a key role in facilitating the extraction of the selected metals from BOD-S (Kinnunen and Hedrich, 2023). As a result, to save time and resource 3 days of bioleaching would be enough to extract metals from BOS-D due to extraction of all elements were nearly reached plateau.

The comparison of the extraction efficiency between bioleaching (t = 3d), chemical leaching (t = 2h), and hybrid leaching (t = 14d) showed that bioleaching was more efficient than ChCl-EG for extraction of the rare earth elements (REEs) (Figure 4-3). The hybrid leaching approach led to an additional extraction up to 27% for REEs. For the other metals, the hybrid leaching approach resulted in an extra extraction ranging between 1% and 12%, except for Cu. Overall, the hybrid leaching approach resulted in the extraction of over 80% of REEs and more than 60% extraction of the remaining metals. Single factor ANOVA was performed to evaluate the significant differences between leaching approaches for each element ($P < 0.01$). Only significant difference was observed for Zn and Mn elements between bioleaching vs chemical and hybrid leaching (Figure 4-3).

In Table 4-2, bioleaching, chemical leaching, and hybrid leaching were compared in terms of process time, extraction efficiency, and ease of implementation (Arya et al., 2020; Gavrilescu, 2022; Moazzam et al., 2021). Chemical leaching with ChCl-EG was the most suitable method to extract critical metals, heavy metals

and others. For REEs extraction, bioleaching could be a preferable option to chemical leaching as it yielded higher extraction rates. While hybrid leaching resulted in greater metal extraction for all elements except Cu, it is seen as a less favourable method for extracting metals from BOS-D. This is because hybrid leaching introduces a more complicated process and demands additional time compared to individual processes.

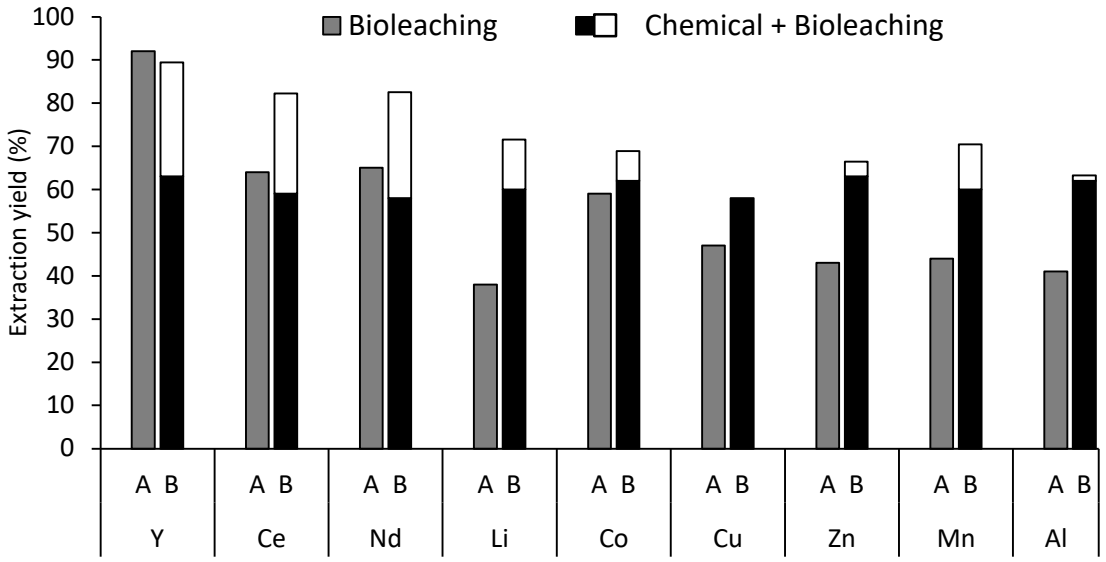

Figure 4-3. Metal extraction yields for Y, Ce, Nd, Li, Co, Cu, Zn Mn and Al from the batch leaching experiments (A: Bioleaching (t = 3d); B: Hybrid leaching process = chemical leaching (t = 2h) + bioleaching (t = 14d); (n=2, SD for all elements ≤ 20%).

Table 4-2. Comparison of the three processes, chemical leaching, bioleaching, hybrid leaching in terms of time, efficiency, and ease. The abbreviation REEs stands for rare earth elements, CRM stands for critical metals.

	Chemical leaching	Bioleaching	Hybrid leaching
REEs	+ +	+ + +	+
CRM	+ + +	+ +	+
Heavy metal and others	+ + +	+ +	+

4.3.2 Scale-up bioleaching

Evolution of ORP values and ferrous iron concentrations during column bioleaching experiments were shown in Figure 4-4. As expected, first two days ORP values of the culture were increased (Figure 4-4a). Supportively, ferrous iron was consumed by *A. ferrooxidans* (Figure 4-4b) as observed in the bioleaching batches. Then, on day 2, columns were started to feed with the culture from the bottom with different recirculation rates, 40 ml/min and 80 ml/min. Compared to the agitation condition (Figure 1a), the decrease in the ORP was slower but still occurred (Figure 4). Following days ORP slightly increase. No significant difference on ORP values and ferrous iron concentration between different recirculation rate was observed.

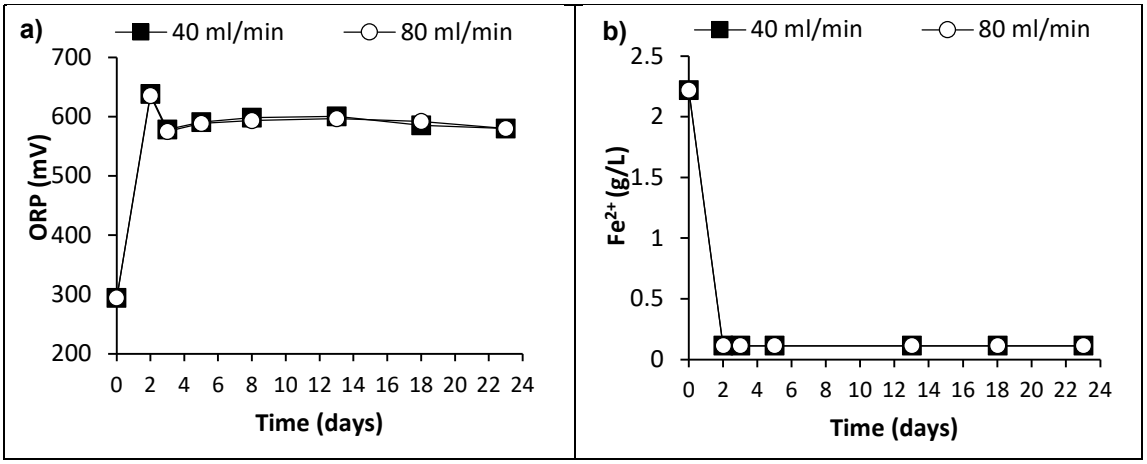

Figure 4-4. Evolution of the ORP values (mV) (a) and ferrous iron concentration (g/L) (b) during column experiments (n=2; SD for all series ≤ 20%).

Figure 4-5 shows the extraction of Y, Ce, Nd, Li, Co, Cu, Zn, Mn, and Al with different recirculation rates over 21 days of column scale bioleaching experiments. After one day of bioleaching metal extraction between 10% and 28% was observed for all elements except Cu. Until Day 6 an increasing extraction was observed for all metals. Day 11 onwards, extraction reached a plateau for most of the elements except REEs. Extraction of REEs continued to increase until the end of the experiment. There was no significant difference between 40 ml/min and 80 ml/min recirculation rates. When it compared to batch scale leaching results, a decrease in the extraction yields of most metals was observed when the bioleaching scaled up, except for a slight increase in Mn and

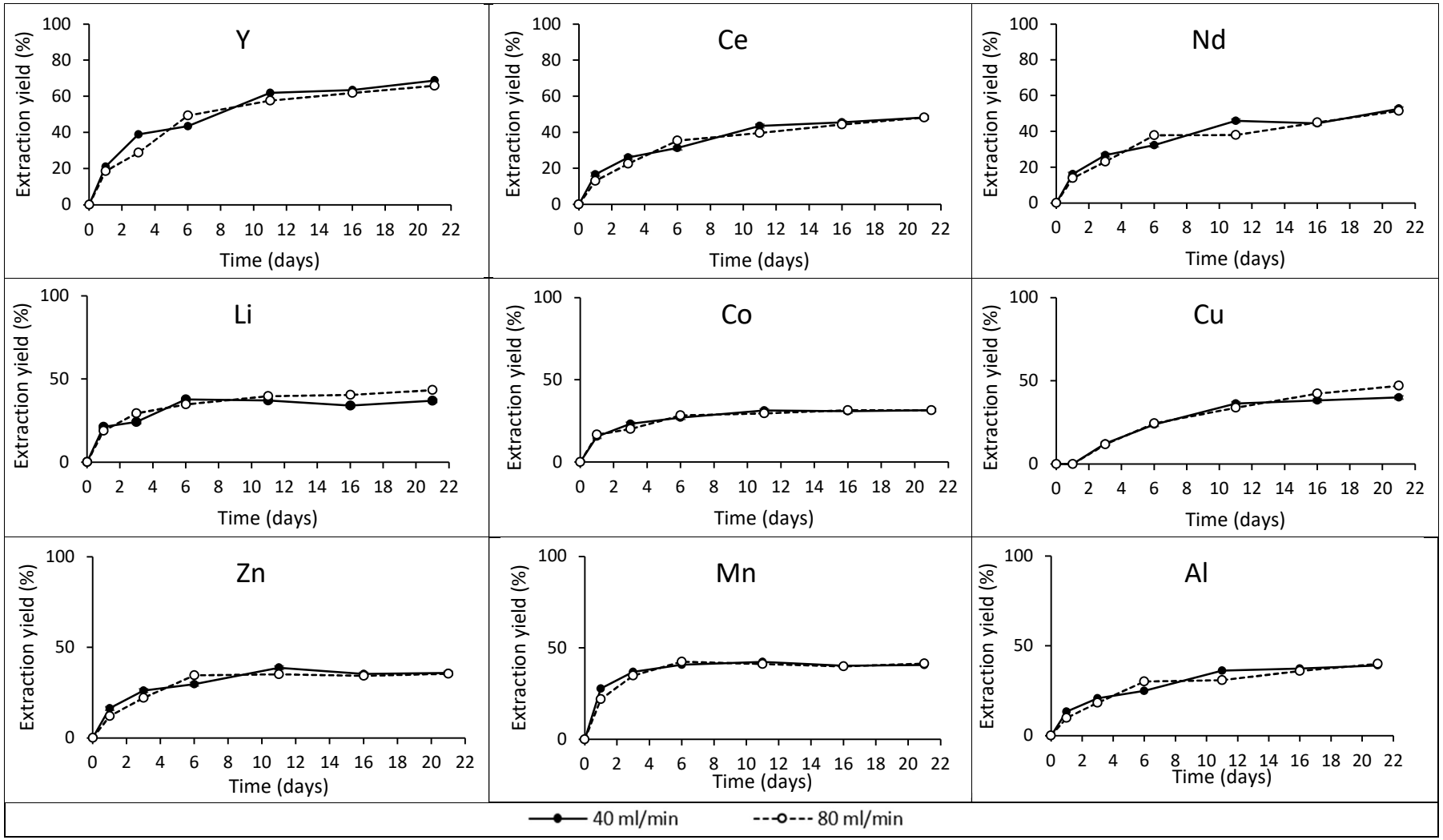

Figure 4-5. Extraction of Y, Ce, Nd, Li, Co, Cu, Zn Mn and Al with different recirculation rates of bioleaching process (t = 0 equals to day 2 in Figure 4-4, which the beginning of the circulation) (n=2; SD for all elements ≤ 20%).

Al. This may have been caused by heat lost and insufficient solid-liquid-gas interaction in the column (Srichandan et al., 2020). More controlled environment is needed to use percolation method as an industrial application. In addition, optimisation of air flow may enhance the metal extraction yield (Zare Tavakoli et al., 2017). Alternatively, stirred tank reactors can be considered as a scale up alternative due to it provides enhanced gas distribution and solid-liquid interaction (Kinnunen and Hedrich, 2023; Gericke et al., 2009).

Table 4-3. Comparison of the extracted amounts of Y, Ce, Nd, Li, Co, Cu, Zn, Mn and Al on day 11 and day 21 (t = 0 equals to day 2 in Figure 4-4, which the beginning of the recirculation) in column scale bioleaching experiments using 40 ml/min recirculation rate (n = 2; SD for all elements ≤ 20%).

	Initial metal concentration	Day 11		Day 21	
Element	Amounts of recovered element (mg/kg)	Metal extraction yield (%)	Amounts of recovered element (mg/kg)	Metal extraction yield (%)	Amounts of recovered element (mg/kg)
REE					
Y	1.3	62	0.80	69	0.89
Ce	3.8	43	1.67	48	1.86
Nd	1.8	46	0.83	53	0.95
Total REE	**7.0**		**3.3**		**3.7**
Critical metals					
Li	4.5	37	1.7	37	1.7
Co	10.2	31	3.2	32	3.2
Cu	126.5	36	46.0	40	50.7
Total critical metals	**141**		**51**		**56**
Heavy metal and others					
Zn	18,300	39	7,089	36	6,555
Al	2,660	36	962	39	1,040
Mn	5,870	42	2,485	41	2,385
Heavy metal and others	**26,830**		**10,536**		**9,980**
Total elements	**26,978**		**10,590**		**10,039**

The amounts of extracted metals from the bioleaching column system, with a recirculation rate of 40 ml/min, at day 11 and day 21 are presented in Al. This may have been caused by heat lost and insufficient solid-liquid-gas interaction in the column (Srichandan et al., 2020). More controlled environment is needed to

use percolation method as an industrial application. In addition, optimisation of air flow may enhance the metal extraction yield (Zare Tavakoli et al., 2017). Alternatively, stirred tank reactors can be considered as a scale up alternative due to it provides enhanced gas distribution and solid-liquid interaction (Kinnunen and Hedrich, 2023; Gericke et al., 2009).

Table 4-3. On day 11, 3.3 mg/kg REEs, 51 mg/kg critical metals and 10,536 mg heavy metals and other metals were extracted. Extracted metal amount were slightly increased for the REEs (up to 7%) until the end of the experiment resulted 3.7 mg/kg REEs extraction (Day 21). At the end of the experiment 69% Y, 48% Ce, 53% Nd, 37% Li, 32% Co, 40% Cu, 36% Zn, 39% Al and 41% Mn dissolved which corresponds to 0.89 mg/kg Y, 1.86 mg/kg Ce, 0.95 mg/kg Nd, 2 mg/kg Li, 3 mg/kg Co, 51 mg/kg Cu, 6,555 mg/kg Zn, 1,040 mg/kg Al, 2,385 mg/kg Mn. A total of 10,039 mg/kg elements were extracted from BOS-D at the end of the column experiment using 40 ml/min recirculation rate, while it was higher on day 11 (10,590 mg/kg). As a result, to save time and resource, operating the bioleaching column system for 11 days would be enough to extract metals from BOS-D, considering that the extraction of the most abundant elements had nearly plateaued.

4.4 Conclusion

This study uncovers significant findings and practical implications regarding the extraction of metals from basic oxygen steelmaking dust (BOS-D). Notably, during the initial leaching stage, bioleaching conducted in batch outperformed control methods, demonstrating its efficacy in extracting various metals. Bioleaching further exhibited promising results with the extraction of REEs and other metals, outperforming the alternatives in certain cases. Chemical leaching, while efficient, proved to be less suitable for REEs extraction. The hybrid leaching approach, combining a deep eutectic solvent and bioleaching, yielded additional REEs extraction. Scaling up the bioleaching process highlighted challenges, with decreased extraction rates observed for most metals. The study findings suggest that for optimal efficiency and resource use, concluding column bioleaching after 11 days is advisable. Furthermore, the research emphasizes the need for

controlled environments when implementing percolation methods at an industrial scale. A stirred tank reactor is proposed as a scalable solution for the industrial application of bioleaching, though further investigation is required to optimise aeration and agitation rates. Overall, this study not only advances our understanding of bioleaching applications for secondary resources but also provides valuable insights into the limitations and opportunities for scaling up this eco-friendly metal extraction process. These findings can inform and guide future research on sustainable metal recovery from industrial waste materials.

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5 TECHNO-ECONOMIC ASSESSMENT OF BIOLEACHING FOR INDUSTRIAL METAL-BEARING BY-PRODUCTS

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Abstract: The exploration of methods to extract metals from metal-bearing wastes and by-products has gained significant attention due to increasing demand, decreasing ore quality, and mounting environmental concerns. In this context, bioleaching has emerged as a sustainable and cost-efficient alternative to pyrometallurgy and hydrometallurgy. While the efficacy of bioleaching in extracting metals from sulfidic ores, tailings, and various metallurgical side streams has been demonstrated, there remains a notable absence of operational plants designed to process secondary resources such as metallurgical slag and dust, fly ash, and e-waste. This is partly due to a lack of comprehensive techno-economic assessment (TEA). This study focused on the economic feasibility of two potential industrial-scale bioleaching technologies for metal recovery from specific metallurgical by-products, mainly basic oxygen steelmaking dust (BOS-D) and goethite. The investigation compared two bioleaching scaling technology configurations, including an aerated bioreactor and an aerated and stirred bioreactor across different scenarios. Results indicated that bioleaching using *Acidithiobacillus ferrooxidans* proved financially viable for copper extraction from goethite, particularly when 5% and 10% pulp densities were used in the aerated bioreactor, and when 10% pulp density was used in the aerated and stirred bioreactor. Notably, a net present value (NPV) of 1,275,499 k$ and an internal rate of return (IRR) of 65% for Cu recovery from goethite were achieved over 20-years after project started using the aerated and stirred bioreactor plant with a CAPEX 119,816,550 $ and OPEX 5,896,580 $/year. It is expected that plant will start to make profit after one year of operation. Aerated and stirred bioreactor plant appeared more reliable alternative compared to the aerated bioreactor plant as the plant consists of 12 reactors which can allow better management and operation in small volume with multiple reactors. Despite these limitations, this TEA

emphasized the significance of selective metal recovery and plant design, and underscored the major expenses associated with the process.

Keywords: Bioleaching, biohydrometallurgy, secondary resources, techno-economic assessment, economic feasibility

5.1 Introduction

The high metal content found in metal-bearing wastes and by-products, such as metallurgical slag and dust, e-waste, and fly ash, is often similar or surpasses that of primary ore. This characteristic renders them invaluable as secondary resources within the circular economy (Kinnunen and Hedrich, 2023). Consequently, several studies are increasingly focusing on the feasibility of extracting metals from these secondary resources, driven by rising metal demands, declining concentrations in primary ores, and the imperative for improved environmental stewardship (Owusu-Fordjour and Yang, 2023; Tezyapar Kara et al., 2023a). While conventional methods such as pyrometallurgy and hydrometallurgy are predominantly used for metal extraction from primary ores, they require a significant amount of energy due to high temperature and pressure requirements, substantial capital investment, and the generation of harmful emissions (Arya and Kumar, 2020; Asghari et al., 2013). Moreover, these technologies are deemed inefficient due to metal losses during processing (Roy et al., 2021a; 2021b). In this context, bioleaching has emerged as an environmentally friendly and economically viable alternative for extracting metals from secondary resources (Owusu-Fordjour and Yang, 2023; Al Sultan and Benli, 2023). Also known as biohydrometallurgy, bioleaching relies on using naturally occurring microorganisms and/or their metabolites to extract metals from solid matrixes (Srichandan et al., 2019; Tezyapar Kara et al., 2023a). While commercial-scale bioleaching is available for metal extraction from sulfidic ores, tailings, and metallurgical side streams (Kinnunen and Hedrich, 2023; Kržanović et al., 2019; Petersen, 2023), there is currently no existing plant specifically designed for other secondary resources (Owusu-Fordjour and Yang, 2023; Thompson et al., 2018). This is partly due to the lack of techno-economic assessment (Vo et al., 2024).

Alipanah et al. (2023) conducted a techno-economic assessment for a potential industrial-scale bioleaching plant capable of processing 10,000 tonnes of black mass annually. They estimated that bioleaching process using a biolixiviant produced from corn stover by *Gluconobacter oxydans* can generate an average of $110M net potential value (NPV) over a period of 30 years. In another study, Irrgang et al., (2021) projected a NPV > $28M from bioleaching zinc sulphide to recover zinc, copper and indium using a thermophilic microbial consortium in continuously stirred tank reactors (CSTR) over 20 years. In this study, the economic feasibility of two potential industrial scale bioleaching technologies, including an aerated and an aerated and stirred bioreactors were evaluated for metal recovery from metallurgical by-products, including basic oxygen steelmaking dust and goethite. The assessment considered different operational conditions such as pulp density, energy source concentration ($FeSO_4.7H_2O$ as a source of Fe^{2+}), inoculum concentration, and pH and evaluated their effects on operational costs. Three scenarios were considered as follows: (1) extracting and recovering all nine elements (Y, Ce, Nd, Li, Co, Cu, Zn, Mn and Al) in elemental form by electrowinning, (2) recovering only Zn in elemental form via electrowinning, (3) recovering only the most abundant element, excluding Zn, in elemental form via electrowinning. Zn recovery was specifically targeted as these metal-bearing by-products are potentially considered as a secondary iron resource due to their high iron content (40-60%); however, their high zinc content (10-100 g/kg) restricts their use in the blast furnace for the steelmaking industry (Xue et al., 2022). The reason is that when zinc charged into a blast furnace, it vaporizes at high temperatures (1600–1650 °C) due to its low boiling point (907 °C). Zinc condenses and accumulates on blast furnace walls that affect the solid and gas flows through the furnace. This can decrease the productivity and damage the furnace linings (Stewart and Barron, 2020; Xue et al., 2022). Zinc also shortens the furnace operating life by attacking refractories in the upper stack of the furnace (Binnemans et al., 2020). So, one of our focuses was evaluating the high Zn extraction conditions. This research provides insights into the potential value of extracted elements, emphasizes the importance of selective metal recovery and process design, and highlights the highest costs of the process.

5.2 Materials and methods

5.2.1 Characterisation of materials and metal values

A bulk mixture of BOS-D from a former iron and steelwork plant stockpiled in Teesside, UK, was received in a large, sealed drum and stored at ambient temperature. Additionally, goethite, a mineral residue from zinc ore refining throughout a hydrometallurgical process, was obtained from the Nyrstar plant in Auby, France and stored in a bucket at ambient temperature. Roughly 3 kg of samples from each by-product were used for analysis and experiments. Both samples were air-dried, ground, and sieved through a 2 mm mesh. The physico-chemical characteristics for BOS-D and goethite have been previously reported by Tezyapar Kara et al. (2023) and given in the supplementary materials (Table S1). The elemental composition of the autoclaved BOS-D (n=2) and goethite (n=3) is given in Table 5. The elemental composition of non-autoclaved materials has been given in supplementary materials (Table S2). In this study, the techno-economic extraction assessment encompassed nine elements, including Yttrium (Y), Cerium (Ce), Neodymium (Nd), Lithium (Li), Cobalt (Co), Copper (Cu), Zinc (Zn), Manganese (Mn), and Aluminum (Al). These elements were selected based on their abundance, significance as critical metals, and inclusion as rare earth elements (REE). Their potential values assuming 100% extraction efficiency along with commodity price are presented in Table 5. Unless stated otherwise, the currency referred to in this study is US dollar ($).

5.2.2 Process description

This technoeconomic assessment evaluated two distinct industrial scale bioleaching technologies, including an aerated bioreactor based on the study of Isildar (2016) and a combined aerated and stirred bioreactor based on the study of Irrgang et al. (2021) (Table 1). The size of the aerated bioreactor is 5,000 m³, with an effective volume of 4,944 m³. The bioreactor is designed to introduce air from the bottom. This configuration serves multiple purposes: (i) aiding the oxidation of the leaching of metals, (ii) supplying the necessary oxygen and carbon dioxide for microbial activity, and (iii) facilitating leachate circulation by creating internal channels within the by-product in the reactor. In this study, it was assumed

that the by-products are ground to a size smaller than 500 µm. Following the completion of bioleaching, the targeted metals are recovered from the leachate in their elemental form through electrowinning. To ensure comparability with the study conducted by Isildar (2016), the initial size of the combined aerated and stirred bioreactor outlined by Irrgang et al. (2021) was doubled (Table 1). The aerated and stirred bioreactor is equipped with state of art technologies such as measurement/sensor technology, cooling, aeration, extraction and electrowinning equipment. The schematic process designs for the aerated bioreactor plant and the aerated and stirred bioreactor plant are shown in Figure 5-1.

The equipment data for the aerated and stirred bioreactor was more current, dating from 2021, in contrast to the aerated bioreactor which dated from 2016. Hence, the cost data for the aerated bioreactor were extrapolated for 2021 using the Chemical Engineering Plant Cost Index (CEPCI) as described in Equation 1 (Bacatelo et al., 2023; Peters and Timmerhaus, 1991; Isildar 2016):

$$C = C_0 x \left(\frac{CEPCI}{CEPCI_0}\right) \quad (1)$$

Where C_0 is cost in 2016, C is cost in 2021, $CEPCI_0$ is CEPCI in 2016 (541.7) and CEPCI is CEPCI in 2021 (677.7). Since the recent CEPCI index is available for 2021, cost data was extrapolated for this year.

Parameters considered for both technologies are summarised in Table 2 and adapted from Irrgang et al., (2021) and Towler and Sinnott, (2007). An overall plant efficiency of 95% was assumed and plant lifespan of 20 years was chosen according to Irrgang et al., (2021) and Isildar, (2016). The leaching duration for the aerated bioreactor was 11 days, whereas for the combined aerated and stirred bioreactor, it was extended to 16 days, as determined from the laboratory experiments. The number of leaching cycles per year was then calculated based on leaching duration and plan capacity. By-product processing quantities were derived by considering leaching cycles and pulp density (PD) (Dewulf, 2022). Based on these assumptions, the processing capacities of the aerated bioreactor were estimated at 1,434 t/y when 1% PD was used, 7,169 t/y with 5% PD, and

14,338 t/y with 10% PD. Similarly, the processing capacities of the aerated and stirred bioreactor were estimated at 1,015 t/y with 1% PD, 5,073 t/y with 5% PD, and 10,146 t/y with 10% PD.

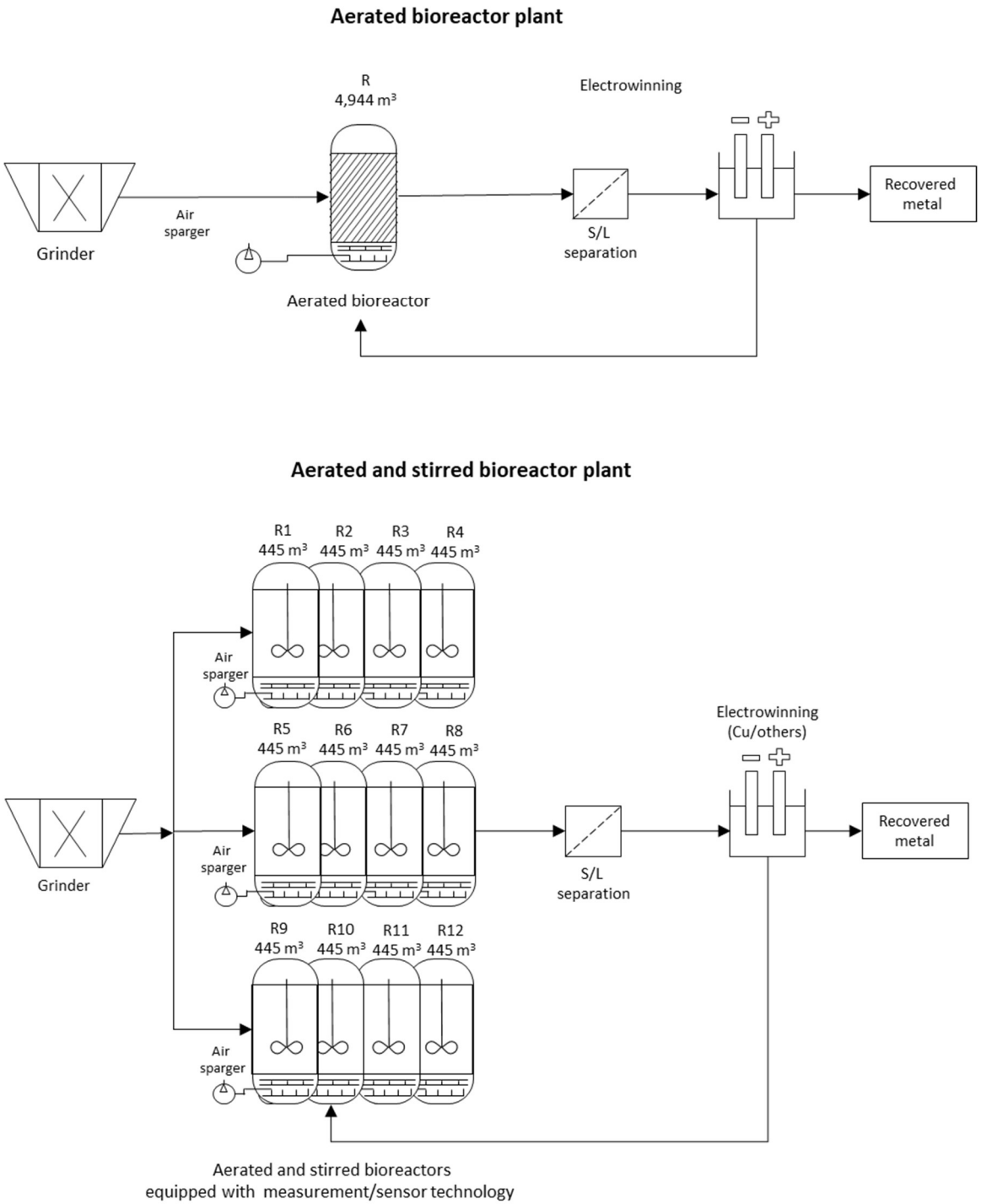

Figure 5-1. Schematic process design for both the aerated and the aerated and stirred bioreactor plants for metal recovery from industrial metal-bearing by-products (Isildar, 2016; Irrgang et al., 2021).

Table 5-1. Details of the equipment requirements and costs for each bioreactor.

Bioreactor type	Process unit	Unit number	Costs ($/unit)	Total costs ($)
Aerated bioreactor (Isildar, 2016)	Grinding	5	27,213	136,065
	Bioreactor (5,000 m^3)	1	9,383	9,383
	Electrowinning cells	9	34,016	306,147
	TPEC			451,596
Aerated and stirred bioreactor (Irrgang et al., 2021)	Grinding	2	282,000	564,000
	Bioreactor (450 m^3)	12	1,695,000	20,340,000
	Measurement/Sensor technology	12	19,217	230,600
	Cooling	16	141,250	2,260,000
	Aeration	26	6,446	167,600
	Extraction	1	3,738,300	3,738,300
	Electrowinning (copper)	1	180,360	180,360
	Electrowinning (other elements)	1	8,126,220	8,126,220
	TPEC			35,607,080

Table 5-2. Parameters and metrics used for the industrial-scale bioreactors.

Bioleaching reactor types		Aerated			Aerated and stirred		
Description	Units	Value					
Annual working hour	h/y	8000					
Annual working day	d	334					
Overall plant efficiency	%	95					
Plant life	y	20					
Bioleaching duration	d	11			16		
Bioleaching cycle	n/y	29			19		
Pulp density	% (w/v)	1%	5%	10%	1%	5%	10%
Plant capacity	t/y	1,434	7,169	14,338	1,015	5,073	10,146
Total processed material	t	28,680	143,380	286,760	20,300	101,460	202,920

5.2.3 Experimental setup and revenues calculations

A series of bioleaching batch experiments were performed for 16 days under different solid concentration, energy source concentration, inoculum concentration (Tezyapar Kara et al., 2023b). Briefly, the batch experiments were performed using 250 mL Erlenmeyer flasks under 100 ml working volume, incubated at 30°C on an orbital shaker at 150 rpm. It was assumed that the orbital shaker could effectively replicate an industrial aerated and stirred bioreactor (Heyman et al., 2019; Irrgang et al., 2021). Additionally, a column experiment was performed for 11 days, focusing solely on BOS-D under specific conditions derived from batch optimisation experiments where leachate was circulated at 40 ml/min. BOS-D was selected as reference by-product to be investigated on column scale instead of

processing both materials due to time and resource constraints. The column was run under submerged conditions, with air supply from the bottom of the columns, aiming to simulate an industrial aerated bioreactor setup (Isildar, 2016). Extracts were analysed with a PerkinElmer's NexION® Inductively Coupled Plasma Mass spectrometer (ICP-MS) using uranium (U) as internal standard as described. Single ICP-MS standard solution sourced from Perkin Elmer for the metals with the analyte concentration of 10 µg/ml were used for calibration.

For the plant revenue calculations, the extraction yields (%) from batch experiments (Table 5) were used to calculate the revenue from aerated and stirred bioreactor assuming that the agitation conditions mimicked the stirred environment present in the aerated and stirred bioreactor (Pourhossein and Mousavi, 2019). On the other hand, the column experiment data (Table 5) was used to derive the extraction yield for the aerated bioreactor plant where for each element the extraction data was proportionally adjusted to compute the revenue. The total cost and revenue were determined based on three distinct scenarios: (1) Extraction and recovery of all nine elements (Y, Ce, Nd, Li, Co, Cu, Zn, Mn, and Al) in elemental form through electrowinning, (2) Recovery of zinc (Zn) only in elemental form via electrowinning and (3) Extraction of the most abundant element, excluding zinc, in elemental form through electrowinning. For BOS-D, the most prevalent element was Mn, while for goethite, it was Cu, excluding zinc. The specific focus on zinc is due its significance, as its presence >1% content restricts the reusability the by-products as secondary iron resources. So, the extraction resulting Zn content <1% in the residue may potentially allow this metallurgical by-product to be considered as secondary resource (Xue et al., 2022). For those conditions additional revenue was added where the by-product was resold as secondary iron resource based on their iron content (Trading Economics, 2023).

5.2.4 Techno-economic assessment

Details of on the total cost breakdown of the bioleaching process including the capital expenditure (CAPEX) and the operational expenditure (OPEX) are provided in Table 3. CAPEX includes equipment purchase and installation costs, piping costs, electrical, buildings, yard improvement, service facilities and land cost. Cost

ranges and assumed values for each of these expense items are derived from Peters and Timmerhaus, (1991). OPEX includes utilities cost such as water and electricity, maintenance and wear, personnel cost, and reagent cost (Irrgang et al., 2021; Thompson et al., 2018). As different equipment was used for the aerated bioreactor and the aerated and stirred bioreactor, specific energy requirements were considered. For instance, the energy requirement for the electrowinning equipment in the aerated bioreactor plant is 1240 kWh per tonne of metal extracted, whereas for the aerated and stirred bioreactor plant, it increases to 3338 kWh per tonne of metal extracted. The assumed costs were set at $2.47 for 1 m^3 of water and $0.36 for 1 kWh of electricity. Because for this study the number of units of the aerated and stirred bioreactor plant was twice of the study of Irrgang et al. (2021), personnel costs were doubled accordingly. Considering the similar capacities of both plants, the personnel costs for the aerated bioreactor plant were assumed to be equivalent. Reagent costs were computed based on the laboratory experiment conditions, and the chemical commodity prices as detailed in Table 4.

The financial analysis was conducted using GoldSim version 14.0 (GoldSim Technology Group, 2018) to forecast cash flow, net present value (NPV), and internal rate of return (IRR) for the three scenarios. To ensure a more comprehensive and realistic feasibility analysis, the model incorporated discount rates and inflation rates (Figure 5-2). Specifically, the model incorporated a discount rate of 10% and an inflation rate of 3.5%, aligning with established practices (Irrgang et al., 2021; GoldSim Technology Group, 2018). Furthermore, the model elements integrated development costs of $10,000 per year for the initial two years (model default). Data for initial capital costs (TFCI), operating costs, and revenues were sourced from Table 1, 3 and 4.

Table 5-3. Overview of the CAPEX and OPEX for each bioreactor type.

CAPEX			
Direct cost		Typical range %	Assumed % of total
	Purchased equipment	15-40	25
	Purchased equipment installation	6-14	9
	Instruments and controls (installed)	2-8	7
	Piping (installed)	3-20	8
	Electrical (installed)	2-10	5
	Buildings (including services)	3-18	5
	Yard improvement	2-5	2
	Services facilities (installed)	8-20	15
	Land	1-2	1
Indirect cost			
	Engineering and supervision	4-21	10
	Construction expense	4-16	12
	Contractor's fee	2-6	2
	Contingency	5-15	8
OPEX			
Utilities cost			
	Water	2.47$/m^3	
	Electricity	0.36$/kWh	
	Grinding[*,**]	38kWh/tonne ore[*]; 60 kWh/tonne ore[**]	
	Electrowinning[*,**]	1240 kWh/tonne metal[*]; 714 kWh/tonne Cu[**]; 3338 kWh/tonne metal[**]	
	Aeration[*]	20 kWh/tonne metal	
	Bioreactor[**]	2525 kWh/tonne metal	
	Extraction[**]	60 kWh/tonne metal	
Maintenance and wear		5% of investment sum per year	
Personnel costs		50 positions, including labour, laboratory, administration.	
Reagent Cost			

[*]Input used for aerated bioreactor; [**]Input used for aerated and stirred bioreactor

Table 5-4. Bioleaching medium components and commodity prices.

Chemical	Chemical formula	Concentration (g/L)	Price ($/t)	Reference
Ammonium sulphate	$(NH_4)_2SO_4$	2	120	Echemi.com (2023)
Potassium phosphate dibasic	K_2HPO_4	0.25	1,270	Echemi.com (2023)
Magnesium sulphate heptahydrate	$MgSO_4.7H_2O$	0.25	150	Echemi.com (2023)
Potassium chloride	KCl	0.1	91	Echemi.com (2023)
Calcium nitrate tetrahydrate	$Ca(NO_3)_2$	0.01	240	Echemi.com (2023)
Iron sulphate heptahydrate	$FeSO_4.7H_2O$	22.2	50	Alibaba.com (2023)
Sulfuric acid	H_2SO_4	272	120	Made-in-China (2023)

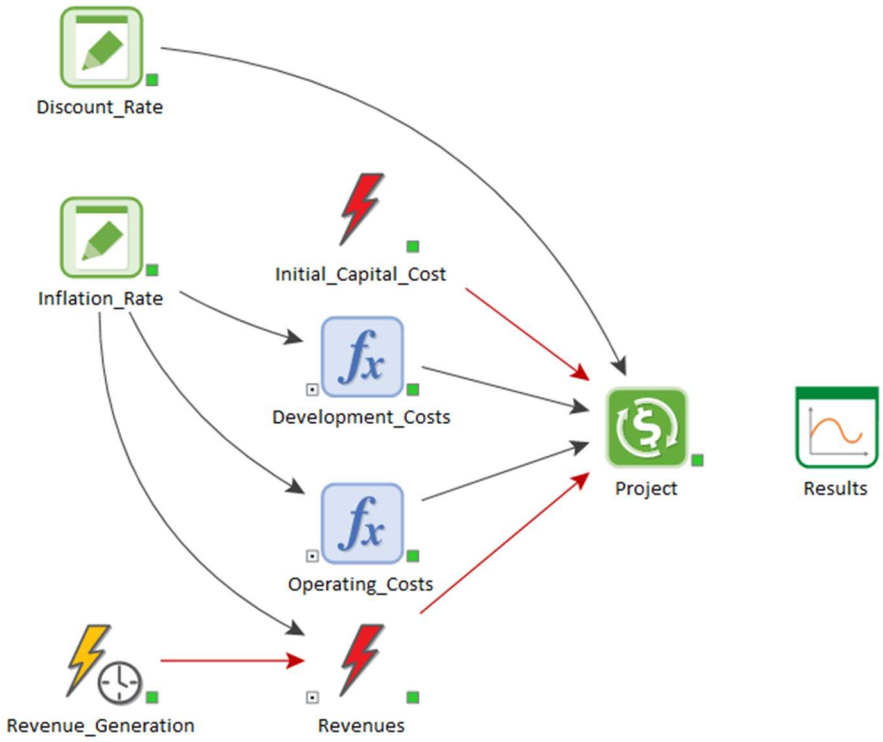

Figure 5-2. Overview of the financial module elements used.

5.3 Results and Discussion

Table 5 indicates the estimated potential values of elements for per tonne processed metallurgical by-product under 100% extraction efficiency. According to this, there is a potential of 133$ value from per processed BOS-D as well as 1,506$ value from per processed goethite The substantial difference between the values of BOS-D and goethite stems from the notably higher initial Copper (Cu) content found in goethite (203,667 g/t) compared to BOS-D (126.50 g/t). Additionally, these values may vary based on fluctuations in market prices and shifts in demand.

Based on varying operational conditions different extraction yields were obtain as shown in Table 6. The leaching kinetics decreased in the column setup (operational condition 1 | 1 | 1 | 1.75) compared to the batch (operational condition 1 | 1 | 1 | 1.50), resulting in decreased dissolution rates (Table 6). This occurrence could be due to less effective interactions between microorganisms, leaching agents, and materials in larger volumes, along with heat dissipation in the column arrangement, in contrast to the batch system.

Table 5-5. Estimated potential values of elements for per tonne processed metallurgical by-product, assuming 100% extraction efficiency.

Elements	Commodity price[1] ($/t)			BOS-D				Goethite			
				Concentration (g/t)	Value of resource ($/t)			Concentration (g/t)	Value of resource ($/t)		
	Min	Max	Average		Min	Max	Average		Min	Max	Average
REE											
Y [2, 5, 6, 8]	26,850	60,000	38,338	1.30	0.03	0.08	0.05	2.30	0.06	0.14	0.09
Ce [2, 5, 6, 8]	5,000	11,000	7,093	3.84	0.02	0.04	0.03	5.60	0.03	0.06	0.04
Nd [2, 4, 5, 6, 8]	46,030	142,600	81,126	1.81	0.08	0.26	0.15	2.40	0.11	0.34	0.19
Total REE				**6.95**	**0.14**	**0.38**	**0.22**	**10.30**	**0.20**	**0.54**	**0.32**
Critical metals											
Li [2, 6, 7, 8]	63,370	115,700	88,208	4.47	0.28	0.52	0.39	3.20	0.20	0.37	0.28
Co [2, 6, 7, 8]	32,800	59,500	42,738	10.16	0.33	0.60	0.43	9.00	0.30	0.54	0.38
Cu [2, 3, 6, 7, 8]	4,530	8,490	6,386	126.50	0.57	1.07	0.81	203,667	922.61	1,729.13	1,300.62
Total critical metals				**141.13**	**1.19**	**2.20**	**1.64**	**203,679**	**923**	**1,730**	**1,301**
Heavy metal and others											
Zn [3, 6, 7, 8]	2,170	2,830	2,466	18,300	39.71	51.79	45.12	61,615	133.70	174.37	151.92
Al [2, 6, 7, 8]	1,470	2,032	1,853	2,660	3.91	5.41	4.93	11,164	16.41	22.69	20.69
Mn [6, 8]	1,580	2,060	1,820	5,870	9.27	12.09	10.68	1,457	2.30	3.00	2.65
Fe [6, 7, 8]	60	400	180	390,500	23.43	156.20	70.29	162,333	9.74	64.93	29.22
Total heavy metal and others				**417,330**	**76**	**225**	**131**	**236,569**	**162**	**265**	**204**
Total elements				**417,478**	**78**	**228**	**133**	**440,259**	**1,085**	**1,996**	**1,506**

[1] Commodity prices of elements form various resources. Average value of each element was determined based on a range of reference demoted by number., [2]Gutiérrez-Gutiérrez et al., (2015); [3] KME (2023); [4] Strategic metals invest (2023); [5] Institute of Rare Earths and Metals (2019); [6] Compound Interest (2019); [7] Alan's Factory Outlet (2021); [8] Leon Becker (2018).

Table 5-6. Extraction yields (%) from batch and column scale experiments, n=2; SD for all series ≤ 20% (Series names represent the values of "solid concentration | energy source concentration | inoculum concentration | pH").

Scale	Condition	Li	Al	Mn	Co	Cu	Zn	Y	Ce	Nd
BOS-D batch	1 | 1 | 1 | 1.50	92	59	54	40	61	61	99	67	80
	1 | 2 | 5 | 1.75	55	43	53	54	34	55	96	76	58
	1 | 3 | 10 | 2.00	55	39	57	52	33	53	32	75	48
	5 | 1 | 5 | 2.00	62	47	51	43	59	61	87	48	59
	5 | 2 | 10 | 1.50	36	53	47	35	59	52	100	58	59
	5 | 3 | 1 | 1.75	39	49	46	40	56	51	100	53	60
	10 | 1 | 10 | 1.75	41	46	46	37	56	51	73	43	38
	10 | 2 | 1 | 2.00	46	43	52	39	55	55	69	35	35
	10 | 3 | 5 | 1.50	31	46	42	34	52	48	80	45	52
Goethite batch	1 | 2 | 1 | 1.50	57	23	86	64	95	35	100	15	23
	1 | 3 | 5 | 1.75	61	20	100	100	80	43	96	14	23
	1 | 4 | 10 | 2.00	64	16	100	100	77	43	96	14	21
	5 | 2 | 5 | 2.00	56	20	35	25	85	34	100	15	20
	5 | 3 | 10 | 1.50	50	23	40	31	77	33	98	15	21
	5 | 4 | 1 | 1.75	56	21	50	39	84	36	99	15	23
	10 | 2 | 10 | 1.75	51	22	29	21	95	33	100	14	20
	10 | 3 | 1 | 2.00	50	22	33	24	100	31	100	14	19
	10 | 4 | 5 | 1.50	45	24	33	24	97	30	93	14	19
BOS-D column	1 | 1 | 1 | 1.75	37	36	42	31	36	39	62	43	46

5.3.1 Potential revenues and profits of the different bioreactors

Figure 5-3 shows the potential revenues resulting from the different operational conditions and different scenarios. Processing BOS-D processing with the aerated bioreactor can generate a potential revenue ranging from $126,284 and $6,308,847. A significant portion of this value originated from zinc recovery, given its predominance in BOS-D. Meanwhile, the potential revenue from the various elements recovered from the goethite ranged between $974,816 and $228,932,495. Most of these revenues were attributable to Cu recovery, the most abundant element in goethite. However, assessing potential revenues alone is insufficient to gauge profitability, necessitating a comprehensive techno-economic assessment involving CAPEX and OPEX. According to this, detailed cost analysis was performed, and the estimated total profits of each scenario are given in Table

7. Notably, no profit was projected from BOS-D processing using the aerated bioreactor. In contrast, profits between $16,029,208 to $142,538,726 from goethite were estimated when 5% and 10% PD was used, respectively. As an illustration, a detailed breakdown of the total costs for recovering all nine elements and Cu from goethite using the aerated bioreactor is provided in Tables 8 and 9. Maintaining a constant plant capacity for each condition resulted in similar expenses for total fixed capital investment (TFCI), water, maintenance, and personnel costs. However, discrepancies were observed in electricity and reagent costs due to varying extraction yields, energy source concentration, and sulfuric acid consumption between the operational conditions. For instance, to recover all elements from goethite, the condition of 10 | 2 | 10 | 1.75 incurred an estimated cost of $27,919,319 in electricity and reagent costs, while the condition of 10 | 3 | 1 | 2.00 incurred a cost of $29,749,939 (Table 8). Although the latter condition had higher costs, the estimated profit of $8.5M was greater compared to the more economical option. Energy source costs are as the highest expense among reagents (Table 8). Similar trends were observed for the scenario where Cu is only recovered, where the condition of 10 | 3 | 1 | 2.00 incurred higher costs but yielded a higher estimated profit of $9.7M compared to the condition of 10 | 2 | 10 | 1.75 (Table 9). Overall, the operational condition of 10 | 3 | 1 | 2.00 presented the highest potential profit for both scenarios. While the recovery of Cu only appeared more profitable due to lower processing costs for other elements, recovering all elements remained profitable, primarily owing to the value of copper. Personnel costs, followed by electricity and reagent costs, constituted the major expenses for the aerated bioreactor plant.

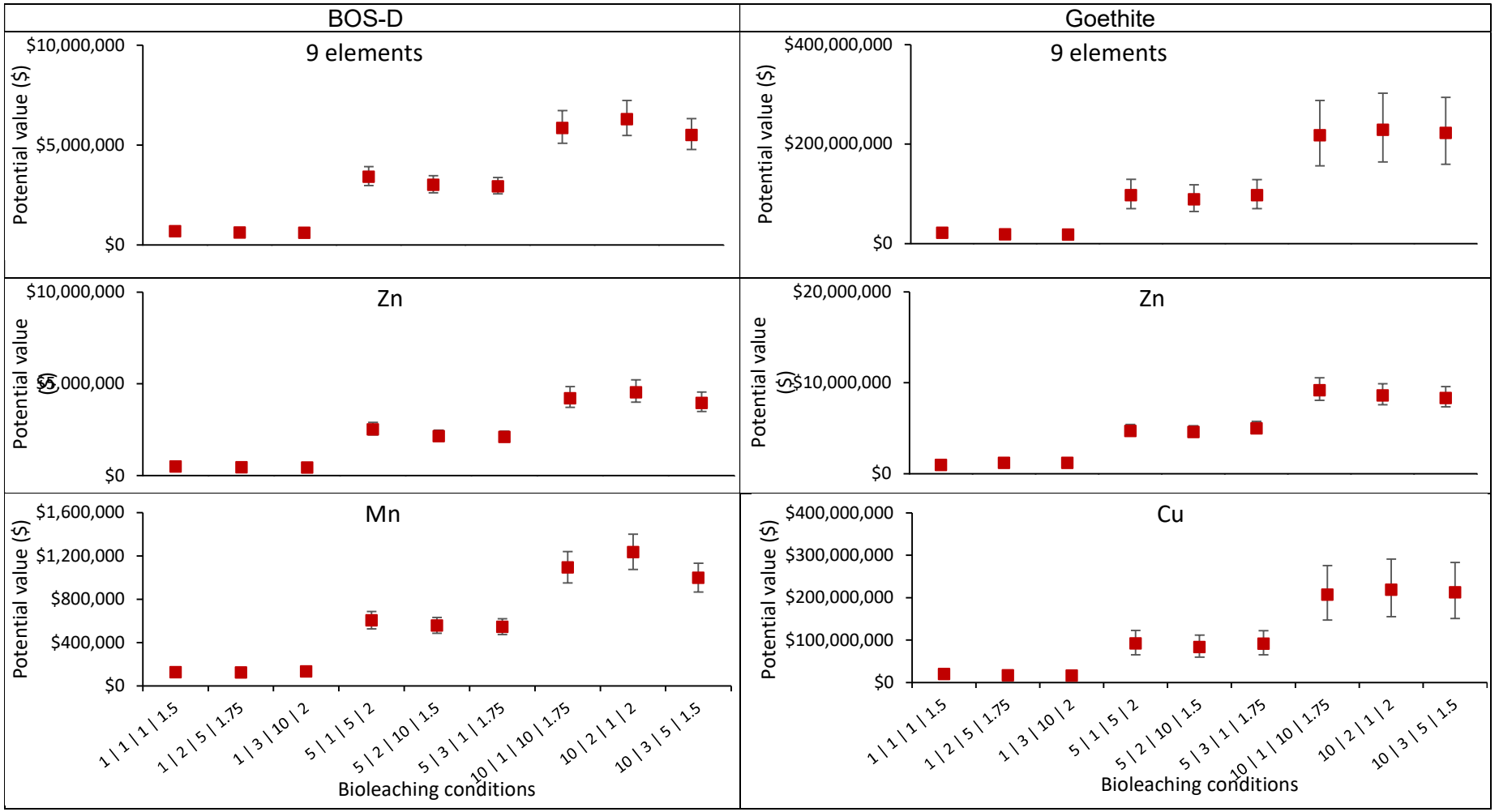

Figure 5-3. Potential revenues from extracted elements from BOS-D and goethite based on aerated bioreactor plant capacity and experimental results. Error bars represent minimum and maximum values based on the commodity prices in Table 5-5 (Series names represent the values of "solid concentration | energy source concentration | inoculum concentration | pH").

Table 5-7. Projected total profit of the aerated bioreactor considering varied operational conditions and extracted metals over 20 years (Conditions names represent the values of "solid concentration | energy source concentration | inoculum concentration | pH").

	PD	Condition	9 elements	Zn	Mn
BOS-D	1%	1 | 1 | 1 | 1.50	-59,989,204	-58,888,484	-59,186,449
		1 | 2 | 5 | 1.75	-60,856,858	-59,740,545	-60,001,936
		1 | 3 | 10 | 2.00	-61,921,111	-60,807,972	-61,049,869
	5%	5 | 1 | 5 | 2.00	-60,564,674	-59,972,624	-61,487,729
		5 | 2 | 10 | 1.50	-65,204,168	-64,588,377	-65,850,243
		5 | 3 | 1 | 1.75	-64,443,612	-63,808,178	-65,046,588
	10%	10 | 1 | 10 | 1.75	-66,601,061	-66,548,469	-69,025,288
		10 | 2 | 1 | 2.00	-65,127,470	-65,157,869	-67,788,633
		10 | 3 | 5 | 1.50	-71,904,057	-71,775,704	-74,128,637
	PD	**Condition**	**9 elements**	**Zn**	**Cu**
Goethite	1%	1 | 2 | 1 | 1.50	-41,833,988	-59,040,455	-40,015,461
		1 | 3 | 5 | 1.75	-45,371,186	-59,734,117	-43,950,914
		1 | 4 | 10 | 2.00	-47,217,192	-60,936,755	-45,836,359
	5%	5 | 2 | 5 | 2.00	**23,389,482**	-57,366,114	**27,011,314**
		5 | 3 | 10 | 1.50	**12,926,739**	-60,589,309	**16,029,208**
		5 | 4 | 1 | 1.75	**19,140,337**	-61,096,553	**22,441,809**
	10%	10 | 2 | 10 | 1.75	**125,017,937**	-57,483,507	**132,766,305**
		10 | 3 | 1 | 2.00	**133,590,932**	-59,043,273	**142,538,726**
		10 | 4 | 5 | 1.50	**124,384,020**	-62,926,100	**133,075,433**

Table 5-8. Cost analysis for recovering all nine elements from goethite using aerated bioreactor over 20 years (Conditions names represent the values of “solid concentration | energy source concentration | inoculum concentration | pH”).

Pulp density	1%			5%			10%		
Condition	1 | 2 | 1 | 1.50	1 | 3 | 5 | 1.75	1 | 4 | 10 | 2.00	5 | 2 | 5 | 2.00	5 | 3 | 10 | 1.50	5 | 4 | 1 | 1.75	10 | 2 | 10 | 1.75	10 | 3 | 1 | 2.00	10 | 4 | 5 | 1.50
CAPEX									
Direct cost	*1,390,915*	*1,390,915*	*1,390,915*	*1,390,915*	*1,390,915*	*1,390,915*	*1,390,915*	*1,390,915*	*1,390,915*
Indirect cost	*578,042*	*578,042*	*578,042*	*578,042*	*578,042*	*578,042*	*578,042*	*578,042*	*578,042*
Total fixed capital investment	1,968,957	1,968,957	1,968,957	1,968,957	1,968,957	1,968,957	1,968,957	1,968,957	1,968,957
OPEX									
Utilities cost									
Water	7,077,594	7,077,594	7,077,594	7,077,594	7,077,594	7,077,594	7,077,594	7,077,594	7,077,594
Electricity									
Grinding	*388,173*	*388,173*	*388,173*	*1,940,866*	*1,940,866*	*1,940,866*	*3,881,732*	*3,881,732*	*3,881,732*
Electrowinning	*1,647,794*	*1,463,775*	*1,410,607*	*7,371,266*	*6,790,370*	*7,391,629*	*16,250,068*	*16,950,311*	*16,495,691*
Aeration	*204,302*	*204,302*	*204,302*	*1,021,508*	*1,021,508*	*1,021,508*	*2,043,017*	*2,043,017*	*2,043,017*
Total electricity	2,240,269	2,056,250	2,003,082	10,333,640	9,752,744	10,354,004	22,174,816	22,875,060	22,420,440
Maintenance and wear	98,448	98,448	98,448	98,448	98,448	98,448	98,448	98,448	98,448
Personnel costs	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000
Reagent Cost	6,300,228	7,165,646	8,368,284	5,154,367	8,270,214	9,099,501	5,744,503	6,874,879	10,543,011
Total cost	62,685,496	63,366,895	64,516,365	69,633,006	72,167,958	73,598,504	82,064,318	83,894,938	87,108,450
Total revenue	20,851,508	17,995,708	17,299,172	93,022,488	85,094,697	92,738,841	207,082,255	217,485,870	211,492,470
Estimated profit	**-41,833,988**	**-45,371,186**	**-47,217,192**	**23,389,482**	**12,926,739**	**19,140,337**	**125,017,937**	**133,590,932**	**124,384,020**

Table 5-9. Cost breakdown for extracting Cu from goethite using aerated bioreactor over 20 years (Conditions names represent the values of "solid concentration | energy source concentration | inoculum concentration | pH").

Pulp destiny	1%			5%			10%		
Condition	1 | 2 | 1 | 1.50	1 | 3 | 5 | 1.75	1 | 4 | 10 | 2.00	5 | 2 | 5 | 2.00	5 | 3 | 10 | 1.50	5 | 4 | 1 | 1.75	10 | 2 | 10 | 1.75	10 | 3 | 1 | 2.00	10 | 4 | 5 | 1.50
CAPEX									
Direct cost	*552,751*	*552,751*	*552,751*	*552,751*	*552,751*	*552,751*	*552,751*	*552,751*	*552,751*
Indirect cost	*229,715*	*229,715*	*229,715*	*229,715*	*229,715*	*229,715*	*229,715*	*229,715*	*229,715*
Total fixed capital investment	782,466	782,466	782,466	782,466	782,466	782,466	782,466	782,466	782,466
OPEX									
Utilities cost									
Water	7,077,594	7,077,594	7,077,594	7,077,594	7,077,594	7,077,594	7,077,594	7,077,594	7,077,594
Electricity									
Crushing	*388,173*	*388,173*	*388,173*	*1,940,866*	*1,940,866*	*1,940,866*	*3,881,732*	*3,881,732*	*3,881,732*
Electrowinning	*12,345*	*14,354*	*14,354*	*25,120*	*28,708*	*35,885*	*41,627*	*47,369*	*47,369*
Aeration	*204,302*	*204,302*	*204,302*	*1,021,508*	*1,021,508*	*1,021,508*	*2,043,017*	*2,043,017*	*2,043,017*
Total electricity	604,819	606,829	606,829	2,987,494	2,991,083	2,998,260	5,966,376	5,972,118	5,972,118
Maintenance and wear	39,123	39,123	39,123	39,123	39,123	39,123	39,123	39,123	39,123
Personnel costs	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000
Reagent Cost	6,300,228	7,165,646	8,368,284	5,154,367	8,270,214	9,099,501	5,744,503	6,874,879	10,543,011
Total cost	59,804,231	60,671,658	61,874,296	61,041,045	64,160,481	64,996,945	64,610,062	65,746,180	69,414,312
Total revenue	19,788,771	16,720,744	16,037,938	88,052,359	80,189,689	87,438,754	197,376,368	208,284,906	202,489,745
Estimated profit	**-40,015,461**	**-43,950,914**	**-45,836,359**	**27,011,314**	**16,029,208**	**22,441,809**	**132,766,305**	**142,538,726**	**133,075,433**

Using the aerated and stirred bioreactor, the potential revenues from recovering various elements from BOS-D and from goethite were estimated at $274,625,691 (Figure 5-4). Despite considering additional revenue from reselling BOS-D as a secondary resource, the configuration with the aerated stirred bioreactor did not yield profitability when CAPEX and OPEX were taken into account (Table 10). Conversely, projected profits ranging from $3,038,728 to $12,977,072 were observed for goethite processing at 10% PD in bioleaching (Table 10), excluding the resale of goethite as a secondary resource. A detailed breakdown of the total costs for recovering Cu from goethite in the aerated and stirred bioreactor is provided in Table 11. Similar to aerated bioreactor plant, where the plant capacity was constant for each condition, several expense items, including total fixed capital investment (TFCI), water, maintenance and wear, and personnel costs, remained consistent across all conditions. Nonetheless, discrepancies were still observed for the electricity and reagent costs due to different extraction yields, energy source concentration, and sulfuric acid consumption among operational conditions. As previously mentioned, despite higher expenses in one operational condition, the estimated profit could be greater owing to the higher revenue obtained. For instance, estimated electricity and reagent costs totalled $58,739,395 for the condition 10 | 2 | 10 | 1.75 (10% PD, 2% energy source concentration, 1% inoculum concentration at pH 1.75), while for the condition 10 | 3 | 1 | 2.00 (10% PD, 3% energy source concentration, 1% inoculum concentration at pH 2.00), costs reached $61,932,323. Although the latter condition incurred higher costs, the estimated profit of nearly $10M was higher compared to the more economical option. Ultimately, condition 10 | 3 | 1 | 2.00 yielded the highest potential profit, amounting to $12,977,072, for Cu recovery using the aerated and stirred bioreactor. For the aerated and stirred bioreactor plant, the highest expense was the electricity costs and personnel expenses.

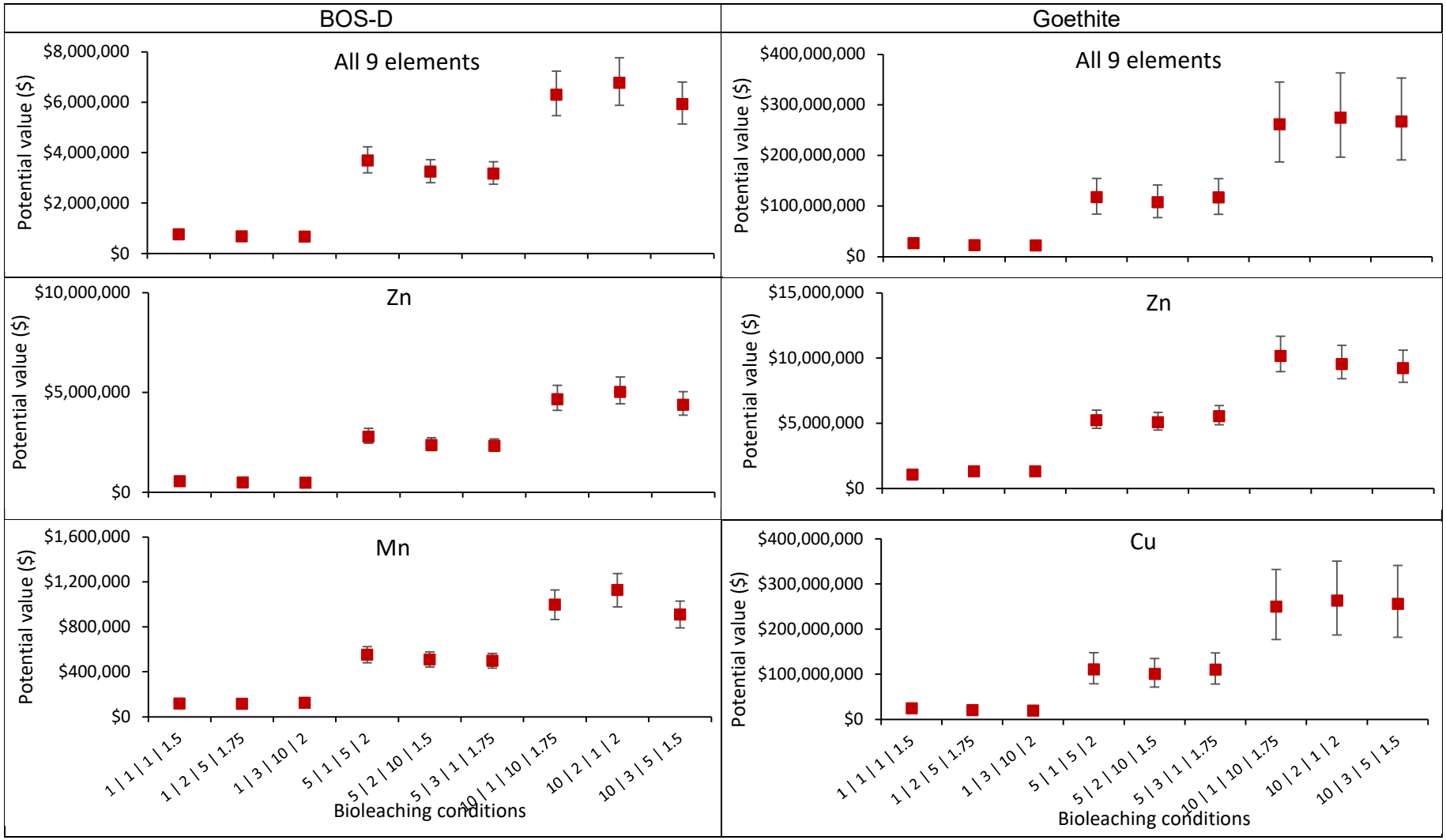

Figure 5-4. Potential revenues from extracted elements from BOS-D and goethite based on bioreactor plant capacity and experimental results. Error bars represent minimum and maximum values based on the commodity prices in Table 5-5 (Series names represent the values of "solid concentration | energy source concentration | inoculum concentration | pH").

Table 5-10. Projected total profit of the aerated and stirred bioreactor considering varied operational conditions and extracted metals over 20 years (Conditions names represent the values of "solid concentration | energy source concentration | inoculum concentration | pH").

	PD	Condition	9 elements	Zn	Mn
BOS-D	1%	1 | 1 | 1 | 1.50	-216,223,828	-215,473,769	-215,700,272
		1 | 2 | 5 | 1.75	-216,805,912	-216,043,963	-216,245,415
		1 | 3 | 10 | 2.00	-217,556,047	-216,794,198	-216,984,513
	5%	5 | 1 | 5 | 2.00	-212,857,345	-212,366,263	-213,508,427
		5 | 2 | 10 | 1.50	-215,961,854	-215,454,985	-216,417,300
		5 | 3 | 1 | 1.75	-215,382,984	-214,867,610	-215,811,728
	10%	10 | 1 | 10 | 1.75	-211,970,706	-211,746,991	-213,635,227
		10 | 2 | 1 | 2.00	-211,160,028	-210,966,610	-212,987,560
		10 | 3 | 5 | 1.50	-215,547,125	-215,285,903	-217,071,389
Goethite	**PD**	**Condition**	**9 elements**	**Zn**	**Cu**
	1%	1 | 2 | 1 | 1.50	-1,172,876,980	-220,668,968	-161,975,242
		1 | 3 | 5 | 1.75	-663,142,533	-220,676,950	-165,636,905
		1 | 4 | 10 | 2.00	-389,763,538	-221,389,742	-167,137,172
	5%	5 | 2 | 5 | 2.00	-132,854,766	-233,772,887	-98,297,827
		5 | 3 | 10 | 1.50	-142,663,300	-234,537,484	-108,058,286
		5 | 4 | 1 | 1.75	-136,221,411	-236,455,777	-101,832,571
	10%	10 | 2 | 10 | 1.75	-29,006,793	-256,211,864	**3,038,728**
		10 | 3 | 1 | 2.00	-19,349,225	-259,142,628	**12,977,072**
		10 | 4 | 5 | 1.50	-27,574,252	-260,706,408	**4,873,966**

Table 5-11. Cost analysis for recovering Cu from goethite using aerated and stirred bioreactor over 20 years (Conditions names represent the values of "solid concentration | energy source concentration | inoculum concentration | pH")

Pulp density	1%			5%			10%		
Condition	1 | 2 | 1 | 1.5	1 | 3 | 5 | 1.75	1 | 4 | 10 | 2	5 | 2 | 5 | 2	5 | 3 | 10 | 1.5	5 | 4 | 1 | 1.75	10 | 2 | 10 | 1.75	10 | 3 | 1 | 2	10 | 4 | 5 | 1.5
CAPEX									
Direct cost	*84,641,049*	*84,641,049*	*84,641,049*	*84,641,049*	*84,641,049*	*84,641,049*	*84,641,049*	*84,641,049*	*84,641,049*
Indirect cost	*35,175,501*	*35,175,501*	*35,175,501*	*35,175,501*	*35,175,501*	*35,175,501*	*35,175,501*	*35,175,501*	*35,175,501*
Total fixed capital investment	119,816,550	119,816,550	119,816,550	119,816,550	119,816,550	119,816,550	119,816,550	119,816,550	119,816,550
OPEX									
Utilities cost									
Water	5,008,458	5,008,458	5,008,458	5,008,458	5,008,458	5,008,458	5,008,458	5,008,458	5,008,458
Electricity									
Crushing	*274,691*	*274,691*	*274,691*	*1,373,453*	*1,373,453*	*1,373,453*	*2,746,907*	*2,746,907*	*2,746,907*
Bioleaching	*3,995,422*	*3,535,486*	*3,405,470*	*17,878,278*	*16,455,978*	*17,911,479*	*39,465,307*	*41,197,992*	*40,091,919*
Extraction	*253,175*	*224,031*	*215,792*	*1,132,881*	*1,042,755*	*1,134,985*	*2,500,772*	*2,610,566*	*2,540,478*
Electrowinning	*998,712*	*843,873*	*809,413*	*4,443,881*	*4,047,063*	*4,412,913*	*9,961,313*	*10,511,852*	*10,219,378*
Total electricity	5,522,000	4,878,081	4,705,366	24,828,494	22,919,249	24,832,830	54,674,299	57,067,316	55,598,682
Maintenance and wear	5,990,827	5,990,827	5,990,827	5,990,827	5,990,827	5,990,827	5,990,827	5,990,827	5,990,827
Personnel costs	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000	45,000,000
Reagent Cost	4,458,355	5,070,768	5,921,814	3,647,487	5,852,416	6,439,260	4,065,096	4,865,007	7,460,760
Total cost	185,796,190	185,764,684	186,443,015	204,291,816	204,587,500	207,087,925	234,555,230	237,748,158	238,875,276
Total revenue	23,820,949	20,127,778	19,305,843	105,993,989	96,529,214	105,255,355	237,593,958	250,725,231	243,749,242
Estimated profit	**-161,975,242**	**-165,636,905**	**-167,137,172**	**-98,297,827**	**-108,058,286**	**-101,832,571**	**3,038,728**	**12,977,072**	**4,873,966**

5.3.2 Comparative financial analysis of Zn recovery from BOS-D and Cu recovery from goethite

For this financial analysis, the focus was on recovering the most abundant elements present in the two metallurgical by-products (Figure 5-5). It is evident that the cash flow and the NPV projections for Zn recovery from BOS-D using the aerated bioreactor exhibit a consistent decline in profits over the 20-year period, showing a cumulative loss exceeding $25,000,000 (Figure 5-5a). Likewise, no profitability was observed within the 19 years after the project started for the aerated and stirred bioreactor plant. However, there is a potential for the plant to become profitable beyond this initial phase (Figure 5-5b).

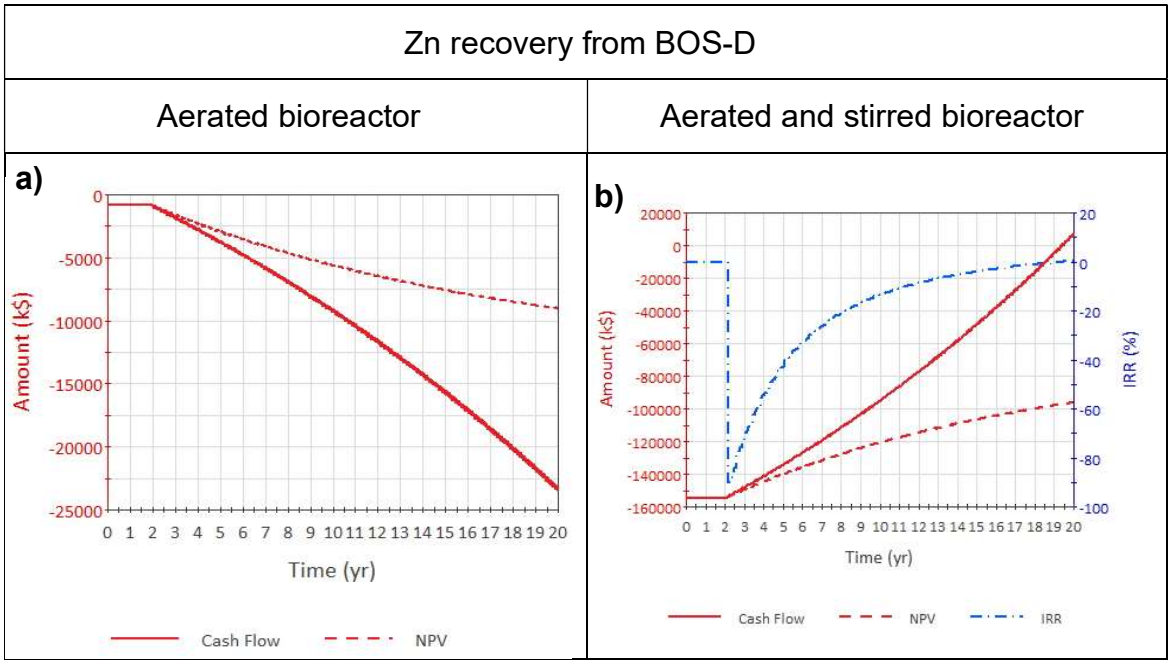

Figure 5-5. Projection of cash flow, net present value (NPV) and internal rate of return (IRR) of Zn recovery from BOS-D over 20 years using aerated bioreactor (a) and aerated and stirred bioreactor (b). Bioleaching condition was 10% PD, 1% energy source concentration, 1% inoculum concentration, pH 2.

In contrast to Zn recovery from BOS-D, the recovery of Cu from goethite is much more profitable (Figure 5-6). After 20 years of operation, the NPV for the aerated and stirred bioreactor plant reached $1,275,499 k with an IRR of 64.9%. Meanwhile, the NPV and IRR for the aerated bioreactor plant were $1,174,310 k and 747.7%, respectively. Despite the close NPV values, the aerated bioreactor plant exhibited an IRR over 10 times higher than the aerated and stirred bioreactor plant due to its lower initial capital and operational costs. It also

suggests that it generates significantly greater returns relative to its initial investment compared to the aerated and stirred bioreactor. This higher IRR implies that, despite both scenarios being profitable, the aerated bioreactor has a more efficient utilisation of investment capital and generates higher returns per unit of invested capital than the aerated and stirred bioreactor over the given time frame. Yet, given the disparity in the equipment data, with the aerated bioreactor data sourced from a study published in 2016 and the aerated and stirred bioreactor data being sourced from a study published in 2021, caution is needed in extrapolating these results. Ultimately, the aerated and stirred bioreactor plant appears to be a more pragmatic alternative when compared to the aerated bioreactor plant as it potentially provides better management with multiple reactors with small volumes.

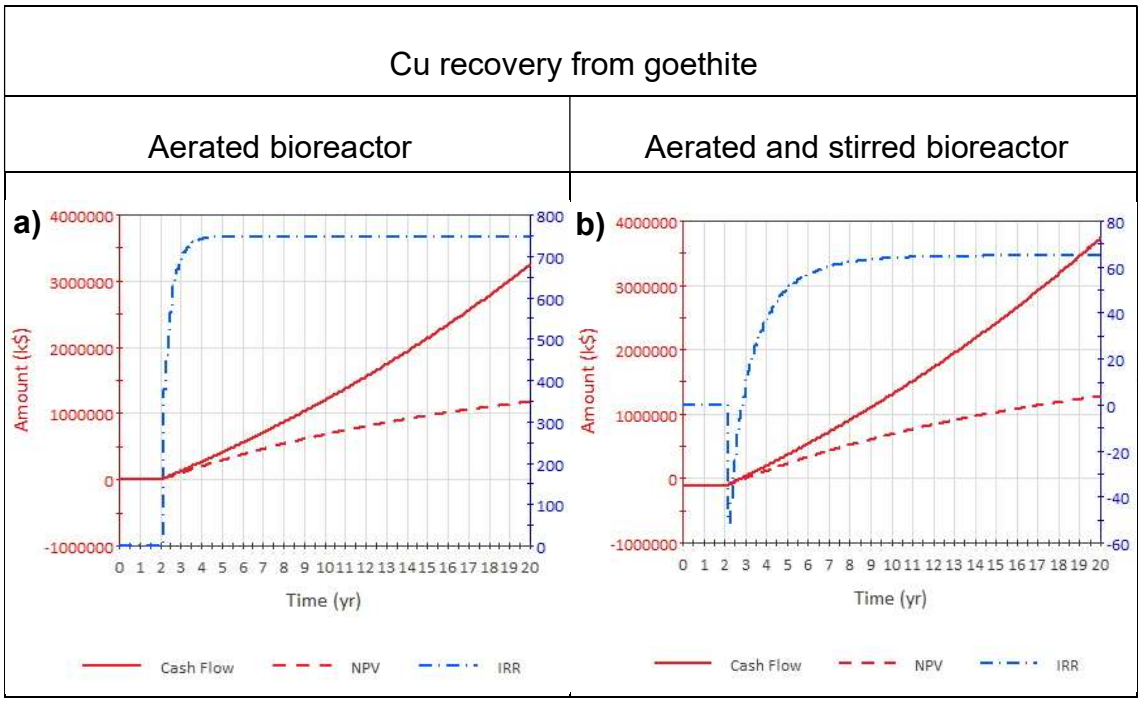

Figure 5-6. Projection of cash flow, net present value (NPV) and internal rate of return (IRR) of Cu recovery from goethite over 20 years using aerated bioreactor (a) and aerated and stirred bioreactor (b). Bioleaching condition was 10% PD, 2% energy source concentration, 1% inoculum concentration, pH 2.

5.4 Conclusion

The technoeconomic evaluation between an aerated bioreactor and an aerated and stirred bioreactor for recovering metals from BOS-D and goethite demonstrated varying profit outcomes. Notably, bioleaching of BOS-D revealed

no estimated profits, whereas goethite bioleaching for Cu and all nine elements resulted in profitable scenarios, particularly when 10% pulp density was used. The aerated bioreactor showed potential profits up to $133.5M and $142.5M for the recovery of all nine elements and solely Cu over 20 years, respectively. In contrast, profits up to $13M were forecasted when the aerated and stirred bioreactor was used with a 10% PD, 3% energy source concentration, 1% inoculum concentration and pH 2. Personnel, electricity, and water costs appeared as major expenses associated with the process.

Using the aerated and stirred bioreactor plant for copper recovery from goethite, financial analysis predicted a NPV of 1,275,499 k$ and an IRR of 64.9% over 20-years after project started. It is expected that plant will start to make profit after one year of operations with a 119,816,550 $ CAPEX and 5,896,580 $/year OPEX. Despite the slightly lower profit projection compared to the aerated bioreactor, the aerated and stirred bioreactor can possibly provide better management and operation as the plant consists of multiple reactors with small volumes.

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6 OVERALL DISCUSSION AND CONCLUSION

6.1 Introduction

This PhD research investigated the feasibility of bioleaching metallurgical by-products. To achieve this aim, (i) relevant literature was reviewed, (ii) batch scale bioleaching optimisation experiments were performed, (iii) methods for enhancing and scaling up the extraction of metals were explored, and (iv) economic feasibility of bioleaching metallurgical by-products on industrial scale was assessed. This chapter provides a summary of the key findings, and an overview of how the different objectives contributed to achieve the aim of the PhD study.

Overall, this research provided valuable knowledge on bioleaching approaches, mechanisms and influencing parameters for the extraction of metals from primary (ores) and secondary resources (metallurgical by-products, electronic wastes, fly ash) (**Chapter 2**). It also highlighted the future needs for industrial scale bioleaching operation as well as provided an optimisation framework from laboratory scale operation to pilot/industrial scale operation. Two metallurgical by-products, basic oxygen steelmaking dust (BOS-D) and goethite, were then investigated on batch scale for the leachability of various metals using bioleaching using *Acidithiobacillus ferrooxidans* (**Chapter 3**). Taguchi L9 (4^3) orthogonal array design was used to evaluate the effect of four operational parameters including pulp density, energy source concentration, inoculum concentration and pH on three levels. The order of importance of the parameters and optimum values were defined for individual metal extraction. Further it compared the effectiveness of three batch systems approaches: bioleaching using *A. ferrooxidans*, chemical leaching using choline chloride-ethylene glycol (ChCl-EG) and a combined approach (**Chapter 4**). Then, scaling up was evaluated through a semi-continuous bioleaching column system where leachate was recirculated at different regimes. Lastly, techno-economic assessment conducted to compare two potential industrial scale bioleaching plant including an aerated bioreactor and an aerated and stirred bioreactor for three different recovery scenarios (**Chapter 5**). It provided overall cost of the bioleaching

process, highlighted where the highest costs are, and then evaluated the plants economic feasibility under inflationist economy. A Summary of the key findings was given in the Figure 6-1. Overall, the findings can inform and guide future research on sustainable metal recovery from industrial metal-bearing by-products.

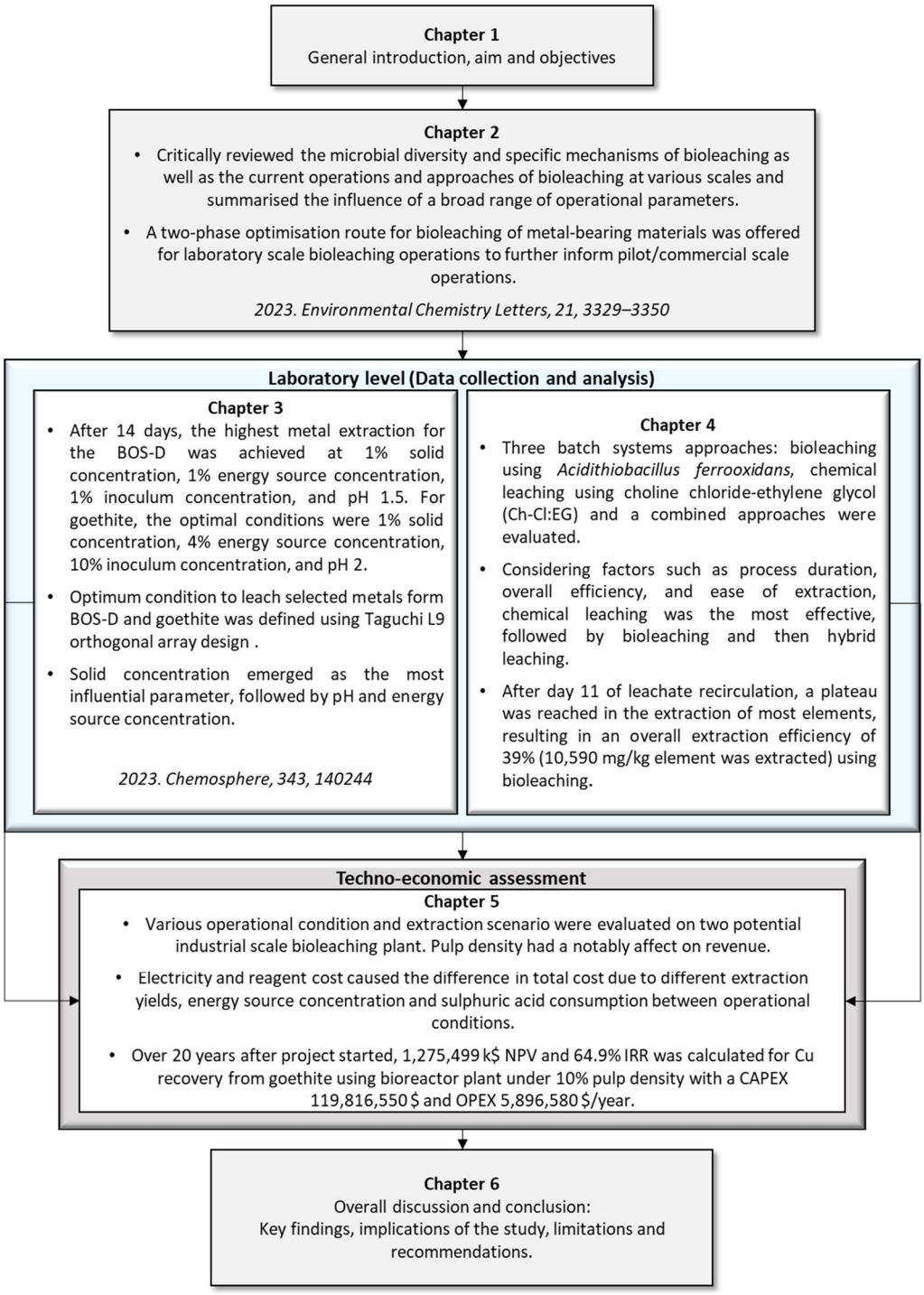

Figure 6-1. Summary of the key findings

6.2 Key findings

Chapter 2, Critical review, highlighted that bioleaching has emerged as a promising cost-effective and environmentally friendly alternative for recovering metals from metal-bearing materials e.g., low-grade ores, tailings. Acidophilic bacteria and fungi appeared as key microorganisms that play an important role in metal dissolution through specific mechanisms. Bioleaching of various metal-bearing materials on various scales e.g., shake flask, shake bioreactor, columns, heap, and stirred-tank reactor has been summarised. Although bioleaching has been successfully applied at a commercial scale for low-grade ores and tailings for decades, slow dissolution kinetics remain a challenge. Recent advances in gene libraries and PCR-based techniques can offer effective on-site monitoring and better understanding of microbe-mineral/waste interactions to enhance the dissolution kinetic. Conversely, bioleaching approaches are still being developed at laboratory scale for other secondary resources such as metallurgical slag and dust, fly ash and e-waste. Differences between secondary resources and metal sulphides such as oxidized and alkaline composition and toxic substances in the materials pose challenges. Other challenges associated with bioleaching of secondary resources are complex structure of materials, adequate solids concentrations and dissolution kinetics. The performance of bioleaching is influenced by pH, solid concentration, energy source, agitation rate, irrigation rate, aeration rate, and inoculum concentration. Simultaneous optimisation of these parameters rather than one-factor-at-a-time methodology reveals the most influencing parameters, and their optimum values, improves extraction yields and encourages the wider application of bioleaching (Naseri et al., 2023; Niu et al., 2016). Therefore, this PhD study proposes a two-step optimisation approach. The first step consists of employing a screening method, such as Plackett-Burman, allowing researchers to understand the most influencing parameters (Amiri et al., 2011; Zare Tavakoli et al., 2017). Then, in the second step, further optimisation can be performed using response surface methodologies such as central composite design, Box–Behnken, or Taguchi orthogonal array design. These methods aim to determine optimal values for the most influencing parameters (Amiri et al., 2011; Jalali et al., 2019; Mo et al., 2019; Nkulu et al., 2013). This

two-step optimisation process is applicable for all types of metal-bearing-materials from laboratory scale bioleaching operations to pilot/commercial scale operations.

In **Chapter 3**, feasibility of extracting metals from two industrial metal-bearing by-products, mainly BOS-D and goethite, were investigated using *A. ferrooxidans*. Several elements including Mn (1,457-5,870 mg/kg), Zn (18,300-61,615 mg/kg), Pb (1,738-24,800 mg/kg), Al (2,660-11,164 mg/kg), Li (3-4 mg/kg), Co (9-10 mg/kg), Y (1-2 mg/kg), and Ce (4-6 mg/kg) were chosen for investigation due to their prevalence as abundant metals and their critical status as REEs. In addition, he high concentrations of Fe in BOF (390,500 mg/kg) and goethite material (162,333 mg/kg) indicate the potential of these by-products as secondary iron resources. However, their high zinc content, >1%, restricts their reuse in steelmaking industry as zinc damages the normal operations of blast furnace (Xue et al., 2022). The XRD analysis results further revealed that magnetite(Fe_3O_4) is predominant in both materials, which is consistent with previous studies (Hoeber and Steinlechner, 2021). The pH values of BOS-D and goethite were 9.40 and 4.30, respectively. Therefore, to prevent jarosite formation and to make a favourable environment for acidophiles, pH needs to be regularly adjusted using 5 M H_2SO_4 between1.50-2.00 as this is the optimal pH range for *A. ferrooxidans*. Four variables at three different levels including solid concentration (1, 5 and 10% w/v), energy source concentration (1, 2 and 3% w/v for BOF optimisation; 2, 3 and 4% w/v for goethite optimisation), inoculum concentration (1, 5 and 10% v/v) and pH (1.5, 1.75, and 2) were evaluated using L9 Taguchi orthogonal array design. The range of parameters for optimisation was selected based on the literature review **(Chapter 2)** and Mo et al., (2019). For the bioleaching of BOS-D, an intermediate energy source concentration of 2.22% was chosen, in accordance with the findings of Chen et al. (2015) and Gomes et al. (2018). Additionally, for the bioleaching of goethite, an energy source concentration of 4.44%, was considered as supported by the research of Jensen and Webb (1995), Roy et al. (2021), and Zeng et al. (2013). Overall, nine different bioleaching condition was tested. After 14 days, the highest metal extraction from BOS-D (11.2 mg Zn /g, 3.2 mg Mn /g, 1.6 mg Al /g, 0.0013 mg Y

/g, and 0.0026 mg Ce /g) was achieved at 1% solid concentration, 1% energy source concentration, 1% inoculum concentration, and pH 1.5. In contrast for goethite, the optimal conditions were 1% solid concentration, 4% energy source concentration, 10% inoculum concentration, and pH 2 resulting in an extraction of 26.6 mg Zn /g, 2.1 mg/g Mn, 1.8 mg Al /g, 0.01 mg Co /g, 0.0022 mg Y /g. These findings are significant, as they demonstrate the potential to extract valuable metals from previously discarded industrial by-products. The extraction of such metals can have substantial economic and environmental implications, while simultaneously reducing waste in the metallurgical industry. Nevertheless, additional research is required to decrease the Zn content to below 1%. This step is essential to fully utilise goethite and BOS-D as secondary iron resources within the steel-making industry. Regarding the abiotic and acid controls, they were solely conducted under a single condition, aligning with one bioleaching condition set at under 1% PD and pH 1.75 as the primary focus of this study was centred around the optimisation of bioleaching processes. After a 14-day period, bioleaching outperformed acid leaching in extracting Co, Y, Ce from BOS-D and Mn, Zn, Li, Co from goethite. In addition, acid leaching required 4 to 10% more acid than bioleaching for BOS-D and goethite, respectively. This results in high operational cost in a potential industrial application. Moreover, during the experimental period, chemical leaching necessitated regular acid adjustments to maintain the desired pH level. In contrast, pH was self-regulated during bioleaching after a couple of days of leaching. Abiotic controls were performed to assess the effect of ferrous iron as a reducing agent. Results showed that the presence of ferrous iron has no additional effect indicating that bioleaching was the main process of Co, Y, Ce extraction from BOS-D and Mn, Zn, Li, Co extraction from goethite.

Chapter 4 investigated methods for enhancing and scaling up the extraction of metals from BOS-D. Initially, the effectiveness of three batch systems approaches, including bioleaching using *A. ferrooxidans*, chemical leaching using choline chloride-ethylene glycol (ChCl-EG) and a combined approach were compared. Bioleaching outperformed the control experiments achieving metal extraction recovery between 38% and 92% within 3 days, with a pulp density of

1% (w/v) demonstrating the key role of *A. ferrooxidans*. Conversely, ChCl-EG leaching, conducted with a pulp density of 12.5% (w/v), successfully dissolved over half amount of all metals (58 - 63%) within two hours. When examining the extraction of REEs at the ppm level, both bioleaching (98% Y, 64% Ce, 66% Nd) and hybrid leaching (89% Y, 82% Ce, 83% Nd) outperformed chemical leaching (63% Y, 59% Ce, 58% Nd). However, considering factors such as process duration, overall efficiency, and ease of extraction, chemical leaching was the most effective, followed by bioleaching and then hybrid leaching. Then, scaling up was evaluated through a semi-continuous bioleaching column system where leachate was recirculated at different regimes over 21 days. After day 11 of leachate recirculation, a plateau was reached in the extraction of most elements, resulting in an overall extraction efficiency of 39% (10,590 mg/kg extracted from an initial concentration of 26,978 mg/kg). This suggests that concluding the column bioleaching process at day 11 can save time, energy, and resources, yielding higher overall metal extraction than extending it to day 21. Interestingly, variations in recirculation rates did not influence the extraction efficiency. Overall, this chapter emphasizes the considerable potential of bioleaching for metal recovery, but it also highlights the need for further optimisation, particularly in parameters such as aeration rate, when transitioning to larger scale systems.

In **Chapter 5**, an evaluation was conducted to assess the economic viability of two potential large-scale bioleaching technologies, mainly an aerated bioreactor and an aerated and stirred bioreactor, aimed at metal recovery in industrial settings. To facilitate a comparative analysis of potential costs and profits for both plants, capacities of 4,944 m^3 for the aerated bioreactor and 5340 m^3 for the aerated and stirred bioreactor were selected to align as closely as possible. The total costs and potential revenue were calculated based on three scenarios: (i) extraction and recovery of all nine elements (Y, Ce, Nd, Li, Co, Cu, Zn, Mn and Al) in elemental form via electrowinning, (ii) exclusive recovery of Zn in elemental form via electrowinning, (iii) recovery only of the most abundant, excluding Zn, in elemental form via electrowinning. The focus on Zn stemmed from its concentration >1% limiting the reuse of these by-products as secondary iron resources. The most abundant element for BOS-D was Mn, while it was Cu for

goethite when disregarding Zn. The primary data for the techno-economic assessment (TEA) was sourced from bioleaching yields obtained from batch-scale (**Chapter 3**) and semi-continuous column-scale laboratory experiments **(Chapter 4**). Varied operational conditions tested in **Chapter 3** resulted in different bioleaching yields, impacting operational costs differently. Among expense items, such as total fixed capital investment (TFCI), water, maintenance, and personnel costs, the electricity and reagent costs made the most substantial difference due to varying extraction yields, energy source concentrations, and sulfuric acid consumption among operational conditions. For instance, higher energy source concentrations and lower pH levels led to increased reagent costs, while higher solid concentrations incurred higher grinding costs, impacting electricity expenses. Pulp density notably affected revenue, despite a lower extraction rate, as increased pulp density correlated with increased revenue, keeping plant capacity constant. Project cash flow, net present value (NPV), and internal rate of return (IRR) for Zn recovery from BOS-D and Cu recovery from goethite were projected over 20 years using GoldSim version 14.0. The results indicated that bioleaching of BOS-D was not profitable. However, copper recovery from goethite showed promising figures, with an NPV of 1,275,499 k$ and an IRR of 65% over 20 years, using the aerated and stirred bioreactor plant with a CAPEX of 119,816,550 $ and OPEX of 5,896,580 $/year. Profitability was expected after one year of operations, following a 2-year development period. Overall, the aerated and stirred bioreactor plant appeared as more reliable alternative compared to the aerated bioreactor plant, due to its configuration of 12 reactors, enabling better management and operations within a smaller volume featuring multiple reactors. Despite these limitations, this TEA emphasized the significance of selective metal recovery and plant design, shedding light on the main expenses associated with the process.

6.3 Research implications

As a part of the critical review (**Chapter 2**), mechanisms of bioleaching and overall influencing parameters together with typical values were provided. In addition, a detailed and generalised bioleaching optimisation flow chart for metal-

bearing-materials was produced as a part of the critical review. The use of this proposed flow chart for bioleaching optimisation in general metal-bearing-materials holds the potential to enhance bioleaching yields in future laboratory scale operations. By providing detailed information and clear decision-making pathways, it will facilitate wider implementation of pilot and large-scale bioleaching operations for various metal-bearing-materials. The flexibility of Taguchi orthogonal array design was assessed through the optimisation of four process parameters for two industrial by-products, namely BOS-D and goethite (**Chapter 3**). It proved to be highly effective in understanding the order of importance of the parameters and determining the optimal values for selected metals. Moreover, selection of bioleaching agent, Fe^{3+} produced by *A. ferrooxidans* using Fe^{2+}, exhibited greater effectiveness than sulphuric acid and Fe^{2+} for Co, Y, Ce from BOS-D and Mn, Zn, Li, Co from goethite. This insight could aid future studies in selecting efficient leaching agents for selectively extracting metals from these by-products. Further comparison among three batch systems, including bioleaching using *A. ferrooxidans*, chemical leaching using ChCl-EG and a combined approach for metal extraction from BOS-D offers clear guidance to decision making regarding method selection based on metal groups including REEs, critical materials and heavy metal and others (**Chapter 4**). The column bioleaching of BOS-D provides valuable insights into an aerated bioreactor system as scaling up alternatives. Both batch and column bioleaching results assisted in performing a TEA to compare two potential industrial scale bioleaching plants including an aerated bioreactor and an aerated and stirred bioreactor for different recovery scenarios (**Chapter 5**). The TEA provides a comprehensive overview of the bioleaching process costs, highlighting where the highest costs are, and allows for the evaluation of the plant's economic feasibility under inflationist economy. Overall, this PhD study will spark further research on bioleaching particularly targeting industrial metal-bearing by-products that are commonly regarded as waste materials.

6.4 Limitations of the research

Performing biometallurgical studies for different industrial metal-bearing by-products on a different scale was a great advantage; however, this also presented some problems. In Chapter 3, optimisation parameters including pulp density, energy source concentration, inoculum concentration and pH were selected based on literature as they appeared some of the important parameters. However, the effect of other influencing parameter for example particle size, nutrient concentration such as ammonium and magnesium, temperature and agitation speed have not been evaluated due to time constraint. For bioleaching study *Acidithiobacillus ferrooxidans* was selected as it is a well-studied microorganism. Although efforts were made by adapting the culture to metallurgical by-products to enhance the bioleaching yields, bioleaching potential of other species such as *Acidithiobacillus thiooxidans,* fungi of mixed culture have not been investigated due to time limitations. As this study did not prioritize the elemental recovery of metals from the lixiviant, this aspect remained unexplored. In terms of techno-economic assessment, calculations of capital and operational expenditures, personnel cost utilities cost were based on rough estimates, potentially carrying large error margins (30–40%) as reported by Dewulf (2022). Lastly, time delays and unforeseen events are inevitable in any research project, however, these challenges are magnified when dealing with systems requiring longer operation times (Freeman, 2017).

6.5 Further research recommendations

As it was described in Chapter 2, rather than using one-factor-at-a-time methodology, multiparameter optimisation is recommended to understand the interactions between parameters and to identify optimal conditions in the process. First, using a screening method such as Plackett-Burman on batch scale operation provides better understanding the effect of many parameters such as pH, solid concentration, energy source, agitation rate, irrigation rate, aeration rate, inoculum concentration, leaching time, temperature, nutrient medium component’s concentration, particle size at the same time. Then, further optimisation using Taguchi orthogonal array design or response surface

methodology on intermediate scale bioleaching which are stirred bioreactor (particle size < 1mm) and column rector (particle size > 1mm) is recommended. As optimisation parameters, top 1 or 2 most influential parameter from Placket-Burman results and intermate scale specific parameters such as irrigation rate for column and aeration rate for both column and stirred bioreactor can be selected.

For this study *A. ferrooxidans* which is an iron and sulphur oxidiser was used for its iron oxidation properties. So, $FeSO_4.7H_2O$ was used as a source of Fe^{2+} to investigate the potential of Fe^{3+} as it is known as a strong leaching agent. For future studies elemental sulphur can also be used to investigate its leachability potential. This may reduce the acid consumption and cost associated with this. In addition to using iron-oxidising bacteria, investigating the potential use of sulphur-oxidising microorganisms such as *A. thiooxidans*, and mixed culture is recommended. Using fungi for metallurgical by-products which has alkaline nature should also be investigated.

During acidophilic bioleaching, precipitation of Fe3+ could lead jarosite formation, this lowers metal extraction and causes co-precipitation of extracted metals (Srichandan et al., 2020). Using iron reducing microorganisms such as *Shewanella oneidensis* can liberate precipitated metals as well as bring ferrous iron into process that can be used by *A. ferrooxidans* again (Husain et al., 2022; Bingjie et al., 2014).

The potential of using spent medium step approach should also be investigated as it will prevent the possible contamination of material processed with microorganisms. Additionally, conducting life cycle assessments and life cycle sustainability assessments is essential to assess and mitigate the environmental impact and cost associated with the bioleaching process. These evaluations will also facilitate the enhancement of pilot studies and collaborative research involving chemists, biologists, and metallurgists. This collaborative effort is crucial to scale up bioleaching for industrial utilisation, specifically targeting metal-bearing materials like metallurgical sludge and dust, e-waste, spent catalysts, and fly ash.

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APPENDICES

Appendix A Ethical Approval Letter

7 January 2021

Dear Miss Tezyapar ,

Reference: CURES/12556/2021
Title: Linking Circular Economy to Recovery and Redevelopment of Brownfield: Potentials for added value materials and soil reuse

Thank you for your application to the Cranfield University Research Ethics System (CURES).

We are pleased to inform you your CURES application, reference CURES/12556/2021 **has been reviewed. You may now proceed with the research activities you have sought approval for.**

If you have any queries, please contact CURES Support.

We wish you every success with your project.

Regards,

CURES Team

Appendix B Bioleaching metal-bearing wastes and by-products for resource recovery: a review

Table B-1. Bioleaching of low-grade ores

Microbial group	Species	Material and elemental content	Operation conditions	Bioleaching yield (%)	Reference
Acidophiles	*Sulfobacillus thermosulfidooxidans strain-RDB* and *Thermoplasma acidophilum.*	low-grade mining ore % (w/w): 8.2% bornite, 0.3% Pentlandite, 0.8% chalcocite, 0.8% linnaeite, 0.3% wurtzite and 0.3% sphalerite. The 20% smithsonite, 3% talc, 25% sphaerocobaltite, 10% azurite	- pH: 2 - Temperature: 25 °C - Period: 112 days - Particle size: <0.1 mm - Inoculum rate: 20% - Irrigation rate: 45–48 L/m^{2}h - Aeration rate: 180–250 L/h - Solid amount: 80000 - Solid/liquid ratio: 4444:1 - Exogenous culture - Bioleaching approach: one-step - Column size: L 85 × ø15 cm - Inoculation place: Column	84% Cu, 76% Co, 72% Ni, 70% Zn, 63% Fe	Ilyas et al., (2012)
Fungi	*Aspergillus Niger*	low-grade pyrolusite ore % (w/w): 8.75 MnO, 40.20 SiO_2, 28.32 Fe_2O_3, 10.47 CaO, 6.04 Al_2O_3, 0.64 TiO_2, 1.09 MgO, 2.36 K_2O, 1.02 PbO+SrO+BaO+ZnO	- pH: 5.6 - Temperature: 25°C - Period: 30 - Particle size: <150 µm - Inoculum rate: 10^7 cells/ml - Agitation speed: 120 rpm - Solid amount: 0.1 g - Pulp density: 0.1 (w/v) - Exogenous culture - Bioleaching approach: two- step	80% Mn	Keshavarz et al., (2021)
Cyanogens	*Bacillus megaterium*	oxidized gold-bearing ore 6.2 mg/kg Au	- pH: 7-10 - Temperature: 35°C - Period: 108 hours - Particle size: < 80 µm - Inoculum rate: 10% (v/v) - Agitation speed: 150 rpm - Solid amount: 1 g - Pulp density: 1% (w/v) - Exogenous culture - Bioleaching approach: -	>80% Au	Gorji, et al., (2020)

Table B-2. Bioleaching of mineral concentration waste e.g., tailing

Microbial group	Species	Material and elemental content	Operation conditions	Bioleaching yield (%)	Reference
Acidophiles	*Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Leptospirillium ferroxidans, Acidiphilium species*	High-pyrrhotite tailings Elements (mg/kg): 58.8 Ni, 1.73 Co, 12 Cu, 0.148 Au, 0.332 Pt, 0.352 Pd, 0.070 Rh, 2.5 Ag	- pH: 2 - Temperature: >40 °C - Period: 10 days - Particle size: 147 µm - Inoculum rate: - - Agitation speed: - - Solid amount: 200 g - Pulp density: 10% (w/v) - Exogenous culture - Bioleaching approach: -	80-90% Ni, 70-80% Co	Cameron et al., (2021)
Fungi	*Aspergillus fumigatus*	Mine Tailings Elements (mg/kg): 2,030 As, 10,300 Fe, 672 Mn, 135 Zn, 90 Pb	- pH: 7.45 - Temperature: 30°C - Period: 15 days - Particle size: - - Inoculum rate: 10^7 spores/ml, 1% (v/v) - Agitation speed: 150 rpm - Solid amount: 1 g - Pulp density: 1% (w/v) - Exogenous culture - Bioleaching approach-	62%As, 58% Fe, 100% Mn, 54% Zn, 88% Pb	Seh-Bardan et al., (2012)
Cyanogens	-	-	-	-	-

Table B-3. Bioleaching of metallurgical (refining) waste e.g., slag, sludge, dust

Microbial group	Species	Material and elemental content	Operation conditions	Bioleaching yield (%)	Reference
Acidophiles	*Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidnas*	Steel slag Element (mg/kg): 290,000 Mg, 200,000 Ca, 11,000 Fe, 6,000 Si, 4,000 Al, 500 P, 10 As, 30 Ba, 1.6 Cd, 100 Cr, 20 Cu, 10 Ga, 4 Li, 200 K, 1,000 Mn, 2 Mo, 300 Na, 40 Ni, 500 Pb, 0.002 Ti, 40 V, 30 Sr, 30 Zn	- pH: 2-2.25 - Temperature: 28 °C - Period: 22 days - Particle size: 4-10 mm - Inoculum rate: 10% - Irrigation rate: 5 mL/min - Aeration rate: - - Solid amount: 100 g - Solid/liquid ratio: 100:1 - Exogenous culture - Bioleaching approach: two-step - Column size: L 35 × ø1.8 cm - Inoculation place: Incubator	100% Al, 64% Cr, 8% V	Gomes et al., (2018)
Fungi	*Aspergillus niger*	Linz-Donawits (LD) converter slag % (w/w): 1.97% V_2O_5	- pH: 6.5 - Temperature: 25°C - Period: 22 days - Particle size: 37 µm - Inoculum rate: 10% (v/v) - Agitation speed: 180 rpm - Solid amount: 1 g - Pulp density: 1% (w/v) - Exogenous culture - Bioleaching approach: two-step	92% V	Mirazimi et al., (2015)
Cyanogens	-	-	-		-

Table B-4. Bioleaching of waste from electric electronic equipment (WEEE)

Microbial group	Species	Material and elemental content	Operation conditions	Bioleaching yield (%)	Reference
Acidophiles	*Acidithiobacillus ferrooxidans*	waste printed circuit boards (WPCBs) Element (mg/kg): 245,000 Cu	- pH: 2.25 - Temperature: 30 °C - Period: 28 days - Particle size: 4-10 mm - Inoculum rate: 1% - Irrigation rate: 40 mL/min - Aeration rate: 20 L/min - Solid amount: 250 g - Solid/liquid ratio: 50:1 - Exogenous culture - Bioleaching approach: two-step - Column size: L 20 × ø 6 cm - Inoculation place: Incubator	95% Cu	Chen et al., (2015)
Fungi	*Penicillium simplicissimum*	mobile phone printed circuit boards (MPPCBs) Element (mg/kg): 210,000 Cu, 2,800 Ni, 600 Au, 1,500, Ag, 47,000 Fe	- pH: 6 - Temperature: 30 °C - Period: 7 - Particle size: 75–149 µm - Inoculum rate: 10^6 spores - Irrigation rate: - Aeration rate: 0.29 L/min - Solid amount: 10 g - Solid/liquid ratio: 10 :1 - Exogenous culture - Bioleaching approach: two-step - Column size: L 55 × ø 7.5 cm - Inoculation place: water bath	97% Cu	Nili et al., (2022)
Cyanogens	*Chromobacterium violaceum*	Electronic waste: Element (mg/kg): 924,000 Cu, 62,600 Au, 5,400 Fe, 3,100 Ag, 2,800 Sn, 2,000 Pb	- pH: 10 - Temperature: 30°C - Period: - - Particle size: 100 µm - Inoculum rate: - Agitation speed: 170 - Solid amount: 0.5 g - Pulp density: 0.5 (w/v) - Exogenous culture - Bioleaching approach: spent medium	30% Au, 96% Cu	Natarajan and Ting, (2015)

Table B-5. Bioleaching of fly ash

Microbial group	Species	Material and elemental content	Operation conditions	Bioleaching yield (%)	Reference
Acidophiles	Mixed acidophilic culture e.g., *Acidithiobacillus ferrooxidans, At. thiooxidans, At. caldus, Leptospirillum ferrooxidans, Sulfobacillus thermo-sulfidooxidans, Sb. thermotolerans* and some members of *Alicyclobacillus genus*	Fly ash (municipal solid waste incineration) Elements (mg/kg): 19,300 Al, 10,300 Mg, 370 Mn, 10,200 Zn, 600 Cu	- pH: 2 - Temperature: 30°C - Period: 21 days - Particle size: - - Inoculum rate: 10% (v/v) - Agitation speed: 320 rpm - Aeration rate: 1 l/min - Solid amount: 5 g - Pulp density: 5% (w/v) - Exogenous culture - Bioleaching approach: two-step	>85% for Al, Mg, Mn, Zn, Cu	Funari et al., (2017)
Fungi	*Aspergillus niger*	Fly ash (municipal solid waste incineration residues) Elements (mg/kg): 19,210 Al, 309 Mn, 6288 Zn, 570 Cu, 2000 Pb, 5240 Fe	- pH: from 11 to 4 - Temperature: 30 °C - Period: - - Particle size: - - Inoculum rate: 10^7 spores - Agitation speed: 120 rpm - Solid amount: 1 g - Pulp density: 1% (w/v) - Exogenous culture - Bioleaching approach: two-step	80–100% for Al, Mn, Zn; 60–70% for Cu, Pb 30% Fe	Wu and Ting, (2006)
Cyanogens	-	-	-	-	-

Table B-6. Bioleaching of spent catalyst

Microbial group	Species	Material and elemental content	Operation conditions	Bioleaching yield (%)	Reference
Acidophiles	*Acidothiobacillus ferrooxidans*	Spent fluid catalytic cracking catalyst Element (mg/kg): 14,000 La, 1,000 Ce, 24,4000 Al, 217,000 Si, 630 Ni, 460 V, 0270 Ti	- pH: 1.5-2.5 - Temperature: 30 °C - Period: 10 days - Particle size: - - Inoculum rate: 10% - Agitation speed: 130 rpm - Solid amount: 1 and 7 g - Pulp density: 1% (w/v) for La and 7% (w/v) for Ce - Exogenous culture - Bioleaching approach: two-step	83% La, 23% Ce	Muddanna and Baral, (2021)
Fungi	*Penicillium simplicissimum*	Spent hydrocracking catalyst Elements (mg/kg): 40 Ag, nt Si, 210,000 Al, 6000 Fe, <100 Ca, 950 Na, 70 Mg, 130 K, nt Ti, 30 Mn, <20 Co, 100 Cr, 45 Cu, 8000 Ni, 90 Pb, 25 V, 77,000 W, 130 Zn, 6800 Mo, <50 Hg, <20 Ga	- pH: 4.5 - Temperature: 30 °C - Period: 30 days - Particle size: <150 µm - Inoculum rate: 10% - Agitation speed: 120 rpm - Solid amount: 3 g - Pulp density: 3% (w/v) - Exogenous culture - Bioleaching approach: two-step	100% W, 100% Fe, 93% Mo, 66% Ni, 25% Al	Amiri, et al., (2011b)
Cyanogens	*Pseudomonas fluorescens*	Spent automotive catalyst Elements (mg/kg): 1,909 Pt, 1,451 Pd, 535 Rh, 34,689 Mg, 4,300 Fe, 921 Ni, 48 Cu, Zn, 233,425 Al, 6,566 Ba, 351 Ca	- pH: 10 - Temperature: 30 °C - Period: 1 day - Particle size: <45 µm - Inoculum rate: - - Agitation speed: 150 rpm - Solid amount: 0.5 g - Pulp density: 0.5(w/v) - Exogenous culture - Bioleaching approach: two-step	44% Pt, 54% Pd, 96% Rh	Karim and Ting, (2022)

Table B-7. Bioleaching of contaminated soil

Microbial group	Species	Material and elemental content	Operation conditions	Bioleaching yield (%)	Reference
Acidophiles	sulphur-oxidizing bacteria	heavy metal-contaminated soil (industrial): Elements (mg/kg): 210 Cu, 625 Zn, 232 Ni, 454 Pb	- pH: 1-2 - Temperature: 30 °C - Period: 3 days - Particle size: 0.84mm - Inoculum rate: 3% - Agitation speed: 200 rpm - Solid amount: 1-7 g - Pulp density: 1-7% (w/v) - Indigenous culture - Bioleaching approach: one-step	83.8% Cu, 82.8% Zn, 77.6% Pb, 73.7% Ni	Chen and Lin, (2010)
Fungi	*Penicillium chrysogenum*	contaminated soil: Elements (mg/kg): 48.4 Cd, 1,848 Cu, 1,889.6 Pb, 5,682 Zn, 3,045 Mn, 103.99 Cr	- pH: 2.5-3 - Temperature: 30 °C - Period: 15 days - Particle size: 2 mm - Inoculum rate: 2% - Agitation speed: 120 rpm - Solid amount: 0.5 g - Pulp density: 5% (w/v) - Indigenous culture - Bioleaching approach:	74% Cd, 59% Cu, 24% Pb, 55% Zn, 57% Mn, 25% Cr	Deng et al., (2013)
Cyanogens	-	-	-	-	-

Table B-8. Compilation of shake flask bioleaching conditions with various microorganisms, applications to various ores/solid wastes, solid amount, bioleaching yield, pH, temperature, period, particle size, inoculum rate, indigenous/exogenous

Shake flask									
Microorganism	**Material**	**Initial metal composition**	**Solid amount (g)**	**Medium composition**	**Operation conditions**	**Bioleaching yield (%)**	**Optimisation of parameters**	**Importance order of the optimisation parameters**	**Reference**
Acidithiobacillus ferrooxidans	EAF steel-making dust	Elements (mg/kg): 12,300 Zn, 547,300 Fe	0.5	9K medium consists of 33.4 g/L $FeSO_4 \cdot 7H_2O$, 0.4 g/L $(NH_4)_2SO_4$, 0.4 g/L $MgSO_4 \cdot 7H_2O$ and 0.4 g/L K_2HPO_4	- pH: 1.3 - Temperature: 30 ± 2 °C - Period: 21 days - Particle size: 0.038 mm - Inoculum rate: 10% - Agitation speed: 150 rpm - Pulp density: 1% (w/v) - Exogenous culture - Bioleaching approach: one-step	37% Fe, 35% Zn	Influence of particle size, solids concentration, pH	1. Particle size (0.038 mm) 2. solids concentration (1% w/v) 4. pH (1.3)	Bayat et al. (2009)
Acidothiobacillus ferrooxidans	spent fluid catalytic cracking catalyst	Elements (mg/kg): 14,000 La, 1,000 Ce, 244,000 Al, 217,000% Si, 630 Ni, 460 V, 270 Ti	1-7	9K medium 0.5 g/L K_2HPO_4, 3 g/L $(NH_4)_2SO_4$, 0.1 g/L KCl and 0.5 g/L $MgSO_4.7\ H_2O$ and additional 10-44 g/L $FeSO_4.7H_2O$	- pH: 1.5-2.5 - Temperature: 30 °C - Period: 10 days - Particle size: - - Inoculum rate: 10% - Agitation speed: 130 rpm - Pulp density: 1% (w/v) for La and 7% (w/v) for Ce - Exogenous culture - Bioleaching approach: two-step	83% La, 23% Ce			Muddana et al., (2021)

Acidithiobacillus ferrooxidans	spent lithium-ion batteries (LIBs)	Elements (mg/kg): 220,500 Co, 30,300 Li, 25,500 Cu, 4400 Mn, 500 Ni, 3,800 Fe, 42,700 Al	5	Modified 9K media consists of 0.50 g/L $(NH_4)_2SO_4$, 0.05 g/L KCl, 0.20 g/L $K_2HPO_4.3H_2O$, 0.50 g/L $MgSO_4.7H_2O$, 0.01 g/L $Ca(NO_3)_2$, and 45.0 g/L $FeSO_4.7H_2O$	- pH: 2 - Temperature: 30 °C - Period: 3 days - Particle size: 100 µm - Inoculum rate: 10% - Agitation speed: 160 rpm - Pulp density: 10% (w/v) - Exogenous culture - Bioleaching approach: -	94% Co, 60% Li			Roy et al., (2021)
Acidithiobacillus ferrooxidans	spent lithium batteries	Elements (mg/kg): 33,700 Li, 485,000 Co, 239,000 Mn, 241,000 Ni, 1,400 Fe	2	9 K medium consists of 3.0 g/L $(NH_4)_2SO_4$, 0.1 g/L KCl, 0.5 g/L K_2HPO_4, 0.5 g/L $MgSO_4.7H_2O$, 0.01 g/L $Ca(NO_3)_2$, 44.8 g/L $FeSO_4.7H_2O$	- pH: 2 - Temperature: 35 °C - Period: 7 days - Particle size: 0.075 mm - Inoculum rate: 5% - Agitation speed: 160rpm - Pulp density: 1% (w/v) - Exogenous culture - Bioleaching approach: -	98.4% Co			Zeng et al., (2013)
Acidithiobacillus sp., Ferrimicrobium sp., Leptospirillum sp., and Acidiphilium sp.	uranium ore	Elements (mg/kg): 307,000 Si, 93,800 Al, 34340 Fe, 21,000 Ca, 3,750 Ti, 33,400 K, 230 Zr, 7,720 Mg, 3,820 S, 3,610 Na, 550 Mn, 2,930 P, 1,140 Mo, 790 Th, 220 Y, 5160 F, 2340 U(Total), 600 U(VI)	0.11	0K medium with Fe^{2+} concentration of 4.60 g/L	- pH: 1.8 - Temperature: 33 °C - Period: 14 days - Particle size: 74 µm - Inoculum rate: 20% - Agitation speed: 160 rpm - Pulp density: 0.11% (w/v) - Indigenous culture - Bioleaching approach: -	90.20% ± 0.70% U	Optimisation parameters response surface methodology (RSM) based Box-Behnken design: pH, pulp density, initial Fe^{2+}, and inoculation percent	1. pH (1.8) 2. pulp density (0.11% w/v) 3. inoculation percent (19.20% v/v) 4. initial Fe^{2+} (4.60 g/L)	Mo et al., (2019)
Acidithiobacillus ferrooxidans (At. ferrooxidans), Acidithiobacillus thiooxidans (At. thiooxidans)	raw petroleum refinery spent catalyst	Elements (mg/kg): 27,000 Ni, 153,100 Al, 2,340 Mo, 87,600 V	10	IEM nutrient medium consists of 20 g/L $FeSO_4{\cdot}7H_2O$	- pH: 1.1 - Temperature: 35 °C - Period: 7 days - Particle size: 2000-5000 µm - Inoculum rate: - - Agitation speed: 160 rpm - Pulp density: 1% (w/v) - Exogenous culture	91% V, 83% Mo, 65% Ni, 0.98% Al			Pathak et al., (2015)

					- Bioleaching approach: two-step				
Sulfobacilllus thermosulfidooxidans	low grade sulphide ore	Elements (mg/kg): 78,000 Fe, 80,000 Cu, 12,000 Ni, 3,000 Co, 8,000 Zn	10	The FeTSB medium composed of (g/L): $MgSO_4$. $7H_2O$, 0.50; $(NH_4)_2SO_4$, 0.15; KCl, 0.05; KH_2PO_4, 0.05; $Ca(NO_3)_2$, 0.01 and TSB, 0.25.	- pH: 1.8 - Temperature: 47 °C - Period: 30 days - Particle size: 120 µm - Inoculum rate: 1% - Agitation speed: 180 rpm - Pulp density: 10% (w/v) - Exogenous culture - Bioleaching approach: -	72% Zn, 68% Co, 78% Cu, 81% Ni, 70% Fe	Optimisation parameters via Taguchi orthogonal experimental array design: pulp density, initial pH, particle size, temperature and agitation	1. pH (1.8) 2. temperature (47°C) 3 agitation (180 rpm) 4. particle size (120 um) 5. pulp density (10 % w/v)	Ilyas et al., (2010a)
Acidithiobacillus ferrivorans and Acidithiosbacillus thiooxidans	various discarded printed circuit boards (PCB) (1st step/2step bioleaching)	Elements (mg/kg): 180,000-230,000 Cu, 2-320 Au, 2,000-50,000 Fe, 10,000-36,000 Al, 930-11,500 Ni, 320-5,000 Zn, 410-9,200 Pb, 140-2,000 Cr	1	Mineral medium containing (g/L: $(NH_4)_2SO_4$ (2.0), $MgSO_4.7H_2O$ (0.25), KH_2PO4 (0.1), KCl (0.1), $FeSO_4.7H_2O$ (8.0), and S0 (10.0)	- pH: 4.5 - Temperature: 23 ± 2 °C - Period: 7-20 days - Particle size: <500 µm - Inoculum rate: 1% - Agitation speed: 150 rpm - Pulp density: 1% (w/v) - Exogenous culture - Bioleaching approach: two-step	>98.4% Cu	-	-	Işıldar et al., (2016)
Pseudomonas fluorescens and Pseudomonas putida	Cu-leached (by A. ferrivorans and A. thiooxidans) PCB material from1st step (2nd step/2step bioleaching)		0.5	nutrient broth medium containing (g/L): meat extract (1.0), yeast extract (2.0), peptone (2.0), and NaCl (5.0) for subculturing and additional various concentrations of glycine (5, 7.5, 10 g/L).	- pH: 7.3-8.6 - Temperature: 30 °C - Period: 2 days - Particle size: <500 µm - Inoculum rate: 1% - Agitation speed: 150 rpm - Pulp density: 0.5% (w/v) - Exogenous culture - Bioleaching approach: two-step	44% Au	-	-	
Acidithiosbacillus thiooxidans	Steel slag	Elements (mg/kg): 8.80 Cr, 8.18 Mo, 3.03 Ba, 2.46 V, 1.08 Pb, 0.741 Zn, 0.719 Cu, 0.14 Ni	-	The basal medium consists of 0.3 g $(NH_4)_2SO_4$, 3.5 g K_2HPO_4, 0.5 g MgSO4.7H2O, and 0.25 g $CaCl_2$, 1.0% (w/v) elemental sulphur	- pH: 1.1 - Temperature: 30 °C - Period: 6 days - Particle size: 324 µm - Inoculum rate: - - Agitation speed: 150 rpm - Pulp density: 0.1% (w/v) - Bioleaching approach: spent medium (according to Hocheng et al., (2014a) it was two-step)	11.55% Mg, 8.58% Zn, 11% Al, 6.30% Cu, 7.22% Mn, 6.01% Fe, 6% Cr	-	-	Hocheng et al., (2014a)

Acidithiobacillus thiooxidans			-	The basal medium consists of 0.3 g $(NH_4)_2SO_4$, 3.5 g K_2HPO_4, 0.5 g MgSO4.7H2O, and 0.25 g $CaCl_2$, 1.0% (w/v) elemental sulphur	- pH: 1.1 - Temperature: 30 °C - Period: 6 days - Particle size: 324 µm - Inoculum rate: - - Agitation speed: 150 rpm - Pulp density: 0.1% (w/v) - Bioleaching approach: spent medium (according to Hocheng et al., (2014a) it was two-step)	11.55% Mg, 8.58% Zn, 11% Al, 6.30% Cu, 7.22% Mn, 6.01% Fe, 6% Cr	-	-	
Aspergillus niger			-	Sucrose medium	- pH: 1.8 - Temperature: 30 °C - Period: 6 days - Particle size: 324 µm - Inoculum rate: - - Agitation speed: 150rpm - Pulp density: 0.1% (w/v) - Bioleaching approach: spent medium (according to Hocheng et al., 2014a it was two-step)	26% Mg, 12.45% Zn, 10% Al, 8.57% Cu, 8.26% Mn, 8.12% Fe, 6.67% Cr	-	-	
Penicillium simplicissimum	spent hydrocracking catalyst	Elements (mg/kg): 64,000 Mo, 24,000 Ni, 240,000 Al %(w/w): 0.49 Na_2O, 50.47Al_2O_3, 33.44 SiO_2, 0.19 CaO, 1.13 Fe_2O_3, 4.05 NiO, 10.24 MoO_3	4	media that consisted of 90 g/L sucrose, 2 g/L $NaNO_3$, 0.36 g/L yeast extract 0.025 g/L $MgSO_4.7H_2O$, 0.025 g/L KCl	- pH: 5-9 - Temperature: 22-35 °C - Period: 30 days - Particle size: <120 µm - Inoculum rate: 2-4% - Agitation speed: 110-160 rpm - Pulp density: 4% (w/v) - Exogenous culture - Bioleaching approach: two-step	98.8% Mo, 46.5% Ni, 13.7% Al	Optimisation parameters via Plackett-Burman and response surface methodology (RSM) based central composite design (CCD): the concentration of carbon source (sucrose), inorganic salts ($NaNO_3$ and KH_2PO_4), growth factors source (yeast extract), cultivation parameters (preculture time, inoculum size, temperature, pH, and shaking rate) and catalyst parameters (particle size and pulp density)	1. yeast extract concentration (0.36 g/L) 2. $NaNO_3$ concentration (2 g/L) 3. pulp density (4.0%, w/v) 4. sucrose concentration (90 g/L)	Amiri et al., (2011a)

Penicillium simplicissimum	spent hydrocracking catalyst	Elements (mg/kg): 40 Ag, nt Si, 210,000 Al, 6,000 Fe, <100 Ca, 950 Na, 70 Mg, 130 K, nt Ti, 30 Mn, <20 Co, 100 Cr, 45 Cu, 8,000 Ni, 90 Pb, 25 V, 77,000 W, 130 Zn, 6,800 Mo, <50 Hg, <20 Ga	1	Czapek yeast extract agar media that consisted of 1.0 g/L K_2HPO_4, 3.0 g/L $NaNO_3$, 0.5 g/L $MgSO_4.7H_2O$, 0.5 g/L KCl, 0.01 g/L $FeSO_4.7H_2O$, 30.0 g/L sucrose, 5.0 g/L yeast extract, 15.0 g/L agar, and trace elements for acclimatisation.	- pH: 4.5 - Temperature: 30 °C - Period: 30 days - Particle size: <150 µm - Inoculum rate: 10% - Agitation speed: 120 rpm - Pulp density: 1% (w/v) - Exogenous culture - Bioleaching approach: one-step	68 W, 39% Fe, 37% Mo, 57% Ni, 17% Al			Amiri et al., (2011b)
			3	Czapek yeast extract agar media that consisted of 1.0 g/L K_2HPO_4, 3.0 g/L $NaNO_3$, 0.5 g/L $MgSO_4.7H_2O$, 0.5 g/L KCl, 0.01 g/L $FeSO_4.7H_2O$, 30.0 g/L sucrose, 5.0 g/L yeast extract, 15.0 g/L agar, and trace elements for acclimatization.	- pH: 4.5 - Temperature: 30 °C - Period: 30 days - Particle size: <150 µm - Inoculum rate: 10% - Agitation speed: 120 rpm - Pulp density: 3% (w/v) - Exogenous culture - Bioleaching approach: two-step	100% W, 100% Fe, 92.7% Mo, 66.43% Ni, 25% Al			
			1	Czapek yeast extract agar media that consisted of 1.0 g/L K_2HPO_4, 3.0 g/L $NaNO_3$, 0.5 g/L $MgSO_4.7H_2O$, 0.5 g/L KCl, 0.01 g/L $FeSO_4.7H_2O$, 30.0 g/L sucrose, 5.0 g/L yeast extract, 15.0 g/L agar, and trace elements for acclimatization.	- pH: 4.5 - Temperature: 30 °C - Period: 30 days - Particle size: <150 µm - Inoculum rate: 10% - Agitation speed: 120 rpm - Pulp density: 1% (w/v) - Exogenous culture - Bioleaching approach: spent-medium step	93% W, 95% Fe, 82% Mo, 64% Ni, 13% Al			

Penicillium chrysogenum	contaminated soil	Elements (mg/kg): 48.4 Cd, 1,848 Cu, 1,889.6 Pb, 5,682 Zn, 3,045 Mn, 103.99 Cr	2.5	Chashi agar medium consists of 30 g/L glucose, 1 g/L, K_2HPO_4, 3 g/L $NaNO_3$, 0.5 g/L $MgSO_4$, 0.5 g/L KCl, 0.01 g/L $FeSO_4$ and 20 g/L agar at pH 7.0	- pH: 7-7.6 - Temperature: 25 °C - Period: 15 days - Particle size: 2 mm - Inoculum rate: 2% - Agitation speed: 120 rpm - Pulp density: 5% (w/v) - Indigenous culture - Bioleaching approach: one-step	49.45% Cd, 35% Cu, 9.36% Pb, 40% Zn	-	-	Deng et al., (2012)
			2.5	Chashi agar medium consists of 30 g/L glucose, 1 g/L, K_2HPO_4, 3 g/L $NaNO_3$, 0.5 g/L $MgSO_4$, 0.5 g/L KCl, 0.01 g/L $FeSO_4$ and 20 g/L agar at pH 7.0	- pH: 3.75-7 - Temperature: 25 °C - Period: 15 days - Particle size: 2 mm - Inoculum rate: 2% - Agitation speed: 120rpm - Pulp density: 5% (w/v) - Indigenous culture - Bioleaching approach: two-step	63% Cd, 56% Cu, 14% Pb and 54% Zn	-		
Penicillium chrysogenum	contaminated soil	Elements (mg/kg): 48.4 Cd, 1,848 Cu, 1,889.6 Pb, 5,682 Zn, 3,045 Mn, 103.99 Cr	2.5	Czapek agar media consists of 1.0 g/L K_2HPO_4, 3.0 g/L $NaNO_3$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L KCl, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 30.0 g/L sucrose and 15.0 g/L agar.	- pH: 2.5-3 - Temperature: 30 °C - Period: 15 days - Particle size: 2 mm - Inoculum rate: 2% - Agitation speed: 120 rpm - Pulp density: 5% (w/v) - Indigenous culture - Bioleaching approach: -	74% Cd, 59% Cu, 24% Pb, 55% Zn, 57% Mn, 25% Cr	-	-	Deng et al., (2013)

Aspergillus niger	spent hydrocracking catalyst	Elements (mg/kg): 64,000 Mo, 24,000 Ni, 240,000 Al %(w/w): 0.49% Na2O, 50.47%Al2O3, 33.44% SiO2, 0.19% CaO, 1.13% Fe2O3, 4.05% NiO, 10.24% MoO4	3	media that consisted of 90.6 g/L sucrose, 1-2 g/L $NaNO_3$, 0.25-075 g/L KH_2PO_4, 0.5-3 g/L yeast extract, 0.025 g/L $MgSO_4.7H_2O$, 0.025 g/L KCl	- pH: 7.22 - Temperature: 22-36 °C - Period: 31 days - Particle size: 150-212 µm - Inoculum rate: 2-10% - Agitation speed: 110-160 rpm - Pulp density: 3% (w/v) - Exogenous culture - Bioleaching approach: two-step	99.5% Mo, 45.81% Ni, 13.9% Al	Optimisation parameters via Plackett-Burman and response surface methodology (RSM) based central composite design (CCD): carbon source (sucrose), inorganic salts ($NaNO_3$, KH_2PO_4), nitrogen source (yeast extract), cultivation parameters (preculture time, inoculum size, temperature, pH, shaking rate) and catalyst parameters (particle size, pulp density)	1. Particle size (150–212 um) 2. sucrose (93.8 g/L) 3. pulp density (3% w/v) 4. pH (7)	Amiri et al., (2012)
Aspergillus Niger	Sn-Pb (63 %-37 %) solder	Elements (mg/kg): 630,000 Sn, 370,000 Pb	0.5	The basal medium consists of 100 g/L sucrose, 1.5 g/L $NaNO_3$, 0.5 g/L KH_2PO_4, 0.025 g/L $MgSO_4.7H_2O$, 0.025 g/L KCl, and 1.6 g/L yeast extract	- pH: - - Temperature: 30 °C - Period: 144 h - Particle size: - - Inoculum rate: - - Agitation speed: 200rpm - Pulp density: 0.5% (w/v) - Exogenous culture - Bioleaching approach: spent medium (according to Hocheng et al., (2014a) it was two-step)	Sn-Pb (99.4 %)	-	-	Hocheng et al., (2014b)
Acidithiobacillus ferrooxidan		Elements (mg/kg): 630,000 Sn, 370,000 Pb	0.5	9K basal medium with 40 g/L $FeSO_4$	- pH: - - Temperature: 30 °C - Period: 144 h - Agitation speed:200 rpm - Pulp density: 0.5% (w/v) - Exogenous culture - Bioleaching approach: spent medium (according	Sn-Pb (4.20 %)	-	-	

					to Hocheng et al., (2014a) it was two-step)				
Aspergillus Niger	Sn-Cu (63 %-37 %) solder	Elements (mg/kg): 630,000 Sn, 370,000 Cu	0.5	The basal medium consists of 100 g/L sucrose, 1.5 g/L $NaNO_3$, 0.5 g/L KH_2PO_4, 0.025 g/L $MgSO_4.7H_2O$, 0.025 g/L KCl, and 1.6 g/L yeast extract	- pH: - - Temperature: 30 °C - Period: 96 h - Agitation speed: 200rpm - Pulp density: 0.5% (w/v) - Exogenous culture - Bioleaching approach: spent medium (according to Hocheng et al., (2014a) it was two-step)	Sn-Cu (99.8 %)	-	-	
Acidithiobacillus ferrooxidans		Elements (mg/kg): 630,000 Sn, 370,000 Cu	0.5	9K basal medium with 40 g/L $FeSO_4$	- pH: - - Temperature: 30 °C - Period: 96 h - Agitation speed: 200 rpm - Pulp density: 0.5% (w/v) - Exogenous culture - Bioleaching approach: spent medium (according to Hocheng et al., 2014a it was two-step)	Sn-Cu (97.9 %)	-	-	
Aspergillus Niger	Sn-Cu-Ag (60 %-37 %-3 %) solder	Elements (mg/kg): 600,000 Sn, 370,000 Cu, 30,000 Ag	0.5	The basal medium consists of 100 g/L sucrose, 1.5 g/L $NaNO_3$, 0.5 g/L KH_2PO_4, 0.025 g/L $MgSO_4.7H_2O$, 0.025 g/L KCl, and 1.6 g/L yeast extract	- pH: - - Temperature: 30 °C - Period: 48 h - Agitation speed: 200 rpm - Pulp density: 0.5% (w/v) - Exogenous culture - Bioleaching approach: spent medium (according to Hocheng et al., (2014a) it was two-step)	Sn-Cu-Ag (97.7 %)	-	-	
Acidithiobacillus ferrooxidans		Elements (mg/kg): 600,000 Sn, 370,000 Cu, 30,000 Ag	0.5	9K basal medium with 40 g/l FeSO4	- pH: - - Temperature: 30 °C - Period: 96 h - Agitation speed: 200 rpm - Pulp density: 0.5% (w/v) - Exogenous culture - Bioleaching approach: spent medium (according to Hocheng et al., 2014a it was two-step)	Sn-Cu-Ag (97.4 %)	-	-	

Indigenous iron-oxidizing microorganisms	sewage sludge	Elements (mg/kg): Dry sludge, 472 Cu, 294 Ni, 1310 Zn, 332 Cr	7.5	9K medium: 33.4 g/L $FeSO_4 \cdot 7H_2O$, 0.4 g/L $(NH_4)_2SO_4$, 0.4 g/L $MgSO_4 \cdot 7H_2O$ and 0.4 g/L K_2HPO_4	- pH: 2.4 - Temperature: 28 °C - Period: 16 days - Particle size: - - Inoculum rate: 10% - Agitation speed: 180 rpm - Pulp density: 3% (w/v) - Exogenous culture - Bioleaching approach: -	64% Cu, 58% Ni, 76% Zn and 52% Cr			Pathak et al., (2009)
				251 ml of sludge (30.2 g/L of the solid), 2% (w/v) of ammonium ferrous sulfate	- pH: 2.7 - Temperature: 28 °C - Period: 16 days - Particle size: - - Inoculum rate: 10% - Agitation speed: 180 rpm - Pulp density: 3% (w/v) - Indigenous culture - Bioleaching approach: -	56% Cu, 48% Ni, 68% Zn and 42% C			
Indigenous iron-oxidizing microorganisms	Pig manure	Elements (mg/kg): Dry sludge, 327 Cu, 1,728.3 Zn, 545 Mn, 3.3 Cr	6	pig manure and additional 4 g/L of Ferrous iron	- pH: 2.4 - Temperature: 28 °C - Period: 12 days - Particle size: - - Inoculum rate: 10% - Agitation speed: 180 rpm - Pulp density: 2% (w/v) - Indigenous culture - Bioleaching approach: -	92.9% Cu, 94.1% Zn, 91.9% Mn, 90.5% Cd			Wei et al., (2020)
				pig manure and additional 10 g/L of elemental sulphur		95% Cu, 96.5% Zn, 93.6% Mn, 92.7% Cd			

Table B-9. Compilation of stirred bioreactor bioleaching conditions with various microorganisms, applications to various ores/solid wastes, solid amount, bioleaching yield, pH, temperature, period, particle size, inoculum rate, indigenous/exogenous

Stirred bioreactor									
Microorganism	**Material**	**Initial metal composition**	**Solid amount (g)**	**Medium composition**	**Operation conditions**	**Bioleaching yield (%)**	**Optimised parameters**	**Importance order of the optimisation parameters**	**Reference**
Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans	raw petroleum refinery spent catalyst	Elements (mg/kg): 27,000 Ni, 153,100 Al, 23,400 Mo 87,600 V	10	IEM nutrient medium consists of 20 g/L $FeSO_4 \cdot 7H_2O$	- pH: 1.1 - Temperature: 35 °C - Period: 7 days - Particle size: 2000-5000 µm - Inoculum rate: - - Agitation speed: 160 rpm - Pulp density: 1% (w/v) - Exogenous culture - Bioleaching approach: two-step	91% V, 83% Mo, 65% Ni, 0.98% Al			Pathak et al., (2015)
Sulphur-oxidizing bacteria	heavy metal-contaminated soil (industrial)	Elements (mg/kg): 210 Cu, 625 Zn, 232 Ni, 454 Pb	50	Water and additional elemental sulphur range of 0.1–0.3% (w/v)	- pH: 1-2 - Temperature: 30 °C - Period: 3 days - Particle size: 0.84mm - Inoculum rate: 3% - Agitation speed: 200 rpm - Pulp density: 1-7% (w/v) - Indigenous culture - Bioleaching approach: one-step	83.8% Cu, 82.8% Zn, 77.6% Pb, 73.7% Ni	Optimisation parameters via response surface methodology (RSM) based central composite design (CCD): solid content and sulphur (substrate) dosage	1. solid content (1% w/v), 2. sulphur (substrate) dosage (0.1% w/v)	Chen and Lin, (2010)
A. ferrooxidans	end-of life vehicles (ELV) Shredder-light-fraction (SLF)	Elements (mg/kg): Combination of two fractions; Shredder-fraction "alt": 29,300Al, 22 As, 4,900 Ba, 39 Cd, 67 Co, 500 Cr, 9,900 Cu,	5	Mineral medium consists of 33.3 g/L $FeSO_4.7H_2O$, 0.4 g/L $(NH_4)_2SO_4$, 0.4 g/L $MgSO_4.7H_2O$, 0.4 g/L KH_2PO_4	- pH: 1.5 - Temperature: 30 °C - Period: 97 days - Particle size: <1mm - Inoculum rate: 10-20% - Agitation speed: 150 rpm - Pulp density: 1% (w/v) - Exogenous culture - Bioleaching approach: two-step	81% Cu, 100% Zn, 55% Ni	-	-	Kremser et al., (2020)
			10			54% Cu, 38% Zn, 91% Ni	-	-	

		95,400 Fe, 33 Li, 2,800 Mn, 150 Mo, 510 Ni, 2,700 Pb, 150 Sb, 270 Sn, 4100 Ti 34 V, 13,100 Zn Shredder-fraction “neu”: 20,600 Al, 19 As, 2,370 Ba, 26 Cd, 52 Co, 700 Cr, 8,420 Cu, 160,000 Fe, 44 Li, 4,540 Mn, 62 Mo, 570 Ni 1,110 Pb, 150 Sb, 250 Sn, 3190 Ti, 44 V, 6040 Zn -							
Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Leptospirillium ferroxidans, Acidiphiliumspecies	High-pyrrhotite tailings	Elements (mg/kg): 351,000 Fe, 5,850 Ni, 173 Co, 150,000 S^{2-}, 1200 Cu, 0.148 Au, 0.332 Pt, 0.352 Pd, 0.070 Rh, 2.5 Ag	200 g	Modified TK Medium (g/L): 0.5 $(NH_4)_2SO_4$, 0.5 K_2HPO_4, 0.5 $MgSO_4{\cdot}7H_2O$, 33.4 $FeSO_4{\cdot}7H_2O$; McCready's medium (g/L): 0.0661 $(NH_4)_2SO_4$, 0.0174 K_2HPO_4, 0.123 $MgSO_4{\cdot}7H_2O$, 33.4 $FeSO_4{\cdot}7H_2O$; ATCC medium 23 (g/L): 0.1 NH_4Cl, 3.0 KH_2PO_4, 0.2 $MgCl_2{\cdot}6H_2O$, 0.1 $CaCl_2$, 5.0 $Na_2S_2O_3{\cdot}5H_2O$	- pH: 2 - Temperature: >40 °C - Period: 10 days - Particle size: 147 µm - Inoculum rate: - - Agitation speed: - - Pulp density: 10% (w/v) - Exogenous culture - Bioleaching approach: -	80-90% Ni, 70-80% Co			Cameron et al., (2021)

Table B-10. Compilation of column bioleaching conditions with various microorganisms, applications to various ores/solid wastes, solid amount, bioleaching yield, pH, temperature, period, particle size, inoculum rate, indigenous/ exogenous

Column									
Microorganism	**Material**	**Initial metal concentration**	**Sample amount (g)**	**Medium**	**Operational parameters**	**Bioleaching yield (%)**	**Optimised parameters**	**Importance order of the optimisation parameters**	**Reference**
Acidithiobacillus ferrooxidans	waste printed circuit boards (WPCBs)	Elements (mg/kg): 245,000 Cu	250	Optimised 4.5 K medium consists of 2.0 g/L $(NH_4)_2SO_4$, 0.25 g/L K_2HPO_4, 0.25 g/L MgSO4.7H2O, 0.1 g/L KCl, 0.01 g/L $Ca(NO_3)_2$, 22.2 g/L FeSO4.7H2O	- pH: 2.25 - Temperature: 30 °C - Period: 28 days - Particle size: 4-10 mm - Inoculum rate: 1% - Irrigation rate: 40 mL/min - Aeration rate: 20 L/min - Solid/liquid ratio: 50:1 - Exogenous culture - Bioleaching approach: two-step - Column size: L 20 × ø6 cm - Inoculation place: Incubator	94.8% Cu	-		Chen et al., (2015)
Acidithiobacillus ferrooxidans	low-grade sphalerite	Elements (mg/kg): 89,000 Zn, 29,000 Pb, 80,000 Fe, 228,000 S, 93,200 Si, 23,000 CaO, and less than 2,000 Cu	410000	Modified medium consists of 3 g/L $(NH_4)_2SO_4$, 0.5g/L $MgSO_4$.7H2O, 0.5g/L K_2HPO_4, 0.1g/L KCl, 0.01g/L Ca $(NO_3)_2$, 0.2g/L yeast extract, ferrous iron from sulfide minerals	- pH: 1.5 - Temperature: 30-42 °C - Period: 120 days - Particle size: 5-30 mm - Inoculum rate: 10% - Irrigation rate: 5L/m^2h - Aeration rate: 45 L/m^2min - Solid/liquid ratio: 1864:1 - Indigenous culture - Bioleaching approach: two-step - Column size: L 220 x ø30 cm - Inoculation place: Incubator	72% Zn	-		Mousavi et al., (2006)

Acidithiobacillus Ferridurans	uranium ore	Elements (mg/kg): 411 As	400	Compositions of Leathen culture were 0.15 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 2 0.01 g/L $Ca(NO_3)$, 0.05 g/L KCl, 0.05 g/ L K_2HPO_4, 14.9 g/L $FeSO_4.7H_2O$. 1 mL of trace elements containing 0.5 g/L Disodium EDTA, 0.2 g/L $FeSO_4.7H_2O$, 0.03 g/L H_3BO_3, 0.02 g/L $CoCl_2.6H_2O$, 0.01 g/L $ZnSO_4.7H_2O$, 3.0 mg/L $Na_2MoO_4.2H_2O$, 2.0 mg/L $NiCl_2 \cdot 6H_2O$ and 1.0 mg/L $CaCl_2.2H_2O$ was added to Leathen culture	- pH: 2.5 - Temperature: 35 °C - Period: 26 days - Particle size: 4.64 mm - Inoculum rate: 10% - Irrigation rate: 1.03 $ml/cm^2/min$ - Aeration rate: 0.78 $ml/cm^2/min$ - Solid/liquid ratio: - - Exogenous culture - Bioleaching approach: two-step - Column size: L 50 × ø5 cm - Inoculation place: -	-	Optimisation parameters response surface methodology (RSM) based Box-Behnken design: particle size, irrigation rate, aeriation rate	1. aeriation rate (0.78 $ml/cm^2/min$) 2. irrigation rate (1.03 $ml/cm^2/min$) 3. particle size (4.64 mm)	Jalali et al., (2019)
Acidithiobacillus Ferridurans	contaminated soil	Elements (mg/kg): 589-48,672 Pb, 50-7,775 Cu, 145-7,754 Zn, 388-10,870	-	9K medium consists of 3 g/L $(NH_4)_2SO_4$, 0.1 g/L KCl, 0.5 g/L K_2HPO_4 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.01 g/L $Ca(NO_3)_2.4H_2O$, 44.2 g/L $FeSO_4.7H_2O$	- pH: 5 - Temperature: 26+-2 °C - Period: 84 days - Particle size: - - Inoculum rate: - - Irrigation rate: - - Aeration rate: - - Solid/liquid ratio: - - Indigenous culture - Column size: L 80 cm - Inoculation place: -	Pb: 78–90%; Cu: 68–84%; Zn: 74–90%; Mn: 85–95%.			Sur et al., (2018)

Acidithiobacillus ferrooxidans GF	low grade copper ore	Elements (mg/kg): 15,870 Cu, 38,920 Fe, 1,330 Zn, 2440 Mg, 23,890 K, 2,830 Ca, 5,740 Pb, 61,350 Al, 371,940 Si, 17,590 S	2000	10 g/L glucose	- pH: 2.5-3.8 - Temperature: 15-25 °C - Period: 102 days - Particle size: 10-15 mm - Inoculum rate: 10% - Irrigation rate: 0.66 L/h - Aeration rate: 35 L/m^2min - Solid/liquid ratio: 800:1 - Exogenous culture - Bioleaching approach: one-step - Inoculation place: Column	20.11% Cu	-		Yang et al., (2013)
Acidithiobacillus ferrooxidans GF	low-grade apatite rich uraninite ore	%(w/w): 0.047%U_3O_8	2000	9K medium contains 44.2 g/L $FeSO_4.7H_2O$	- pH: 1.7 - Temperature: room temperature °C - Period: 40 days - Particle size: <150µm: 100 g at the top; and 5mm-4mm: 1.9 kg in rest - Inoculum rate: 10% - Irrigation rate: 3 L/h - Solid/liquid ratio: 200:1 - Indigenous culture - Bioleaching approach: two-step - Column size: L 75 × ø6.5 cm - Inoculation place: Incubator	57% U	-		Abhilash et al., (2013)
Leptospirillum ferrooxidans						66% U	-		

Acidithiobacillus ferrooxidans strain ZT-94	low grade uranium ore	Elements (mg/kg): 230 U, %(w/w): 42.05 Fe_2O_3, 26.39 SiO_2, 22.22 MgO, 2.35 CaO, 2.21 Al_2O_3, 0.63 K_2O, 0.11 Na_2O, 0.71 P_2O_5,	3000	9K medium contains 10 g/L $FeSO_4.7H_2O$	- pH: 2 - Temperature: 25 °C - Period: 19 days - Particle size: 5 mm - Inoculum rate: 10% - Irrigation rate: 0.34 L/m^2min - Aeration rate: 210 L/m^2min - Solid/liquid ratio: - - Exogenous culture - Column size: L 50 × ø7.5 cm - Inoculation place: Incubator	63.85% U	Optimisation parameters response surface methodology (RSM) based Box-Behnken design: particle size, irrigation rate and aeration rate	aeration rate (210 L/m^3/min) > particle size (5 mm) > irrigation rate respectively (0.34 L/m2/min)	Zare Tavakoli et al., (2017a)
Acidithiobacillus ferrooxidans (ZT-94)	low grade uranium ore	Elements (mg/kg): 300 U %(w/w): 42.05 Fe_2O_3, 26.39 SiO_2, 22.22 MgO, 2.35 CaO, 2.21 Al_2O_3, 0.63 K_2O, 0.11 Na_2O, 0.71 P_2O_5, 29,600 S	3000	9K medium contains 2.89 g/L $FeSO_4.7H_2O$	- pH: 1.45 - Temperature: 25-40 °C - Period: 21days - Particle size: 2.5-12.5 mm - Inoculum rate: 6% - Irrigation rate: 0.75 ml/min - Aeration rate: 420 ml/min - Solid/liquid ratio: - - Exogenous culture - Column size: L 50 × ø7.5 cm - Inoculation place: Incubator	90.27%	Optimisation parameters via Plackett-Burman and response surface methodology (RSM) based central composite design (CCD): irrigation rate, aeration rate, the concentration of initial ferrous, pH, temperature, inoculation percent and particle size	the concentration of initial ferrous (2.89 g/L) > pH (1.45) > aeration rate (420 mL/min) > inoculation percent (6% v/v)	Zare Tavakoli et al., (2017b)
Acidithiobacillus ferrooxidans (ZT-94)	low grade uranium ore	Elements (mg/kg): 300 U %(w/w): 42.05 Fe_2O_3, 26.39 SiO_2, 22.22 MgO, 2.35 CaO, 2.21 Al_2O_3, 0.63 K_2O, 0.11 Na_2O, 0.71 P_2O_5, 29,600 S	3000	Modified medium contains 2.89 g/L Ferrous iron	- pH: 1.45 - Temperature: 35 °C - Period: 21days - Particle size: 2.5 mm - Inoculum rate: - - Irrigation rate: 0.75 ml/min - Aeration rate: 420 ml/min - Solid/liquid ratio: - - Exogenous culture - Column size: L 50 × ø7.5 cm - Inoculation place: Incubator	100% U	Stepwise Optimisation parameters: aeration rate, pH initial Ferrous iron concentration, temperature	aeration rate (100 L/h), pH (1.8), initial Fe^{2+} concentration (4 g/L) and temperature (35 °C)	Zare Tavakoli et al., (2017c)

Acidithiobacillus thiooxidans	spent catalyst	Elements (mg/kg): 29,300% Ni, 172,400% Al, 15,700% Mo, 153,300% V	500	0 K medium contains 1.5% (w/v) of elemental sulphur (S^0).	- pH: 1 - Temperature: 35 °C - Period: 13 days - Particle size: 2-6 mm - Inoculum rate: - - Irrigation rate: 14 mL/min - Solid/liquid ratio: 33:1 - Exogenous culture - Bioleaching approach: two-step - Column size: L 20 × ø5 cm - Inoculation place: Incubator	82.9% Ni, 53.6% V, 33.4% Al, 22.7% Mo	-		Pathak et al., (2019)
Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidnas	steel slag	Elements (mg/kg): 29,4000 % Mg, 20,0000 Ca, 1,1000 Fe, 6000 Si, 4000 Al, 500 P, 10 As, 30 Ba, 1.6 Cd, 100 Cr, 20 Cu, 10 Ga, 4 Li, 200 K, 1000 Mn, 2 Mo, 300 Na, 40 Ni, 500 Pb, 0.002 Ti, 40 V, 30 Sr, 30 Zn	100	Modified 9K medium $FeSO_4.7H_2O$ 22.5 g/L and 10.0 g/L S^0	- pH: 2-2.25 - Temperature: 28 °C - Period: 22 days - Particle size: 4-10 mm - Inoculum rate: 10% - Irrigation rate: 5 mL/min - Solid/liquid ratio: 100:1 - Exogenous culture - Bioleaching approach: spent-medium step (according to Gomes et al. 2018 it was two-step) - Column size: L 35 × ø1.8 cm - Inoculation place: Incubator	95% Al, 84% Cr, 8% V	-	-	Gomes et al., (2018)
					- pH: 2-2.25 - Temperature: 28 °C - Period: 22 days - Particle size: 4-10 mm - Inoculum rate: 10% - Irrigation rate: 5 mL/min - Aeration rate: - - Solid/liquid ratio: 100:1 - Exogenous culture - Bioleaching approach: two-step (according to Gomes et al. 2018 it was one-step) - Column size: L 35 × ø1.8 cm - Inoculation place: Incubator	100% Al, 64% Cr, 8% V	-	-	

Acidithiobacillus spp., Leptospirillum spp., Sulfobacillus spp., Ferrimicrobium spp. and archaea Ferroplasma spp.	Zijinshan copper sulphide ore	Elements (mg/kg): 2800 Cu, 2,7100 Fe %(w/w) 6.52% Pyrite, 0.21% Digenite, 0.12% Covellite, 0.11% Enargite, 0.40% Limonite, 7.86% Alunite, %8.60 Dickite, 0.65% Mica, 75.28% Quartz	3000	Acid Mine Drainage contains 0.52 g/L Ferrous iron	- pH: 1.2 - Temperature: 60 °C - Period: 240 hours - Particle size: 10 mm - Inoculum rate: - - Irrigation rate: 6 h/d - Aeration rate: - - Pulp density: - - Indigenous culture - Column size: L 200 × ø20 cm	92.9% Cu, 51.7% Fe	-	-	Wu et al., (2016)
				Raffinate contains 21.8 g/L Ferrous iron	- pH: 1.5 - Temperature: 60 °C - Period: 240 hours - Particle size: 10 mm - Inoculum rate: - - Irrigation rate: 6 h/d - Aeration rate: - - Pulp density: - - Indigenous culture - Column size: L 200 × ø20 cm	90% Cu, 40% Fe	-	-	
Acidithiobacillus ferrooxidans and Leptospirillum ferrooxidans	complex sulfide ore	Elements (mg/kg): 2900 Ni, 5300 Zn, 2000 Cu, 3500 Co %(w/w): 61.2% pyrrhotite (FeS), 24.3% pyrite (FeS2), 5% pentlandite [$(Fe_x/ Ni_{9-x})_9S_8$], 6.5% alabandite (MnS), 2.4% chalcopyrite ($CuFeS_2$).	9000	Basal salt each contains 0.4g/L K_2HPO_4, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, supplemented with either ferrous ion (4.5 g/L), elemental sulphur (1% w/v) or black schist ore powder (1% w/v) from the Sotkamo deposit	- pH: 1.5 - Temperature: 25 °C - Period: 140 days - Particle size: 17% 8–12 mm, 38% 3.15–8 mm and the rest 0.020–3.15 mm - Inoculum rate: 4% - Irrigation rate: 10 Lm^2/h $ml/cm^2/min$ - Aeration rate: (8–11) m^3/m^2h - Solid/liquid ratio: 1800 - Exogenous culture - Bioleaching approach: one-step - Column size: L 80 × ø10 cm - Inoculation place: column	59% Ni, 52% Zn, 12% Cu 13% Co	-		Halinen et al., (2009)
Sulfobacilllus thermosulfidooxidans and *Thermoplasma acidophilum*	electronic scrap	Elements (mg/kg): 80,000 Fe, 89,000 Cu, 20,000 Ni, 31,500 Pb, 30 Ag, 13 Au, 7,500 Al, 82,000 Zn, 6.5 Sn	10000	FeTSB medium composed of (g/L): $MgSO_4.7H_2O$, 0.50; $(NH_4)_2SO_4$, 0.15; KCl, 0.05; KH_2PO_4, 0.05; $Ca(NO_3)_2$, 0.01 and TSB, 0.25.	- pH: 2 - Temperature: 45 °C - Period: 280 days - Particle size: 100 to 120 µm - Inoculum rate: 10% - Irrigation rate: 50 mL/min - Aeration rate: 150–200 L/h - Solid/liquid ratio: 5000:1 - Exogenous culture	80% Zn, 86% Cu, 74% Ni, 64% Al	-		Ilyas et al., (2010b)

					- Bioleaching approach: one-step - Column size: L 58 × ø13 cm - Inoculation place: column				
Sulfobacillus	low-grade sphalerite	Elements (mg/kg): 89,000 Zn, 29,000 Pb, 80,000 Fe, 228,000 S, 93,200 Si, 23,000 CaO, and less than 2,000 Cu	410000	Modified medium consists of 0.4 g/L $(NH_4)_2SO_4$, 0.4g/L $MgSO_4$.7H2O, 0.4g/L K_2HPO_4, ferrous iron from sulfide minerals	- pH: 1.5 - Temperature: 30-42 °C - Period: 120 days - Particle size: 5-30 mm - Inoculum rate: 10% - Irrigation rate: 5L/m^2h - Aeration rate: 45 L/m^2min - Solid/liquid ratio: 1864:1 - Indigenous culture - Bioleaching approach: two-step - Column size: L 220 × ø30 cm - Inoculation place: Incubator	85% Zn	-		Mousavi et al., (2006)
indigenous bacteria	Mine Tailing Soil	Elements (mg/kg): 4023 As	370+-5	solution containing 100 mM of glucose and lactate, at a ratio of 2:1 (v/v) as carbon sources	- pH: - - Temperature: 27+-2 °C - Period: 28 days - Particle size: - - Inoculum rate: - - Irrigation rate: - - Aeration rate: 18.2 mL/h N_2 (anaerobic) - Solid/liquid ratio: - - Indigenous culture - Column size: L 20 × ø4 cm	As: 17.3+-15.6%			Lee et al., (2009)
Sulfobacillus thermosulfidooxidans strain-RDB and Thermoplasma acidophilum.	low-grade mining ore	%(w/w): 8.2 bornite, 0.3 Pentlandite, 0.8 chalcocite, 0.8 linnaeite, 0.3 wurtzite and 0.3 sphalerite. The 20 smithsonite, 3 talc, 25 sphaerocobaltite, 10 azurite	80000	FeTSB medium contains 0.25 g/L TSB	- pH: 2 - Temperature: 25 °C - Period: 112 days - Particle size: <0.1 mm - Inoculum rate: 20% - Irrigation rate: 45–48 L/m^2h - Aeration rate: 180–250 L/h - Solid/liquid ratio: 4444:1 - Exogenous culture - Bioleaching approach: one-step - Column size: L 85 × ø15 cm - Inoculation place: Column	84% Cu, 76% Co, 72% Ni, 70% Zn, 63% Fe	-		Ilyas et al., (2012)

Table B-11. Comilation of pilot scale heap/dump bioleaching conditions with various microorganisms, applications to various ores/solid wastes applications to various ores/solid wastes, solid amount, bioleaching yield, pH, temperature, period, particle size, indigenous/exogenous

Pilot scale heap-dump						
Microorganism	**Material and elemental content**	**Ore amount**	**Operational conditions**	**Bioleaching yield**	**Elemental recovery**	**Reference**
Leptospirillum ferrooxidans and Acidithiobacillus ferrooxidans	Zijinshan copper sulphide ore Elements (mg/kg): 6,500 Cu, 26,000 S, 24,300 Fe, 380 As	15,839 tons	- pH: 2-2.5 -Temperature: 20-35 °C - Period: 270 days - Particle size: 30 mm - Irrigation rate: 15–20 L/m^{2}h - Indigenous culture -Heap height: 4.5m	80.58% Cu, 12.53% S, 28.36% Fe, 50% As	300 t/a copper cathode	Renman et al., (2006)
		13,075 tons	- pH: 2-2.5 -Temperature: 20-35 °C - Period: 270 days - Particle size: 50 mm - Irrigation rate: 15–20 L/m^{2}h - Indigenous culture -Heap height: 4.5m	65.94% Cu, 19.22% S, 28.86% Fe, 32% As		
		-	- pH: 2-2.5 -Temperature: 20-35 °C - Period: 270 days - Particle size: 30 mm - Irrigation rate: 15–20 L/m^{2}h - Indigenous culture -Heap height: 10 m	80.72% Cu	1000 t/a copper cathode	
A. ferrooxidans, Ferroplasma, Acidiferrobacter, Nitrospira	Paodaoling gold ore Elements (mg/kg): 0.85 Au, 0.19 As, 22,700 S, 32,100 Fe	15,000 tons	- pH: 1.5 -Temperature: 40 °C - Period: 80 days - Particle size: 10 mm - Irrigation rate: 35.7 L/m^{2}h - Exogenous culture -Heap height: 4.5	78% Au, 42% As	-	Li et al., (2020)

A. ferrooxidans dominated mixed culture	low grade chalcopyrite Elements (mg/kg): 3000 Cu	1,000 tons	- pH: 1.8 -Temperature: 25 °C - Period: 383 days - Particle size: 12 mm - Irrigation rate: 10m3/h - Exogenous culture -Heap height: 2.8m	30% Cu	-	Panda et al. (2012)
A. ferrooxidans and L. ferriphilum	low grade, granite-type uranium ore	4,854 tons	- pH: 1-2 -Temperature: 12-40 °C - Period: 85 days - Particle size: 8 mm - Irrigation rate: - - Exogenous culture -Heap height:-	88.3% U	-	Wang et al., (2017)
A. ferrooxidans, A. thiooxidans, A. caldus, L. ferrooxidans, Sulfobacillus thermosulfidooxidans, Thiobacillus thioparus, T. denitrificans, Halothiobacillus neapolitanus, Acidiphilium, Pseudomonas, Bacillus, Aerobacter, Caulobacter, Cladosporium, Penicillium, Trichoderma, Rhodotorula, Algae	low-grade ores and rock mass run-of-mine mining wastes	120000 tons	- pH: 2.1-3.7 -Temperature: 12.0-12.2 °C - Period: 14600 days - Particle size: < 100μm-300 mm (90% <50mm) - Irrigation rate: 10–50 l/t 24 h - Indigenous culture -Dump height: 12m	71.4% Cu		Groudeva et al., (2010)

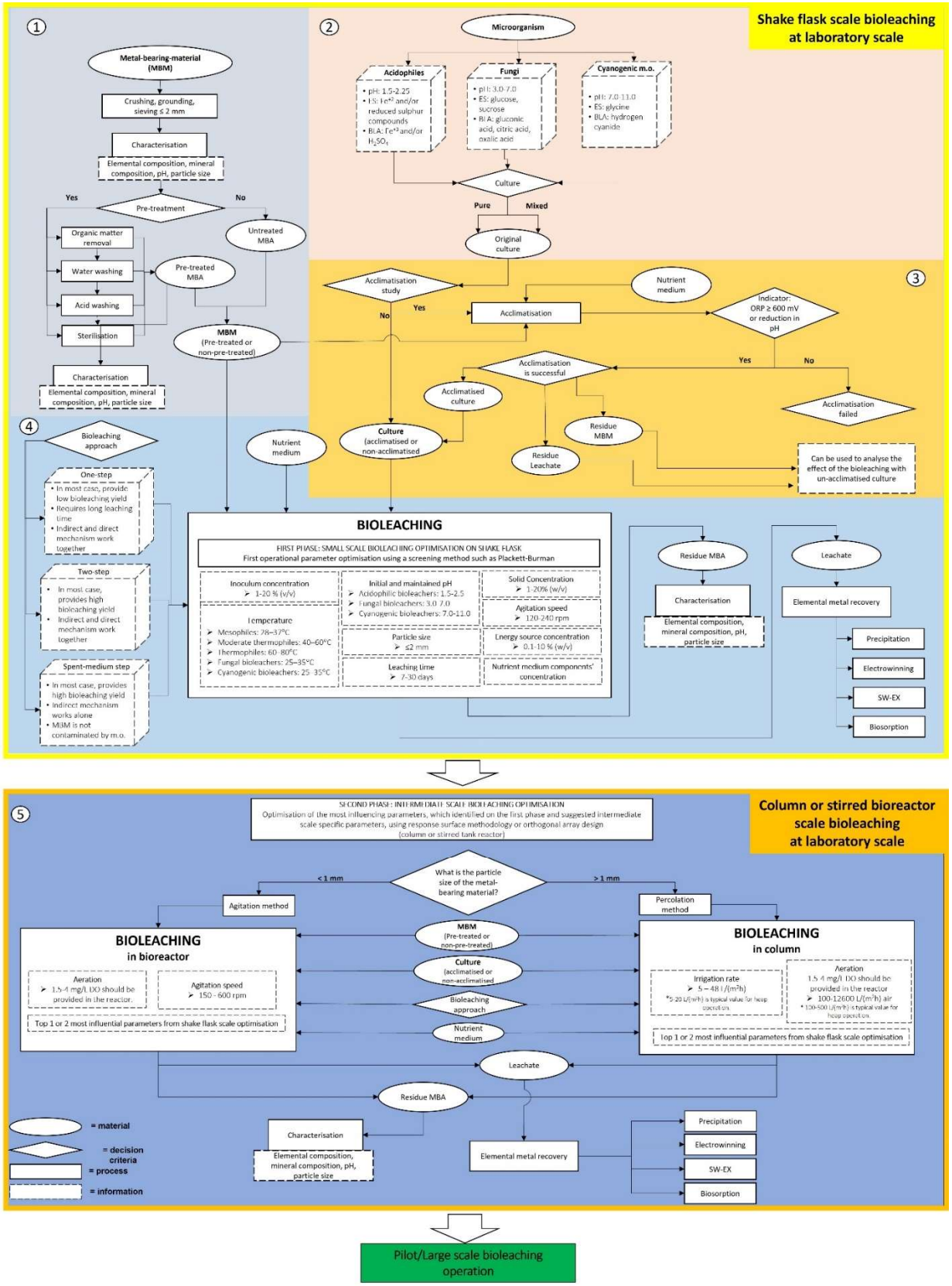

Figure B-1. Two-phase optimisation routes for bioleaching of metal-bearing materials.

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Appendix C Unlocking The Hidden Value Of Industrial By-Products: Optimisation Of Bioleaching To Extract Metals From Basic Oxygen Steelmaking Dust And Goethite

Table C-1. Elemental composition of the non-autoclaved BOS-D (n=2) and goethite (n=3)

Element	BOS-D		Goethite		BOS-D	Goethite
REE	mg/kg	RSD (%)	mg/kg	RSD (%)	Difference between autoclaved and non-autoclaved material (%)	
Y	1.26	2	2.6	14	3	-13
La	-	-	2.6	15	-	-44
Ce	4.09	8	6.5	13	-8	-16
Pr	0.42	7	0.7	16	-5	-17
Nd	1.89	12	2.8	12	-5	-17
Sm	0.28	31	0.5	18	7	-25
Eu	0.01	141	0.1	35	83	-25
Gd	0.27	7	0.5	22	10	-25
Tb	-	-	0.01	89	-	0
Dy	0.18	11	0.45	17	10	-13
Ho	-	-	0.04	50	-	0
Er	0.06	20	0.24	16	40	-20
Tm	-	-	-	-	-	-
Yb	0.05	20	0.27	19	50	-35
Lu	-	-	-	-	-	-
Sc	-	-	-	-	-	-
Critical metals						
Li	5.52	6	3.7	11	-38	-16
Co	9.73	2	9.9	14	3	-10
Sb	12.25	1	769	11	-2	-10
Cu	126.5	1	23600	13	0	-16
Heavy metals						
Cr	167	4	258.7	14	-6	-16
Ni	58.7	2	39.5	15	1	-16
Zn	18950	0	70948.6	13	-4	-15
As	16.9	4	5840	12	-6	-13
Cd	63.85	1	479.6	12	-3	-13
Hg	0.03	141	82.2	10	97	-10
Pb	1772.97	0	28166.7	12	-2	-14
Others						
Be	0.14	2	0.82	11	63	-53
B	20.8	10	-	-	13	-
Mg	4967.3	8	614.3	14	2	-20
Al	2865	8	12896.9	14	-8	-16
Si	-	-	-	-	-	-
V	56.5	14	47.6	14	-11	-16
Mn	6060	0	1686.7	14	-3	-16
Fe	399500	1	188667	14	-2	-16
Ga	13.7	6	259	13	-5	-16
Mo	7.87	6	83	13	-8	-16
Ba	57	15	30.7	29	-6	0

Table C-2. L9 experimental design and extraction yields of Mn, Zn, Pb, Al, Li, Co, Y and Ce from BOS-D (n=3; SD for all elements ≤ 20%).

Condition no.	Experimental scheme	Extraction yield (%)							
		Mn	**Zn**	**Pb**	**Al**	Li	Co	Y	Ce
C1	$A_1B_1C_1D_1$	54	61	13	59	92	40	99	67
C2	$A_1B_2C_2D_2$	53	55	0	43	55	54	96	76
C3	$A_1B_3C_3D_3$	57	53	0	39	55	52	32	75
C4	$A_2B_1C_2D_3$	51	61	0	47	62	43	87	48
C5	$A_2B_2C_3D1$	47	52	0	53	36	35	100	58
C6	$A_2B_3C_1D_2$	46	51	0	49	39	40	100	53
C7	$A_3B_1C_3D_2$	46	51	0	46	41	37	73	43
C8	$A_3B_2C_1D_3$	52	55	0	43	46	39	69	35
C9	$A_3B_3C_2D_1$	42	48	0	46	31	34	80	45

Table C-3. L9 experimental design and extraction yields of Mn, Zn, Pb, Al, Li, Co, Y and Ce from Goethite (n=3; SD for all elements ≤ 20%).

Condition no.	Experimental scheme	Extraction yield (%)							
		Mn	**Zn**	**Pb**	**Al**	Li	Co	Y	Ce
C1	$A_1B_1C_1D_1$	86	35	-	23	57	64	100	15
C2	$A_1B_2C_2D_2$	100	43	-	20	61	100	96	14
C3	$A_1B_3C_3D_3$	100	43	-	16	64	100	96	14
C4	$A_2B_1C_2D_3$	35	34	-	20	56	25	100	15
C5	$A_2B_2C_3D1$	40	33	-	23	50	31	98	15
C6	$A_2B_3C_1D_2$	50	36	-	21	56	39	99	15
C7	$A_3B_1C_3D_2$	29	33	-	22	51	21	100	14
C8	$A_3B_2C_1D_3$	33	31	-	22	50	24	100	14
C9	$A_3B_3C_2D_1$	33	30	-	24	45	24	93	14

Table C-4. R^2 values from kinetic models applied to the best overall condition for BOS-D; 1% solid concentration, 1% energy source concentration, 1% inoculum concentration, and pH 1.5 ($A_1B_1C_1D_1$), and goethite; 1% solid concentration 1% solid concentration, 4% energy source concentration, 10% inoculum concentration, and pH 2 ($A_1B_3C_3D_3$).

		Chemical reaction	Product layer diffusion
	Metal	$kt = 1 - (1 - F_t)^{\frac{1}{3}}$	$kt = 1 - \frac{2}{3}F_t - (1 - F_t)^{\frac{2}{3}}$
BOS-D	Mn	**0.72**	**0.72**
	Zn	**0.79**	0.78
	Al	0.69	**0.74**
	Li	**0.95**	0.89
	Co	**0.73**	0.72
	Y	**0.78**	0.70
	Ce	0.90	**0.94**
Goethite	Mn	**1.00**	**1.00**
	Zn	0.83	**0.86**
	Al	**1.00**	0.95
	Li	0.36	**0.37**
	Co	**1.00**	**1.00**
	Y	0.09	**0.10**
	Ce	**0.34**	0.33

F_t represents the fraction of metal mobilized; t is the leaching time, and k the rate constant

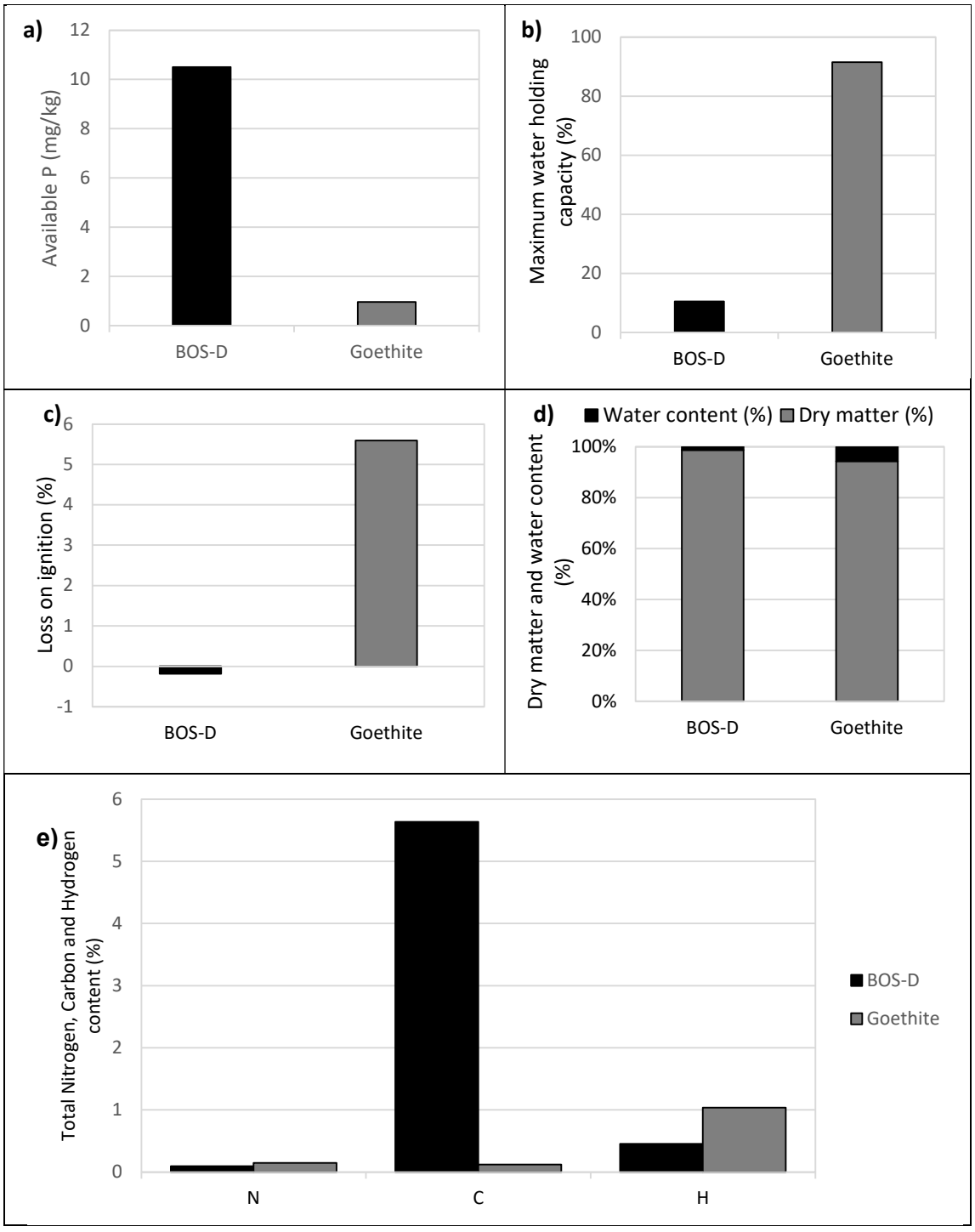

Figure C-1. Characterisation results of BOS-D and goethite (n=3; SD for all elements ≤ 20%), a) available phosphorus concentration, b) maxiumum water holding capacity, c) loss on ignation, d) dry matter and water content, e) total nitrogen, carbon and hydrogen content.

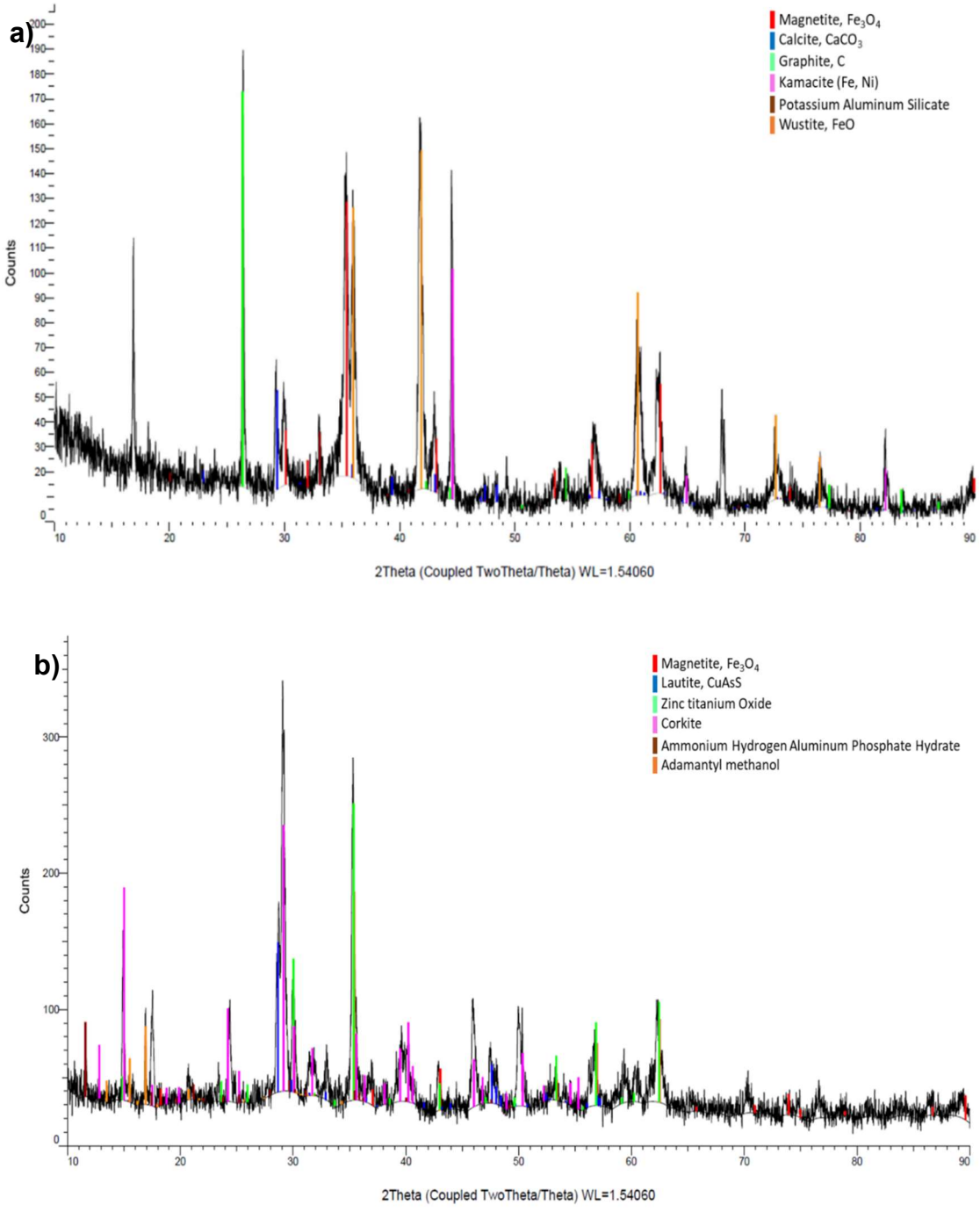

Figure C-2. Results of XRD analysis of autoclaved before bioleaching of BOS-D (a) and goethite (b).

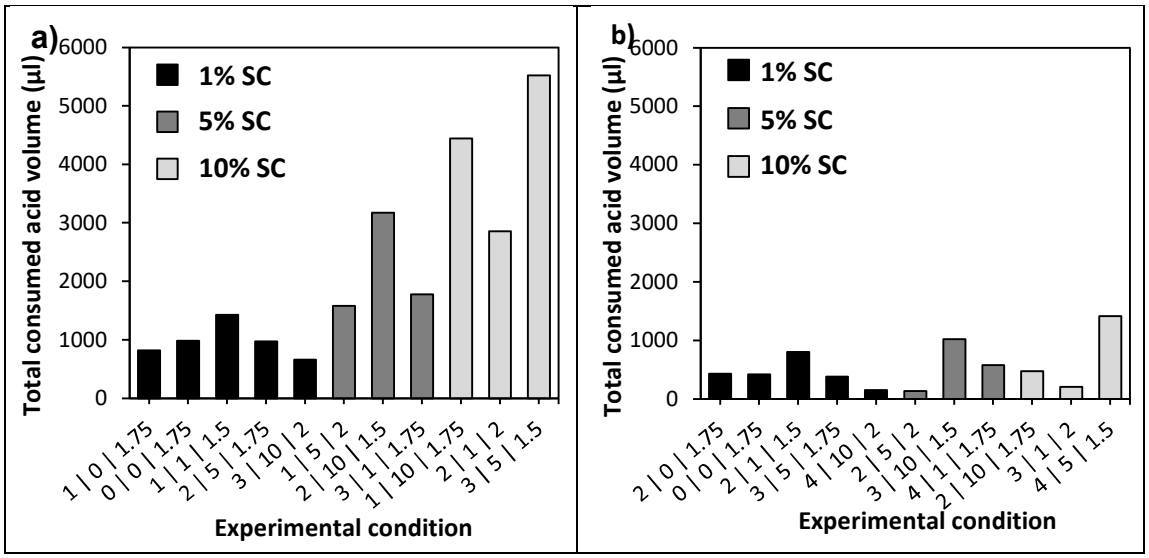

Figure C-3. The cumulative acid consumption over the experiments for a) BOS-D and B) goethite (RSD for all elements ≤ 20%). Series names represent the values of "energy source concentration | inoculum concentration | pH". Abbreviation of SC stands for solid concentration (% w/v).

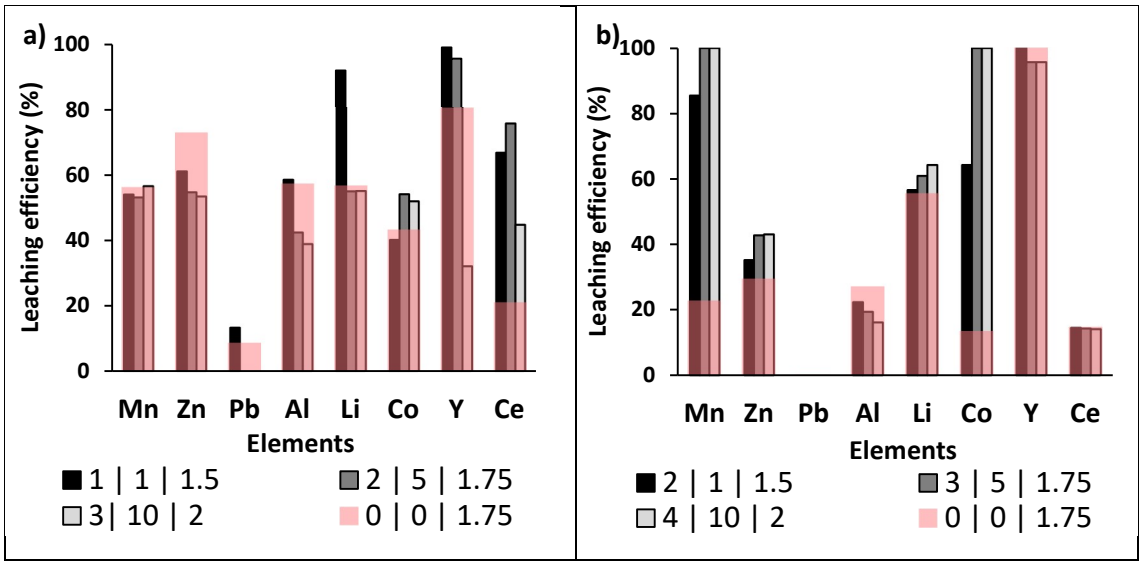

Figure C-4. Bioleaching yield (%) of selected elements after 14 days of bioleaching of BOS-D (a) and goethite (b) materials for 1% (w/v) solid concentration (n=3 for BOS-D; n=2 for goethite; SD for all elements ≤ 20%). Series names represent the values of "energy source concentration | inoculum concentration | pH".

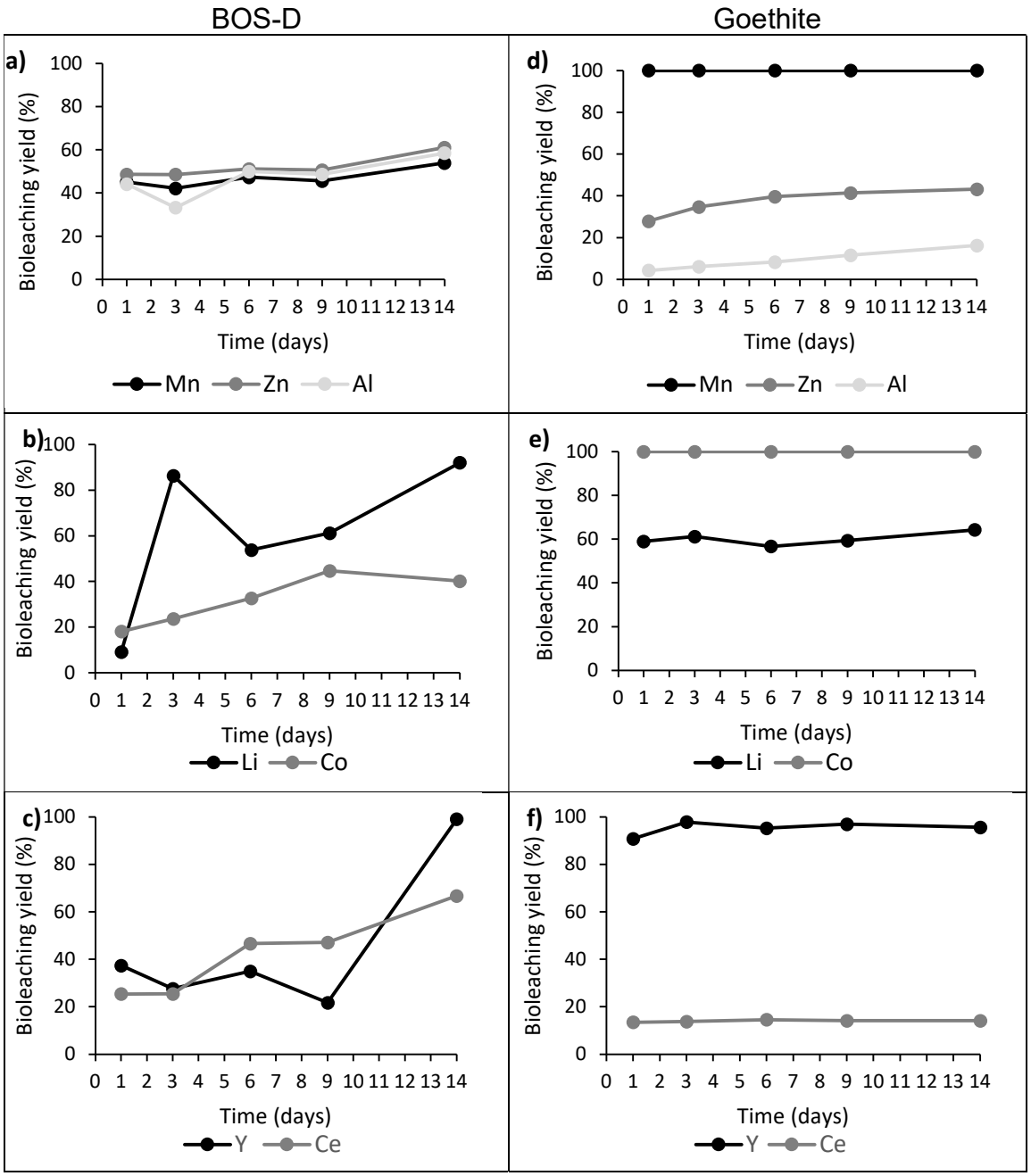

Figure C-5. Bioleaching yields of metals in best overall conditions (n=3 for BOS-D; n=2 for goethite; SD for all elements ≤ 20%); BOS-D (a,b,c); 1% solid concentration, 1% energy source concentration, 1% inoculum concentration, and pH 1.5 ($A_1B_1C_1D_1$), and goethite (d, e, f); 1% solid concentration 1% solid concentration, 4% energy source concentration, 10% inoculum concentration, and pH 2 ($A_1B_3C_3D_3$).

Appendix D Techno-Economic Assessment Of Bioleaching For Industrial Metal-Bearing By-Products

Table D-1. Characterisation results of BOS-D and goethite (n=3; SD for all elements ≤ 20%).

		BOS-D	Goethite
Parameter	Unit	Value	
Available P	mg/kg	10.5	0.96
Loss of ignition	%	-0.19	5.59
Dry matter content	%	99	94
Water content	%	1	6
Maximum water holding capacity	%	10.5	91.5
Total N	%	0.09	0.15
Total C	%	5.63	0.12
Total H	%	0.45	1.04

Table D-2. Elemental composition of the non-autoclaved BOS-D and goethite (n=2; RSD for all elements ≤ 20%).

	BOS-D	Goethite
Element	mg/kg	mg/kg
Y	1.26	2.6
Ce	4.09	6.5
Nd	1.89	2.8
Li	5.52	3.7
Co	9.73	9.9
Cu	126.5	23,600
Zn	18,950	70,948.6
Al	2,865	12,896.9
Mn	6,060	1,686.7