



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. Optimizing 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 · Biohydrometallurgy · Biomining · Metal bearing material · Electronic waste · Multivariate optimisation

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,

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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 from Li-ion batteries, and the production of hazardous gases (Roy et al. 2021a, 2021b). 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 (Fig. 1 and Table 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 Supplementary Material Tables S1–S7). 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 Supplementary Material Tables S8–S11). Commercial scale operations are typically conducted ex-situ using vat, agitated tank reactors, heap, and dump methods (Natarajan 2018; Zambak 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).

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 sheds light on the remaining challenges for scale up alternatives and proposes an optimisation framework for future bioleaching research.

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 Supplementary Material for details).

Acidophilic bioleaching

Acidophilic microorganisms, mainly bacteria, are often used to extract metals from sulphidic ores e.g., pyrite. Most common bacteria are *Acidithiobacillus ferrooxidans*

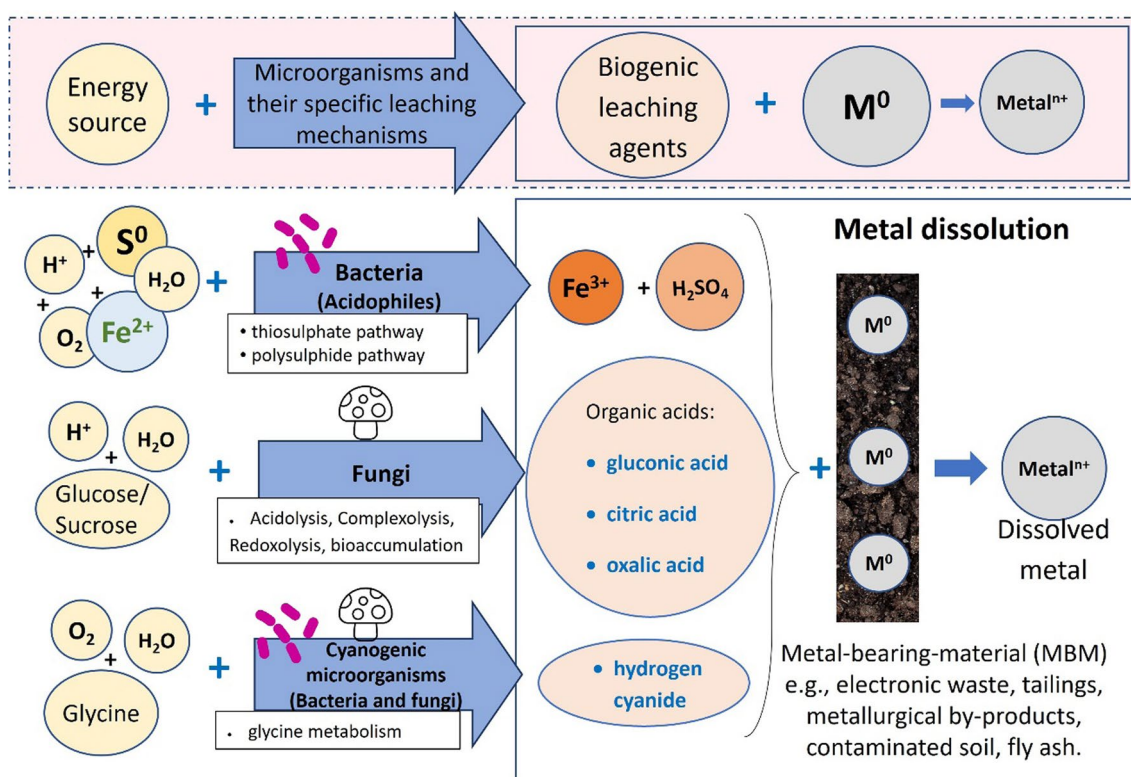
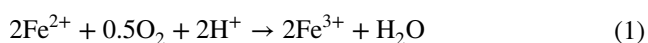
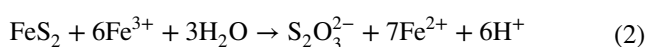


Fig. 1 The bioleaching process. At the top, the figure shows inputs for bioleaching process which are energy sources depending on microorganisms' type and microorganisms with their specific leaching mechanism. Microorganisms produce biogenic leaching agents which

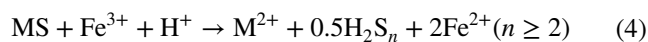
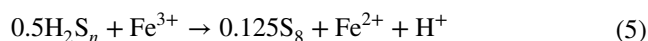
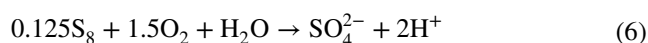
can be ferric iron, sulphuric acid, organic acids, and hydrogen cyanide. Then, biogenic leaching agents react with metal-bearing materials to dissolve metals. M^0 represents a metal in elemental state, M^{n+} represents a metal in ionic form is metal in elemental state

and *Acidithiobacillus thiooxidans* (Table 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.

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 Eqs. (1–3)



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 Eqs. (4–6):



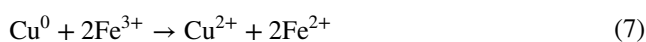
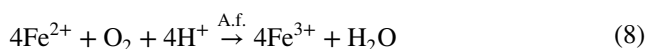
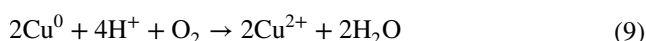
Besides sulphidic form, metals are found as oxide and organically bound forms also present as hydroxides, carbonates, and silicates. Almost all acidophiles can manage to solubilise metals from these metallic forms at suitable conditions (Srichandan et al. 2020). Spent catalyst, fly ash and metallurgical 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,

Table 1 Common microorganisms used for bioleaching applications

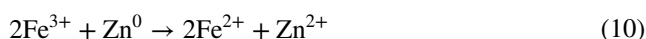
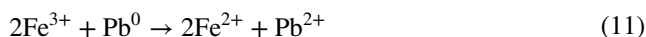
Microbial groups	Species	Microbial leaching mechanism	pH range and temperature	Energy source	Biogenic leaching agents	References
Acidophiles	<i>Acidianus brierleyi</i> , <i>A. infernus</i> , <i>Acidimicrobium species</i> , <i>Acidithiobacillus ferrooxidans</i> , <i>A. thiooxidans</i> , <i>A. caldus</i> , <i>Brevibacillus sp.</i> , <i>Ferroplasma acidiphilum</i> , <i>F. acidarmanus</i> , <i>Leptospirillum ferrooxidans</i> , <i>L. ferriphilum</i> , <i>Metallosphaera sedula</i> , <i>Sulfobacillus thermosulfidooxidans</i> , <i>S. thermotolerans</i> , <i>S. acidophilus</i> , <i>S. metallicus</i> , <i>S. acidocaldarius</i> , <i>S. solfataricus</i> , <i>S. brierleyi</i>	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 ₂ O ₃ ²⁻ , H ₂ S	F ⁺³ H ₂ SO ₄	Figueroa-Estrada et al., (2020) Ma et al., (2021) Rodrigues et al., (2018) Tian et al., (2022)
Fungi	<i>Aspergillus niger</i> , <i>A. flavus</i> , <i>Penicillium simplicissimum</i> , <i>P. chrysogenum</i> ,	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: <i>Pseudomonas fluorescens</i> , <i>P. aeruginosa</i> , <i>P. Caldus</i> , <i>P. putida</i> , <i>P. aeruginosa</i> , <i>Chromobacterium violaceum</i> Fungi: <i>Clytocybe sp.</i> , <i>Poly-sporus sp.</i> , <i>Marasmius oreades</i>	Glycine metabolism	pH 7.0–11.0 25–35 °C	Glycine	Hydrogen cyanide	Arab et al., (2020) Liu et al., (2016) Srichandan et al., (2020)

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 h 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 Eqs. (7–9):



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 Eqs. (10–11):

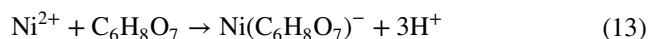


Fungal bioleaching

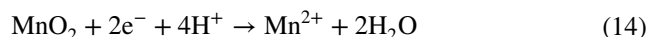
Aspergillus niger and *Penicillium simplicissimum* are the most common types of fungi found in bioleaching processes (Table 1). They produce various organic acids by using organic carbons such as glucose and sucrose as an energy source. The production of organic acids allows to dissolve metals from its 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 Eqs. 12 and 13 and as follows (Asghari et al. 2013):



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):



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).

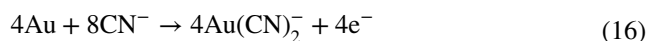
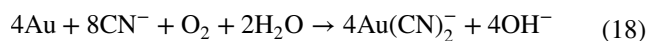
Cyanogenic bioleaching

Cyanogenic microorganisms use organic carbon as energy source and mostly belong to Proteobacteria while there are few fungal species (Table 1). Their ability to produce cyanide, which is a good leaching agent, from glycine ($\text{NH}_2\text{CH}_2\text{COOH}$) 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 fluorescens* 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):



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 $[\text{Cu}(\text{CN})_2]^-$, $[\text{Ag}(\text{CN})_2]^-$, $[\text{Au}(\text{CN})_2]^-$ (Pourhossein et al. 2021). The equations proposed in this process are represented by Eqs. (16–18) as follows:

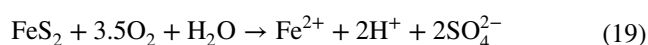
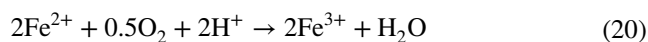


Bioleaching mechanisms, approaches, and processes parameters

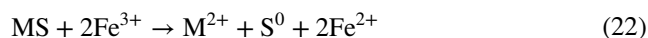
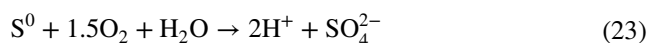
Bioleaching mechanisms

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 (Eqs. 19 and 20) and indirect (Eqs. 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 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 Eqs. (19–23) (Chen and Lin 2010; Sand et al. 2001):

Direct mechanism



Indirect mechanism



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 (Eqs. 19–20) and the free cells in the solution simultaneously mediate dissolution (Eqs. 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.

Bioleaching operations and approaches

Scale of bioleaching applications

Methods in bioleaching are mainly classified as agitation leaching for fine particles and percolation leaching process for coarse particles (Fig. 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 S8). 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 (Fig. 2). Stirring method is used in commercial scale agitated tank reactor (ex-situ) (Maluckov, 2017; Zambak, 2012; Table 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 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

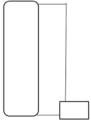
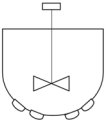
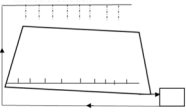
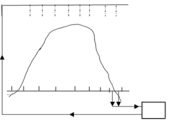
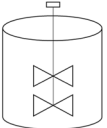
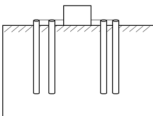
Small scale		
Shake flask		
	Particle size: 120 μm - 2mm Solid concentration: 0.1 - 20 % (w/v) Materials: low grade polymetallic ores, mine tailings, metallurgical waste (slag sludge and dust), fly ash, spent catalyst, waste electric electronic equipment (WEEE), contaminated soil, sewage sludge.	Table S8
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 S10
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 S9
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)

Fig. 2 Bioleaching operations at different scales. Operations are sorted into three categories including small scale, intermediate scale and large scale

Table 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	Gericke et al. (2009)
Sino Gold Mining Limited & Guizhou	Jinfeng	China	790	–	2006	
Golden Star Resources	Bogoso	Ghana	820	–	2006	
Centroservice	Suzdal	Kazakhstan	196	–	2005	Abhilash et al. (2015)

Table 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	Gericke et al. (2009)
Straits Resources	Whim Creek and Mons Cupri	Australia	17,000	2006	
Barrick Gold Corporation	Zaldivar	Chile	150,000	1998	

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). 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 m³) (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).

Bioleaching approaches

There are three approaches to operate microorganisms for bioleaching including: one-step (Fig. 3a, b c), two-step (Fig. 3d, e f) and spent-medium step (Fig. 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 (Fig. 3b). Thus, microbial growth and metal dissolution begin simultaneously. In this approach, microorganisms are however often adversely

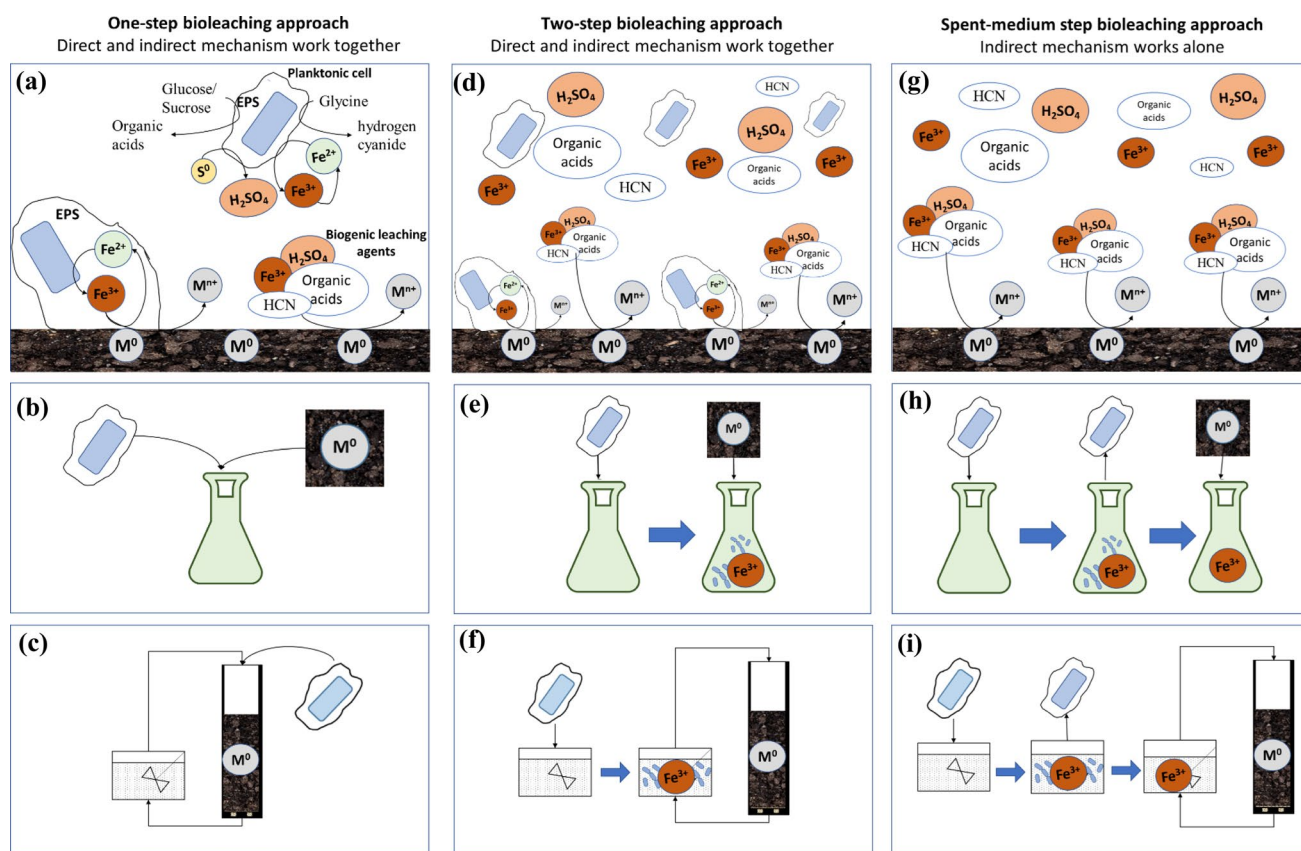


Fig. 3 Bioleaching approaches used for metal solubilisation **a** mechanism of the one-step bioleaching approach; in this approach, both microorganisms and metal-bearing material are added to the medium at the same time, and the bioleaching process is carried out during the microorganisms culturing **b** 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

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 (Fig. 3e), first, microorganisms are added into a fresh growth medium. Then, cell cultures are incubated for 2–7 days or even 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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 3f; Mousavi et al. 2006). Another option is the spent-medium step (Fig. 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.

Conditions and parameters controlling the 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.

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 microorganism 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: 1–10 mm (vat leaching); 10–25 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).

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. 2022; 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, $\leq$ 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 min (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 h, 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.

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 microorganism used for bioleaching (Table 1), and can be defined by conducting an optimisation study (Table 4). To avoid low dissolution yield, maintaining pH at optimum level by adding sulphuric acid is recommended (Chen et al. 2015; Roy et al. 2021a, 2021b). For the bioleaching employing fungi and cyanogenic bacteria pH of the culture medium can be adjusted by 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

Table 4 Bioleaching yield, in %, for different metals by pH optimisation

Material	Microorganisms	Element	pH	Bioleaching yield (%)	Operational conditions	Class	Reference
Dewatered electroplating sludge	Mixed acidophilic strains including <i>Acidithiobacillus ferrooxidans</i> and <i>Acidithiobacillus thiooxidans</i>	Cu	1.5	97	Temperature: 30 °C Period: 24 h Particle size: $\leq 75 \mu\text{m}$ 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	<i>Aspergillus flavus</i>	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	<i>Pseudomonas balearica</i>	Au	7	46	Temperature: 30 °C Period: 7 days Particle size: $150 \leq \mu\text{m}$ 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			

metal-bearing-material to leaching media (Muddanna and Baral 2021; Roy et al. 2021a, 2021b). 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. 2021a, 2021b). 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*.

Solid concentration

Solid concentration is expressed 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 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) (Fig. 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. BIOPRO™ used 117,200 m³ capacity solution pond to bioleach 810,000 tons of refractory sulfidic ore in commercial heap process (Rawlings and Johnson 2007).

Energy source choice and concentration

The type of energy source is dependent on the selected microorganisms (Table 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 Fe³⁺ and H₂SO₄ (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 H₂SO₄ and oxidation/reduction by Fe²⁺/Fe³⁺, whereas Zn, Cd and Pb followed acid dissolution mechanism. Others for instance spent Li-Ion batteries or waste printed circuit boards require Fe²⁺, Fe³⁺ or H₂SO₄

only (Chen et al. 2015; Ghassa et al. 2020; Roy et al. 2021a, 2021b).

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.

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 for 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 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 *Sulfobacillus 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 S8; Table S10). When the effect

Table 5 Optimal energy source concentration for various microorganisms. Conc stands for concentration

Material	Microorganisms	Energy source	Optimisation values % (w/v)	Optimal conc. or selected conc. % (w/v)	Bioleaching yield (%)	Type	Reference
Sewage sludge	Indigenous sulphur oxidising bacteria (<i>Acidithiobacillus ferrooxidans</i> , <i>A. thiooxidans</i> and <i>Thiobacillus thioparus</i>)	Elemental sulphur	0.1, 0.2, 0.3, 0.55, 1.0	0.1–0.3%	> 60% Cu, Zn, Pb, Ni	Bacteria	Chen and Lin (2010)
	Indigenous iron oxidizing bacteria	Ammonium ferrous sulphate (source of Fe ²⁺)	–	2	56% Cu, 48% Ni, 68% Zn, 42% Cr	Bacteria	Pathak et al. (2009)
		Ferrous sulphate (source of Fe ²⁺)	–	2	64% Cu, 58% Ni, 76% Zn, 52% Cr	Bacteria	
Low grade uranium ore	<i>A. ferrooxidans</i>	Ferrous sulphate (source of Fe ²⁺)	0.5, 1, 1.5, 2	1.45	90%	Bacteria	Zare Tavakoli et al. (2017b)
Low grade uranium ore	<i>A. ferrooxidans</i>	Ferrous sulphate (source of Fe ²⁺)	1, 2, 4	2	100%	Bacteria	Zare Tavakoli et al. (2017c)
Spent refinery catalyst	<i>Aspergillus niger</i>	Sucrose (source of organic C)	9, 11, 13, 15, 17	9.40	100% Mo, 46% Ni, and 14% Al	Fungi	Amiri et al. (2012)
Power plant residual ash	<i>A. niger</i>	sucrose (Source of organic C)	5, 7.5, 10, 12.5	10.20	83% V	Fungi	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	Bacteria	Arab et al. (2020)
Printed circuit boards	<i>Pseudomonas aeruginosa</i>	Glycine	0.06, 0.1, 0.2, 0.3, 0.34	0.10	90% Ag	Bacteria	Merli et al. (2022)

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 et al. 2021; Jagannath et al. 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.

Aeration rate and temperature

Aeration provides an efficient transfer of O₂ and CO₂ for microorganism 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 from waste printed circuit boards. Natarajan (2018) reported the typical aeration rates for a heap operation are 0.1–0.5 m³/m²h.

Maintaining temperature at desired level based on employed microorganisms is important to achieve efficient metal dissolution (Table 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).

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; Işildar 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. (2017a, b, c) 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.

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 optimized, the catalyst addition can be evaluated to increase further the bioleaching yield.

Industrial application needs and 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 CO₂ 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 Tongguan-shan 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 (Tables 2, 3, and 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 ($\leq 5\%$ 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 (Fig. 4) (see Supplementary Material Document 2 for the detailed flow chart). Our procedure modified from Potysz et al. (2018) the “flowsheet to develop optimized 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 et al. 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. 2021a, 2021b).

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

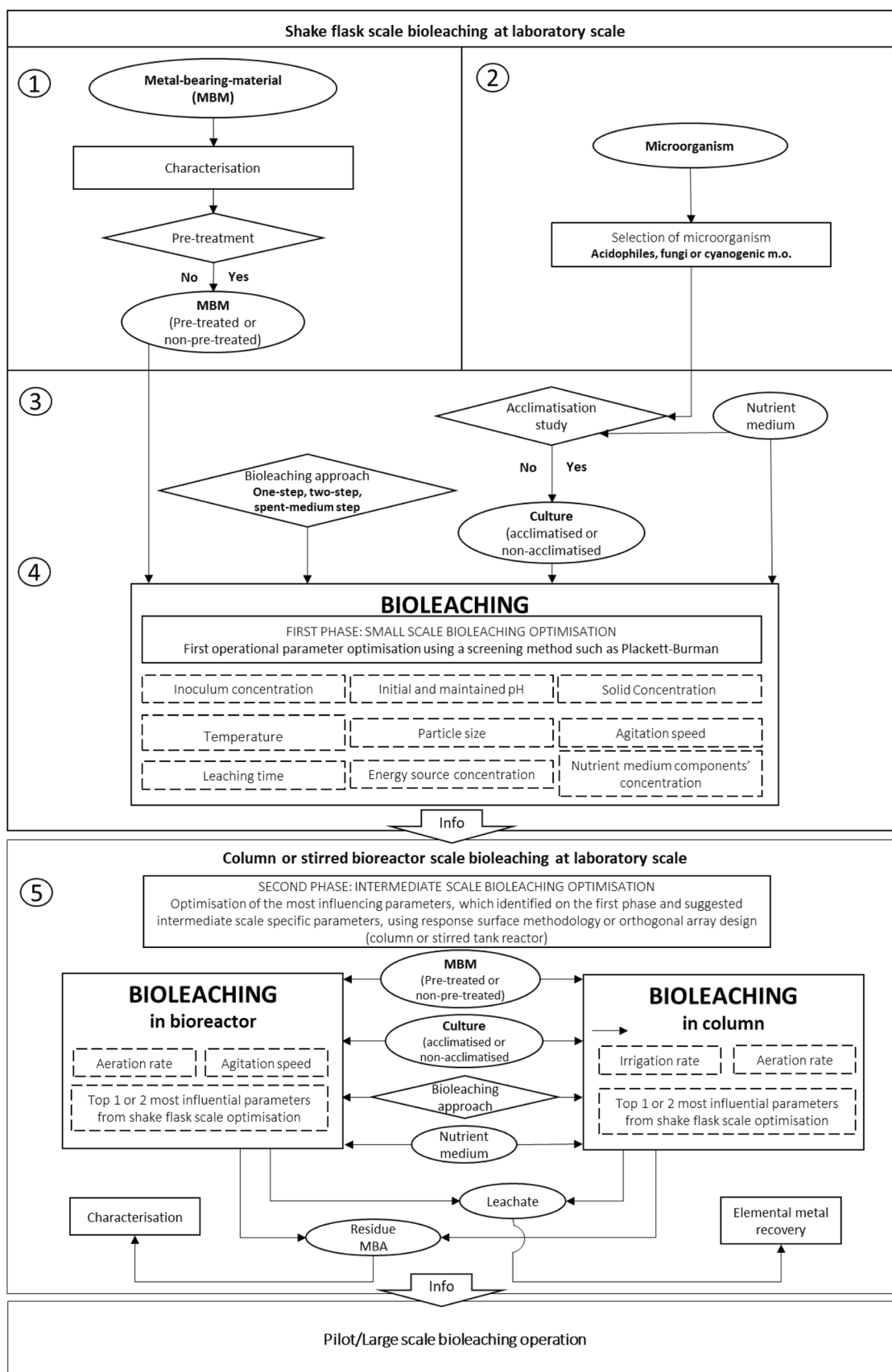


Fig. 4 Two-phase optimisation routes for bioleaching of metal-bearing materials. Flow chart covers characterisation and pre-treatment of metal-bearing materials, microorganism selection and acclimatisation followed by first phase of optimisation which is on shake flask scale. In the second phase of optimisation,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

during bioleaching of fluidized catalytic cracking catalysts 44% of the cost was due to the using of raw energy source for microorganism, 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).

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 optimization 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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Data availability All the data supporting this study are included within the article and/or supporting materials.

Declarations

Conflict of interest The authors declare the following conflict of interests: Stuart T. Wagland is an associate editor for Environmental Chemistry Letters.

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