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Current Opinion in Biotechnology

Review

Biotechnological solutions for critical mineral recovery from unconventional feedstocks

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Secure and sustainable metal recovery from unconventional feedstocks is needed to meet the mineral demands of energy, defense, and electronic technologies. Here, we highlight the potential to leverage nature's ability to extract and differentiate metal ions in biotechnologies that could become the next generation of mining and refining. We describe bulk and trace processes and then discuss the advances and opportunities of two key bioprocesses: microbially mediated solubilization of metal ions from solid matrices (termed 'bioleaching') and bio-based separation of solubilized ions via selective adsorption to proteins. Both biotechnologies have advantages such as reduced energy input for leaching low-grade feedstocks and reduced organic solvent demand for separating ions with similar physiochemical properties but require more development for industrial scale recovery from unconventional feedstocks. Innovation in biological science and engineering may bring timely solutions to key challenges toward recovering critical minerals from unconventional feedstocks.

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Current Opinion in Biotechnology 2025, **95**:103336

This review comes from a themed issue on **Energy Biotechnology**

Edited by **Gregg Beckham** and **Alissa Bleem**

Available online xxxx

https://doi.org/10.1016/j.copbio.2025.103336

An introduction to critical minerals

Modern energy, defense, and advanced electronic technologies rely heavily on metals across the periodic table, including lithium, alkaline earth metals, transition and post-transition metals, and rare earth elements (REEs) [1]. Demand for these materials is accelerating rapidly to match the pace of the efficient energy technology and data center expansions [2]. However, global primary reserves are geographically concentrated, leading to high import reliance in the United States (Figure 1). Accordingly, the US Department of Energy has designated metals and materials with high US supply chain risk and high importance to energy as 'critical' [1], though we note that the criticality designation is subject to time and perspective (both application and geographic).

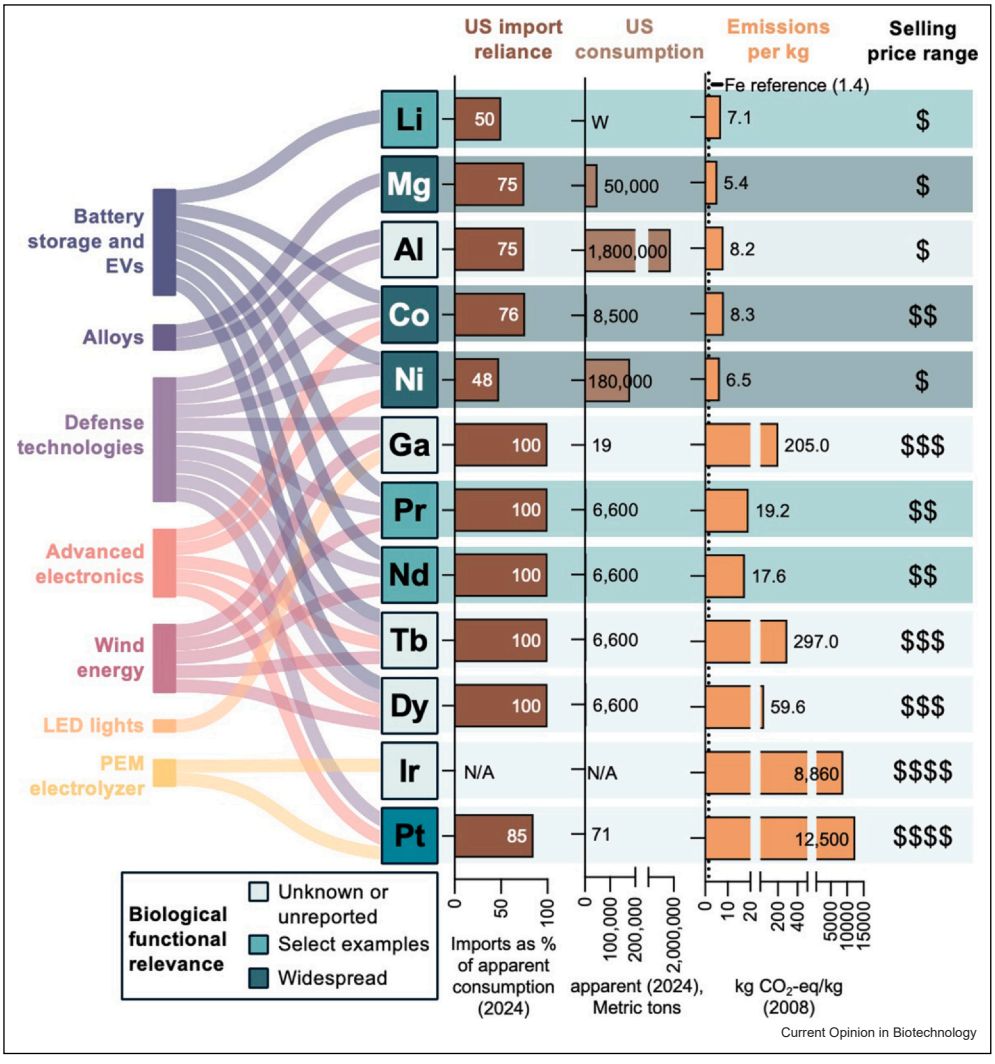
Mineral processing requires high energy input, generates copious greenhouse gas emissions, and can have substantial negative impact on societal equity [1]. Leaching metal ions from solid matrices requires harsh chemicals, and organic solvent-based separations for ions with similar physiochemical properties, such as the lanthanide series, have high chemical, capital, and waste disposal costs; this is especially true for energy transition materials, which have greater than fourfold higher cradle-to-gate emissions compared to iron [2] (Figure 1). Adding to the challenge, virgin mineral ('primary feedstock') mining operations typically recover a single metal (e.g. silver, gold) or a subset of metals (e.g. copper and cobalt, zinc and gallium, REE), often leaving low-level concentrations of the target metals and other valuable secondary minerals in the tailings (e.g. tellurium or arsenic from a copper mining operation).

Unconventional feedstocks have the advantages of local sourcing and avoiding the costs, wastes, and emissions associated with mining of primary feedstocks. Unconventional materials may include wastes streams and secondary feedstocks and are "any resource from a secondary byproduct of anthropogenic processes or in-situ geologic deposits that are distinctive from conventional critical mineral and material deposits" [3] (p. 1). Example secondary feedstocks include produced water, coal fly ash, and mine tailings; example waste feedstocks

include end-of-life batteries, magnets, and electronic waste. New technologies to recover metals from unconventional feedstocks with reduced energy inputs and favorable economics are urgently needed to address mounting environmental, energy security, and national defense challenges.

Here, we highlight the potential to leverage nature's ability to extract and differentiate metal ions in biotechnologies as the next generation of mining and refining [4]. First, we introduce metals in the context of biological systems. Second, we explore the progress and prospects for microbially mediated leaching (termed 'bioleaching') as an approach to extract metal ions from low-grade feedstocks with reduced energy inputs compared to traditional hydro- and pyro-metallurgy. Finally, we describe the advances and opportunities for protein-based metal separations as an adsorption-based separations approach with reduced chemical demand compared to conventional solvent extraction. We conclude that innovative research and development within and across the fields of natural product discovery, biolixiviant

Figure 1



Summary of key metals used in advanced technologies. Key metals used in selected energy, defense, and electronic technologies are indicated by flows (not proportional). Only metals designated as US Department of Energy Critical Materials in the 2023 assessment [1] are displayed in this figure. Elements are ordered by atomic number, and the color of each box indicates our qualitative assessment of the extent to which the element is known to be utilized in biological processes. US import reliance as a percentage of apparent consumption in 2024 [84], US apparent consumption in 2024 [84], and cradle-to-gate kg CO_2-eq emissions per kg material from 2008 data [85]. Li consumption was withheld (W) in the U.S.G.S. report 'to avoid disclosing company proprietary data' [84]. Ir import and consumption data were not available. Approximate selling prices $: $0–20/kg; $$: $21–200/kg; $$$: $201–2000/kg; $$ $$: $2001–200 000/kg. REE prices from MineralPrices.com; all others from U.S.G.S. [84]. EV, electric vehicle; LED, light-emitting diode; PEM, polymer electrolyte membrane.

design, bioinorganic chemistry, (metallo)protein engineering, microbiology, and metabolic engineering may bring timely solutions to the urgent need for sustainable and secure critical mineral supply chains.

An introduction to metallobiology in the context of critical mineral recovery

Metals play diverse roles in cellular homeostasis, with nearly half of the proteins (as determined by crystallization) requiring metal cofactors for structure or function [5]. Across the tree of life, organisms generally use between 10 and 14 different metals to catalyze reactions, support cellular structures, signal across membranes, and maintain redox balance [6]. Proteins mediate metallobiology, serving as scaffolds, transporters, and chaperones [6,7]. In the 1940s, Irving and Williams established that divalent transition metals have highly predictable relative stabilities regardless of the ligand, following $Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$ (coined the 'Irving-Williams series') [8]. Biological systems must work against this series to ensure proper metalation of lower stability ions through clever sensing, transport, and other homeostasis processes [6,7]. Capdevila *et al.* provide a comprehensive review of the structural and biochemical underpinning of specificity in metal-binding proteins and implications for cell homeostasis [9]. Notably, however, a mechanistic understanding of these processes is largely lacking for many metals of interest.

Interactions between proteins and metals can be roughly divided into bulk and trace categories, corresponding to the type of processes supported and the relative abundance of the metals involved [6]. Bulk processes, such as cell signaling, structural stabilization, and maintenance of membrane polarity, typically involve Group I and Group II metals (sodium, magnesium, potassium, and calcium), which are relatively earth abundant and not redox active [6]. While sodium and potassium are typically found in their free ion form, interacting primarily with transporter proteins and ion channels, magnesium and calcium bind to organic chelators (e.g. citric acid and other carboxylic acids, inositol phosphates) and protein ligands [6]. Proteins that bind magnesium and calcium are often promiscuous, binding other species of metal that have similar size and charge distribution [10]. Natural and engineered bulk metal-binding proteins have been shown to bind other metals, most notably REEs (Table 1). Bulk metal-binding proteins tend to be highly expressed and have high binding capacities [11], making them amenable to applications such as bioleaching and separation of metals from nonmetals in solution.

Trace metals are less earth abundant and critical to specific processes, such as catalysis and redox chemistry [6]. Many critical metals are used as trace elements in biological systems and have corresponding evolved binding proteins presented in Table 1. Trace metals in a given organism may interact with transporters, chaperones, and regulatory proteins in addition to their target enzyme [7]. Depending on their role in this cascade, proteins that bind trace metals tend to have higher specificity and affinity due to the relative scarcity of the target metals [7]. Even when metals do not serve as cofactors, microorganisms can interact with trace heavy metals through biosorption, bioprecipitation, sequestration, transport, and chelation reactions [6]. Finally, recent expansions of known REE-dependent enzymes and regulatory switches have challenged the view that REEs, especially the light REEs, are biologically inert [12], raising intriguing questions as to whether other metallobiochemistries exist and how widely distributed in biological systems these once-considered exotic metals may be. Separation of similar metals in solution, which requires high specificity, is a potential application for trace metal–binding proteins. For example, trace metal–binding proteins have been shown to be effective as chromatography ligands for REE separation [13].

In environments where redox-active metals are highly abundant, chemoautolithotrophic bacteria derive energy from redox-active metals, such as iron [14] and manganese [15], in a bulk process using periplasmic metal-interacting proteins. Other bacteria have been shown to reduce metals, such as iron, manganese, cobalt, chromium, palladium, gold, silver, vanadium, molybdenum, and mercury [16] using promiscuous periplasmic proteins. While binding is not an essential function of metabolic metal oxidation proteins, their ability to distinguish between metals using redox potential is useful in whole cell bioleaching of critical metals such as copper and uranium [14]. Metal reduction proteins are often promiscuous, enabling deployment in a variety of purification schemes to separate metals by solubility of different oxidation states [16].

Engineered proteins can have properties of bulk metal–binding proteins for trace metals and vice versa, making them a versatile choice for critical mineral processing. Table 1 contains a nonexhaustive list of proteins that have been engineered using various strategies to bind critical metals, including those that do not interact with any known natural proteins. Harnessing the power of metallobiology will enable facile separations of critical minerals in industrial contexts, from bulk bioleaching to polishing separations, discussed in the next two sections.

Advances and opportunities in bioleaching

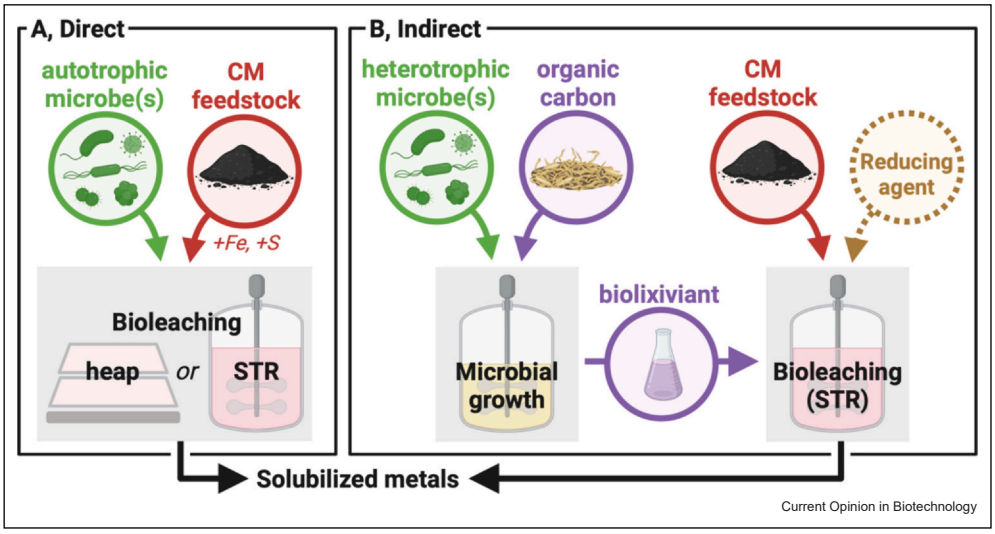
Bioleaching is the microbially mediated solubilization of metal ions from solid matrices [25]. Bioleaching can occur by different mechanisms, using different types of microorganisms, and via different process schemes. This section discusses the considerations of each mechanism and trophic class, as the pairing of mechanism,

Table 1

Selected (non-comprehensive) natural and engineered metal-protein interactions. Z, atomic number; HTP, high throughout.

Metal	Protein	Natural/engineered (engineering strategy)	Protein function	Selectivity	Metal role	Ref.
Lithium (Z = 3)	NhaA	Natural	Transporter	Na and Li	Transporter	[66]
Aluminum (Z = 13)	Transferrin	Natural	Binding	Nonspecific, accepts Al, In, evolved for Fe	Substrate	[67]
	Aluminum-binding peptide	Engineered (HTP screening/ directed evolution)	Binding	Unknown	Structural	[68]
Vanadium (Z = 23)	Table 5 of the cited review	Natural	Binding, enzymes, transport	Depends (highest in transport proteins)	Catalytic metal	[69]
Chromium (Z=24)	Chromium-binding peptide	Engineered (HTP screening/ directed evolution)	Binding	High	Structural	[70]
Cobalt (Z = 27)	Table 1 of the cited review	Natural	Enzymes	High	Catalytic metal	[71]
	NRAMP	Natural	Transporter	Transition metals	Transporter	[72]
Nickel	NikA	Natural	Binding, transport	High	Substrate	[73]
Zinc, copper, iridium, iron	Artificial metalloenzymes	Engineered (computational modeling, rational design)	Catalytic	Unknown	Catalytic	[74]
Gallium (Z = 31)	Transferrin	Natural	Binding	Nonspecific, accepts Al, In, evolved for Fe	Substrate	[67]
	Gallium-binding peptide	Engineered (HTP screening/ directed evolution)	Binding	Distinguishes Ga and As	Structural	[75]
Molybdenum (Z = 42)	Molybdenum-binding peptide	Engineered (HTP screening/ directed evolution)	Binding	Unknown	Structural	[76]
Tellurium (Z = 52)	Phycocyanin, allophycocyanin	Natural	Redox chemistry	Unknown	Catalytic metal	[77]
REEs (Z = 39, 57–71)	*M.ex*-Lanmodulin	Natural	Binding	High (low between REEs)	Structural	[55]
	Hans-LanM	Natural	Binding	High (distinguishes between heavy and light REEs)	Structural	[51]
	RTX	Natural	Binding	Low (binds Ca, all REEs, other metals)	Structural	[11]
	Adh	Natural	Enzyme	High	Catalytic metal	[78]
	LanD	Natural with rational design	Binding	High selectivity within the light REEs	Structural	[8]
	TFD-EE	Engineered (computational modeling)	Binding	Tight binding of lanthanides. Binds Eu-Yb with similar affinity. Appears to have some selectivity over Ce.	Structural	[79]
	Lanthanide-binding peptide	Engineered (HTP screening/ directed evolution)		REEs over calcium	Structural	[58]
	Lanthanide-binding coiled coils	Engineered (rational engineering)	Binding	REE selectivity changes based on location within coiled coil	Structural	[80]
Rhenium (Z = 75)	Mutant ModA/WtpA	Engineered (computational modeling)	Transport	Also binds Mo, W, Cr	Substrate	[63]
Platinum (Z = 78)	Unknown	Natural	Oxidoreductase	Low (reduces ferric, shown to reduce noble metals)	Substrate	[81]
	Platinum-binding peptide	Engineered (HTP screening/ directed evolution)	Binding	Unknown	Structural	[82]
Gold (Z = 79)	GBP-1	Engineered (HTP screening/ directed evolution)	Binding	Unknown	Structural	[83]

Figure 2



Simplified overview of example bioleaching inputs and general process flows. **(a)** In a direct autotrophic scheme, chemolithoautotrophic acidophiles utilize atmospheric CO_2 and Fe or S present in the critical material (CM)-containing feedstock as carbon and energy sources, respectively. Bioleaching reactions occur with living microbes and CM feedstocks in the same heap or STR. **(b)** In an indirect heterotrophic scheme, heterotrophs convert organic carbon inputs, such as lignocellulosic sugars, to a biolixiviant. In a second step, the biolixiviant is applied to the CM feedstock, often with the addition of a reducing agent. In both cases, pure or mixed cultures can be used, though culture stability in direct schemes can be challenged by microbes endogenous to the CM feedstock. STR, stirred tank reactor. This figure was made in part with Biorender.com.
Figure concept adapted from Alipanah et al. [27].

microorganism(s), and process scheme is paramount to maximize bioleaching efficiency with the fewest additional inputs and costs. The microbial mechanisms for bioleaching are reviewed extensively elsewhere [14,17,18] but can be broadly classified into acidolysis (proton donation from sulfuric, phosphoric, or organic acids), complexolysis or chelation (e.g. amino/organic acid, surfactant, or ligand metal coordination), and redoxolysis (e.g. metal oxidation/reduction) [17]. Notably, multiple classes can be at play in a given bioleaching reaction, and elucidating exact mechanisms in complex feedstocks are often active areas of research. For example, copper tailings rich in iron and sulfur invite the use of chemoautolithotrophic bioleaching (Figure 2). The microorganisms oxidize the sulfur in pyrite to sulfuric acid and the ferrous to ferric iron; the lower pH combined with ferric iron's electron donation to minerals promotes metal oxidation and solubilization. For nonsulfidic unconventional feedstocks, this process would require supplemental electron donors (e.g. pyrite, sulfur) that can be oxidized to acid. Alternatively, unconventional feedstocks, such as petroleum cracking catalysts, devoid of organics and iron, may be well suited for indirect heterotrophic bioleaching via complexolysis (Figure 2). Redoxolysis, an energy-conserving electron transfer process, requires that reduced iron and sulfur are added and has been shown successfully for autotrophic bioleaching of e-waste [19], in one example, recovering 79%, 39%, and 29% of the copper, nickel, and zinc, respectively, from printed circuit boards [20]. This approach would not work with secondary waste that has been oxidized (e.g. municipal and coal incineration ash); therefore, the application of redoxolysis for bioleaching metals will depend on the reductant and the oxidation state of the target metals.

Autotrophic bioleaching is typically conducted with chemolithoautotrophic acidophiles, which use iron and/or sulfur as an energy source and CO_2 as a carbon source (Figure 2a), including the classic biomining bacteria *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. Autotrophic bioleaching has been commercialized for copper, nickel, zinc, gold, and cobalt from iron- and sulfur-ore, including chalcopyrite, sphalerite, and laterite [21]. For example, stirred tank reactors (STR)-based bioleaching is practiced by Metso-Outotec for gold extraction in bioreactors as large as 1500 cubic meters [22]. These processes have advantages at small or remote mines to reduce capital and operational expenditures, as well as at larger mines with ore metal concentrations below the cutoff grade for conventional pyro-electrochemical leaching [21,23]. Conversely, the bioleaching of metals (e.g. REE, lithium) from nonsulfidic end-of-life and other secondary wastes (e.g. magnets, batteries) has not been industrialized but is gaining traction due to the increasing secondary feedstock availability and

potential to offset imports via reclamation from domestic wastes [17,18,24].

Heterotrophic bioleaching requires the addition of organics as the carbon and energy source using the microbe to generate a leaching agent, termed a biolixiviant (Figure 2b). The biolixiviant is usually an organic acid or mixture thereof; some organic acids (e.g. gluconic, citric) and bioligands (e.g. metallophores) contain carboxy or carbonyl structures that can also chelate or complex with target metals for extraction from the solid [25]. Heterotrophic acidophiles that can use organic carbon waste (e.g. agriculture, food, or municipal solid waste) for growth and biolixiviant production are ideally suited for feedstocks with co-located or easily available organic waste, potentially lowering environmental impact from reduced waste disposal and increasing profit [26]. For example, Alipanah et al. determined that organic acid-based biolixiviant from *Gluconobacter oxydans* grown on corn stover waste could recover 57–100% of the metals from spent lithium-ion batteries, generating a net present value of $110M with eight times lower CO_2 production [27]. Bioleaching with mixed autotrophic with heterotrophic communities is an emerging approach [28] that has been shown to improve leaching efficiency of some metals likely due to synergy of leaching mechanisms [18] or heterotrophic consumption of organics that might be inhibitory to acidophilic autotrophs [29]. However, more research is needed to better determine the efficacy of mixed autotrophic and heterotrophic bioleaching.

There has been increasing interest in the use of genetic engineering to improve bioleaching. *A. ferrooxidans*, a key member of the consortia found in copper bioleaching environments, has been genetically modified to alter features such as mineral oxidation, quorum sensing, and oxidative stress responses [30]. For example, Jung et al. used CRISPR interference to knock down genes involved in the electron transport chain, leading to a > 3-fold increase in chalcopyrite oxidation [31]. Overexpression of the REE-chelating metallophore methylolanthanin [32] in the phyllosphere-dwelling *Methylobacterium extorquens* increased REE bioaccumulation from magnet swarf by over 20-fold, whereas deletion of a gene involved in the depolymerization of polyphosphate granules, where REEs are stored, increased bioaccumulation by 50-fold [33]. While these studies highlight the potential of genetic engineering to overcome bioleaching bottlenecks, additional strategies are needed to ensure the genetic stability and microbial longevity of these engineered strains in non-sterile biomining environments.

Recent research has highlighted the potential to improve bioleaching via modulating biolixiviant production rates and composition. For example, Aston et al. used metabolic modeling to optimize biolixiviant production in *G. oxydans* via modulated carbon:nitrogen substrate ratios, resulting in decreased cost and a 36% increase in bioleaching efficiency [34]. Schmitz et al. leveraged a genomic mutant library to engineer *G. oxydans* for increased biolixiviant production, improving REE bioleaching to 610 μg REE g^{-1} allanite ore [35]. Finally, Li et al. characterized a microbial metabolome and determined that, in addition to organic acids, lipids and benzenoid molecules improved bioleaching [36]. Future works using metabolomics, genome-scale modeling, and metabolic engineering are likely to improve mechanistic understanding and pave the way to increased bioleaching efficiency, reduced costs, and minimized substrate usage and waste generation [37].

Regardless of the mechanism or microbe, leveraging technoeconomic analysis to guide bioprocess development will be crucial for bioleaching processes to gain full industrial acceptance. This involves optimizing parameters, including biolixiviant concentration, pH and oxidation/reduction potential, incubation aeration, temperature and duration, metal mineralogy or composition, and particle size and density [17], to ensure the process is cost-effective and efficient [38]. Design of experiment and thermodynamic modeling [24,39] are expedient tools toward optimization. Organic acid concentrations and pulp density have been found to be primary factors influencing economic outcomes [39,40], with temperature becoming an important secondary factor for metal solubility [39,40].

Advances and opportunities in bio-based separations

The goal of bio-based separation is to enable the selective capture of a metal ion or group of metal ions of interest from a source material (typically in an aqueous form) through a reversible-binding mechanism. Here, we place significant emphasis on selectivity, given the complex geochemistry of waste solutions and the similar physicochemistry properties among many of the metal ion constituents; however, favorable binding and release kinetics are also desirable for fast and efficient separation process development. Separation can potentially be accomplished with a wide range of biomolecules, although most examples to date have either employed cell surface functional groups (composed of lipids, polysaccharides, and proteins) or proteins [41,42]. Metalloproteins are attractive candidates for this task given their potential for high affinity and selectivity, kinetic lability, and multiple handles for tuning metal binding selectivity, including through interactions distant from the coordination site. These attractive properties combined with the engineerability of proteins, their amenability to high-throughput assays (although not yet realized for metal binding selectivity), and rapid advances in artificial

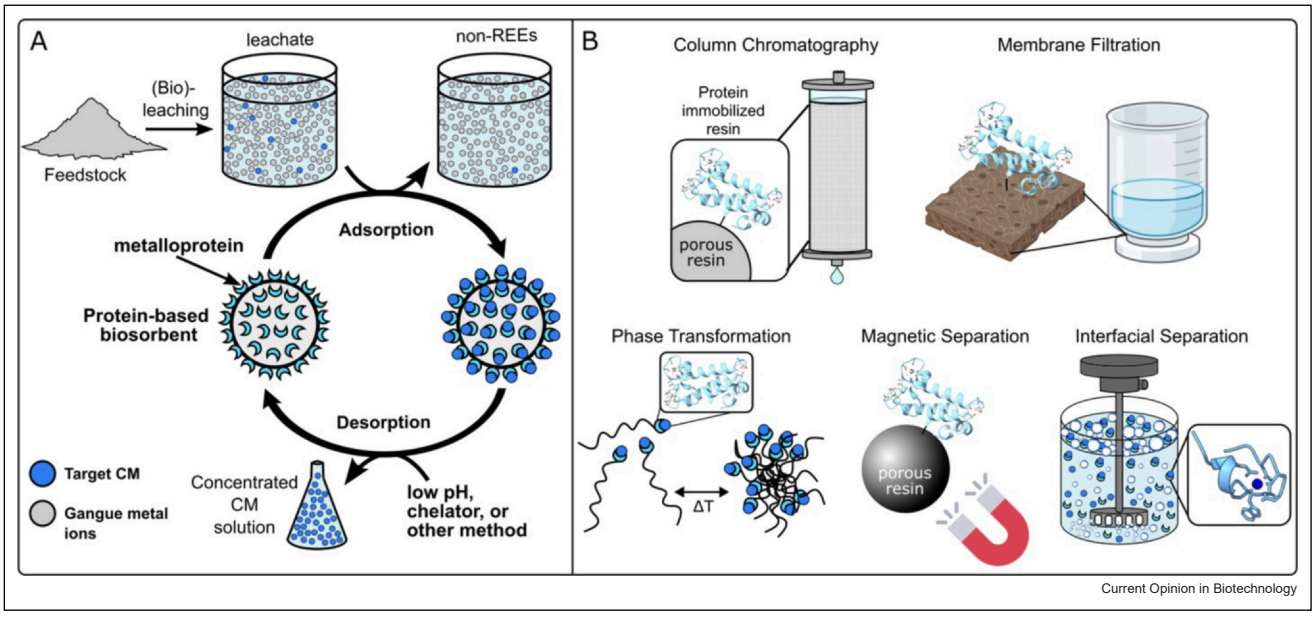
intelligence/machine learning (AI/ML)-based protein design provide a potentially powerful platform for metal capture applications. For these reasons, we focus the bulk of this section on key developments and challenges in protein-based CM recovery.

When starting from a solid feedstock, a (bio)-leaching step is required prior to protein-based metal ion separation. Leachate preprocessing steps may also be required depending on the feedstock and protein used in separations (Figure 3a). For example, since the pH of leachates is often acidic following leaching, a pH adjustment step (e.g. NaOH addition) is necessary when employing pH-sensitive coordinating functional groups, such as carboxylic acids [24]. The necessity of additional preprocessing steps (e.g. impurity removal) depends on the selectivity of the metalloprotein. The high selectivity achievable with proteins represents an advantage over traditional approaches as it can increase the effective capacity, the fraction of the metal binding capacity applied toward a metal ion of interest, and reduce the number of steps required to achieve a separation effect.

Metal ion separations are typically achieved through attachment of the metalloprotein to an abiotic or biotic scaffold that facilitates separation from the bulk solution. A common approach is immobilization on porous resin for flow-through metal ion capture using column chromatography (Figure 3a) [13,43]. Chromatography is particularly effective for separation of similar ions given that separation factors can be efficiently propagated over the theoretical plates of the column (assuming sufficient exchange kinetics). Alternatives to porous resin include membranes [44], magnetic beads [45], cell surfaces [46], hydrogels [47], phase transitioning proteins [48], or even forgoing a scaffold altogether through an interfacial separation mechanism [49] or through intracellular expression in a metal tolerant host, avoiding the cost of protein purification [50] (Figure 3b). For each of these options, there is a need to better understand the potential tradeoffs in terms of process cost, scalability, and robustness. Another important consideration is the metal ion selectivity of the scaffold, which can be a limitation of cell surface approaches, given the abundance of charged cell surface functional groups that adsorb metal ions.

Desorption of bound metal ions is commonly mediated by lowering the pH to protonate coordinating residues or by using metal ion chelators. This can be performed in a nonselective manner, where all bound metal ions are eluted simultaneously, with selectivity driven purely by the adsorption step [13,45,48]. Alternatively, incremental pH or chelator concentration steps can be used to sequentially desorb bound ions [13,43,51]. Here, proteins

Figure 3



Overview of a protein-based bio-hydrometallurgical process for CM extraction. **(a)** A solid feedstock is subjected to (bio)-leaching, and the resulting pregnant leach solution is conditioned (pH adjustment, impurity removal) prior to exposure to a protein-based biosorbent for selective metal ion capture. A desorption step is used to generate a purified CM concentrate and to regenerate the biosorbent for subsequent extraction cycles. **(b)** Different strategies employed in the literature for metalloprotein immobilization and application in CM extraction. This figure was made in-part with Biorender.com.

can be strategically paired with chelators having distinct metal binding selectivity to overcome limitations of the protein-binding multiple metals or to magnify the separation effect through a tug-of-war-like process [43]. When choosing a desorbent, it is important to also consider the impact on protein stability, chelator cost and recyclability, and the technical and economic implications for downstream metal ion processing. Alternative, chemical-free methods of desorption (e.g. light or temperature) may be possible and would be interesting to explore. In addition, the overlap in chelators used in desorption and bioleaching provides a natural point of integration between these two bioextraction processes.

The majority of metal ion recovery studies have focused on REEs due to the availability of exemplary REE-binding proteins (reviewed in Refs. [12,42,52,53]). A well-studied example is the protein lanmodulin, which is part of a REE uptake pathway in methylotrophic bacteria [54,55]. Lanmodulin binds light REEs tightly and selectively, enabling grouped recovery of REEs from a range of secondary feedstocks [13,43,45,48] and the separation of certain REEs (Dy^{3+}, Y^{3+}, Sc^{3+} [13,43]). While most work has focused on lanmodulin from *Methylobacterium extorquens*, the discovery of a variant from *Hansschlegelia quercus* (*Hans*-LanM [51]) with higher light versus heavy REE selectivity highlights the value of exploring this diverse family of proteins and trafficking pathways. Indeed, recent structural and biochemical characterization of the small periplasmic protein, LanD, enabled a selective dimerization process for the enrichment of high-value Nd^{3+} and Pr^{3+} over low-value La^{3+} [56]. Calcium-binding proteins, which bind REEs (in many cases tighter than Ca^{2+} [57]), represent another promising chassis. Lanthanide-binding tags, short peptides derived from calmodulin and engineered for tighter REE binding through combinatorial engineering [58], were among the earliest proteins used in REE recovery (Table 1). More recently, the RTX domain of the adenylate cyclase protein from *Bordetella pertussis* was found to bind Nd^{3+} and Dy^{3+} at an astounding pH < 1 from a simulated magnet leachate [11], which could be advantageous for REE recovery from acid leachates. These proteins provide a promising platform for engineering to further enhance the intra-REE selectivity, which is necessary for efficient separation of adjacent lanthanides.

For many metal ions, adequate solutions do not exist in nature or have yet to be discovered, and selectivity must be engineered. For example, proteins that have evolved to bind the battery metal ions cobalt, nickel, and manganese typically operate under conditions where the intracellular pool of free copper/zinc is tightly controlled [59], a luxury not afforded in industrial metal ion extraction applications. Recent work by Tezcan [60] and Cotruvo [61] has identified strategies for engineering proteins that overcome the Irving-Williams series by selectively binding Co^{2+}/Ni^{2+} or Mn^{2+} over Cu^{2+}, providing a potential path for selective recovery of battery metal ions. A foundational example for targeting non-biological metals (i.e. those without known biological roles) is the super uranyl-binding protein, where coordination site mining and structure-guided metal-binding optimization yielded a protein with sufficient affinity and selectivity for uranium recovery from seawater [62]. More recently, a rhenium-binding protein was developed through computational redesign of the active site of the molybdenum/tungsten transport protein to favor the perrhenate oxyanion [63]. These examples highlight the design flexibility of proteins for engineering selective metal interactions.

The rapid expansion of AI/ML-based design tools is likely to accelerate the pace of metalloprotein engineering and design. While there have been initial successes (Table 1), this remains a challenging design task, given the sub-angstrom level differences in ionic radii between relevant metal ions and the importance of kinetic lability for application; merely designing a tight metal-binding protein is not sufficient. A major bottleneck for using selectivity as an objective function is the laborious nature of current experimental approaches to quantify metal-binding selectivity, making it difficult to generate the large and diverse data sets needed to understand sequence–structure–selectivity relationships. High-throughput assays for screening metal-binding selectivity of proteins are needed. Of additional interest would be clever approaches to link metal binding affinity and/or selectivity to cell viability for use in selections or adaptive laboratory evolution campaigns. Finally, mixed quantum-classical simulations (e.g. quantum mechanics/molecular mechanisms/molecular dynamics) may prove useful in reducing experimental burden in addition to refining metalloprotein designs.

While the immobilization of proteins for metal separations dates to the 1990s [64], biomolecular-based metal ion capture has yet to be performed at an industrial scale. Significant advances in metalloprotein discovery, characterization, and design, combined with advances in low-cost production of recombinant proteins at scale [65], are poised to accelerate industrial adoption, with several companies working toward commercialization of protein-based metal ion extraction technologies. However, important work remains to demonstrate scalability, process economics that rival current chemical approaches, durability under industrial conditions, and the transition from batch to continuous processes (reviewed in Ref. [52]).

Conclusion and outlook

Biohydrometallurgical metal extraction and separation have inherent advantages compared to the pyro- and hydro-technological incumbents but are not yet fully

leveraged. Many fundamental questions related to microbe–metal interactions remain unexplored, including the breadth and prevalence of understudied metals in biological systems. The energy biotechnology community holds relevant expertise and toolsets to answer many of these questions and bring timely solutions to the urgent need for secure and sustainable metal supply chains. Improved connections across the scientific fields of study relevant to these endeavors, including the early evaluation and/or development of integrated processes informed by technoeconomic and lifecycle analyses, will be beneficial, as has been true in the advancement of other biotechnologies.

CRediT authorship contribution statement

Hannah S. Zurier: Writing, reviewing, and editing. **Scott Banta:** Conceptualization, writing, reviewing, and editing. **Dan Mcfarland Park:** Conceptualization, writing, reviewing, and editing. **David W. Reed:** Conceptualization, writing, reviewing, and editing. **Allison Z. Werner:** Conceptualization, writing, reviewing, and editing.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

This work was authored in part by the National Renewable Energy Laboratory (NREL), operated by Alliance for Sustainable Energy, LLC, for the US Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. A.Z.W. was supported by the Laboratory Directed Research and Development Program at NREL. Contributions from DMP were performed under the auspices of the DOE by Lawrence Livermore National Laboratory under Contract DEAC52-07NA27344 (LLNL-JRNL-2004310) and funded by the Critical Materials Innovation Hub funded by the DOE, Office of Energy Efficiency and Renewable Energy, Advanced Materials & Manufacturing Technologies Office. HSZ, SB, and DMP acknowledge funding by the DARPA Environmental Microbes as a Bioengineering Resource program (contracts FA8650-22-C-7213 and DE-AC52-07NA27344). Distribution Statement A. Approved for public release: distribution is unlimited. The views, opinions, and/or findings expressed are those of the authors and should not be interpreted as representing the official views or policies of the Department of Defense or the US Government. DWR work is conducted under DOE Idaho Operations Office Contract DE-AC07-05ID14517, with partial funding coming from the Critical Materials Innovation Hub.

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