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Review

Harnessing the potential of the microbial sulfur cycle for environmental biotechnology

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The sulfur cycle is a complex biogeochemical cycle characterized by the high variability in the oxidation states of sulfur. While sulfur is essential for life processes, certain sulfur compounds, such as hydrogen sulfide, are toxic to all life forms. Micro-organisms facilitate the sulfur cycle, playing a prominent role even in extreme environments, such as soda lakes, acid mine drainage sites, hot springs, and other harsh habitats. The activity of these micro-organisms presents unique opportunities for mitigating sulfur-based pollution and enhancing the recovery of sulfur and metals. This review highlights the application of sulfur-oxidizing and -reducing micro-organisms in environmental biotechnology through three illustrative examples. Additionally, it discusses the challenges, recent trends, and prospects associated with these applications.

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Introduction

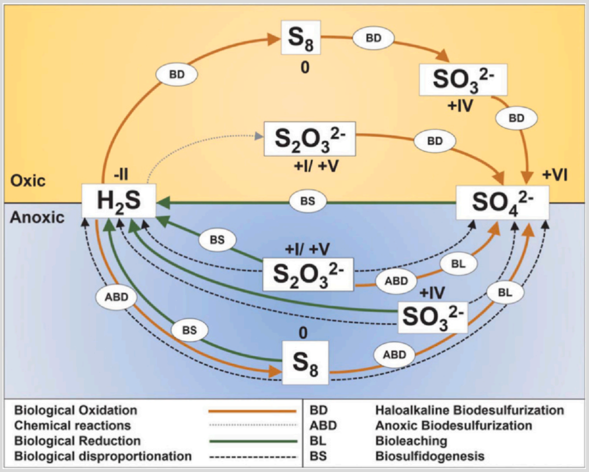
The sulfur cycle involves a series of oxidized and reduced sulfur states that can occur through chemical and biological processes. The sulfur atom cycles between the oxidation states +6 in sulfate and -2 in sulfide (Box 1). Sulfur molecules act as electron donors for aerobic respiration, electron acceptors for anaerobic respiration and anaerobic light-dependent CO_2 reduction, and fermentation substrates [1]. In natural environments, sulfate-reducing micro-organisms (SRM) contribute 29% of organic matter sequestration and play an important role in the biogeochemical cycling of sulfur [2]. SRM use sulfate as an electron acceptor in utilizing both organic and inorganic compounds [3].

Approximately 80–90% of sulfide formed can be re-oxidized to various intermediates or completely oxidized to sulfate by sulfide- and sulfur-oxidizing micro-organisms (SOM). The remaining sulfide is deposited as iron complexes (e.g. FeS, FeS_2) [2]. The intermediates, such as elemental sulfur, sulfite, and thiosulfate, can undergo further oxidation to sulfate by SOM, reduction to sulfide by thiosulfate reducing, sulfur-reducing and sulfite-reducing micro-organisms or disproportionation to sulfide and sulfate by sulfur-disproportionating micro-organisms (SPM). Although organosulfur compounds are prevalent in natural environments, this review focuses on inorganic sulfur compounds.

In man-made systems, such as wastewater treatment plants processing industrial waters, the mineralization or anaerobic digestion of organic compounds by micro-organisms is an important process. For industries that utilize sulfate or sulfuric acid, the water treatment leads to sulfide formation via the activity of SRM. Sulfide is toxic and highly corrosive because it and other reduced sulfur compounds, such as bisulfide and persulfide, react with metals, forming metal sulfides, consequently leading to corrosion [4•].

Sulfur-related micro-organisms have been successfully employed to mitigate industrial and environmental issues [5•]. Specific micro-organisms have been isolated from diverse environments, physiologically characterized, and further studied to understand the molecular mechanisms involved

Box 1 The sulfur cycle and key reactions of biotic and abiotic conversions in (I) biodesulfurization (BD), (II) bioleaching (BL), and (III) biosulfidogenesis-based (BS-based) metal removal processes.



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The figure illustrates the microbial sulfur cycle with examples of its applications in environmental biotechnology. Under neutral to alkaline conditions, H_2S is present in the soluble form HS^- that can be oxidized by SOMs to form sulfur or sulfate depending on the abundance of the electron acceptor O_2 under oxic (BD) and NO_3^- under anoxic conditions (ABD). In BD, the H_2S gas is converted to elemental sulfur by SOM in a controlled setting. Under acidic conditions, the oxidized sulfur compounds are reduced by SRM forming H_2S, known as BS. Subsequently, the H_2S reacts with metal compounds, forming metal sulfides, and this conversion is used to extract valuable metals from contaminated waters. In a reverse conversion, the metal sulfides are oxidized to metal sulfate by SOM under acidic and oxic conditions. This process is applied in BL, where metal sulfides from the ores are dissolved as metal sulfates and thereby extracted. The chemical reactions of the conversions are shown below.

I) General reactions involved in hydrogen sulfide oxidation in a BD process

Biological oxidation of HS^- under (micro)oxic conditions

$2\ HS^- + O_2 \rightarrow 2\ S° + 2\ OH^-$

$HS^- + 2\ O_2 \rightarrow SO_4^{2-} + H^+$

Chemical oxidation of HS^- under (micro)oxic conditions

$2\ HS^- + 2\ O_2 \rightarrow S_2O_3^{2-} + H_2O$

Thiosulfate disproportionation

$S_2O_3^{2-} + OH^- \rightarrow SO_4^{2-} + HS^-$

Biological oxidation of HS^- under anoxic conditions

$5\ HS^- + 2\ NO_3^- + 7\ H^+ \rightarrow 5\ S° + N_2 + 6\ H_2O$

$3\ HS^- + 2\ NO_2^- + 5\ H^+ \rightarrow 3\ S° + N_2 + 4\ H_2O$

$5\ HS^- + 8\ NO_3^- + 3\ H^+ \rightarrow 5\ SO_4^{2-} + 4\ N_2 + 4\ H_2O$

$3\ HS^- + 8\ NO_2^- + 5\ H^+ \rightarrow 3\ SO_4^{2-} + 4\ N_2 + 4\ H_2O$

II) General reactions involved in BL of metals (M) from metal sulfide ores

BL lixiviant Fe^{3+} formation by iron-oxidizing bacteria

$2\ Fe^{2+} + 2\ H^+ + 1/2\ O_2 \rightarrow 2\ Fe^{3+} + H_2O$

Indirect oxidation of metal sulfide with Fe^{3+}

Polysulfide pathway

$MS + Fe^{3+} + H^+ \rightarrow M^{2+} + 1/2\ H_2S_n + Fe^{2+}$ (where $n \geq 2$)

$1/2\ H_2S_n + Fe^{3+} \rightarrow 1/8\ S_8 + Fe^{2+} + H^+$ (where $n \geq 2$)

$1/8\ S_8 + 3/2\ O_2 + H_2O \rightarrow SO_4^{2-} + 2\ H^+$

Thiosulfate pathway

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

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

III) General reactions involved in BS and metal (M) precipitation

BS with H_2

$SO_4^{2-} + 4\ H_2 + H^+ \rightarrow HS^- + 4\ H_2O$

$S° + H_2 \rightarrow HS^- + H^+$

Metal sulfide formation

$M^{2+} + HS^- \rightarrow MS + H^+$

BS sulfur disproportionation

$4\ S° + 4\ H_2O \rightarrow SO_4^{2-} + 3\ HS^- + 5\ H^+$

in sulfur conversions [6,7]. Additionally, applying state-of-the-art omics techniques has identified novel taxa of sulfate reducers and sulfide oxidizers from different environments and revealed insights into their genetic diversity, novel functional genes, and metabolic pathways [8–12•].

In this review, we present three examples of sulfur bacteria's application in environmental biotechnology (Box 1) and discuss the challenges and prospects associated with these applications.

Example 1: biodesulfurization of sulfide-containing gas streams

Haloalkaline biodesulfurization (BD) is an eco-friendly, cost-effective process that converts toxic sulfide gas to elemental sulfur using haloalkaliphilic SOM under microoxic conditions [8,13]. The microoxic conditions inhibit the complete oxidation of sulfide to sulfate (Box 1), which is energetically preferred by chemolithoautotrophic SOMs under ambient oxygen supply and control the formation of sulfur (Box 1). Some notable SOMs include obligated chemolithoautotrophs such as *Thiomicrospira*, *Thioalkalivibrio*, *Thioalkalibacter*, *Thiohalophilus*, and facultative autotrophs such as *Alkalilimnicola*, *Alkalispirillum* [14,15].

The generated sulfur is a nontoxic compound with several downstream applications [16]. Commercially known as 'Thiopaq', the process removes > 99% of sulfide from sour gas streams [17]. The process involves (1) an absorber column for absorbing sulfide gas in a haloalkaline solution, (2) a bioreactor for oxidizing sulfide to elemental sulfur, and (3) a sulfur recovery section for retrieving the elemental sulfur particles from the process solution [17]. The process converts ~90% of sulfide to sulfur and the rest to sulfate and thiosulfate. Unlike sulfur, these latter compounds are undesirable as they decrease the alkalinity, increasing caustic consumption and bleed water formation.

A recent innovation, 'Thiopaq Ultra', is being studied at the pilot scale ([18]), and it incorporates an additional anoxic bioreactor between the absorber and oxic bioreactor (Figure 1, [18]). The new configuration has a higher selectivity for sulfur formation (~97%) and depicts a ~90% reduction in caustic, make-up water consumption and bleed formation [13,18]. This leads to a significant decrease in the operational costs of the BD process. In addition, the operational stability is enhanced due to a better ability for sulfur to settle, resulting in easier separation and, consequently, a better sulfur recovery [18,19••].

Molecular biological analyses have elucidated the presence and activity of a shared microbial community in both full- and pilot-scale Thiopaq Ultra BD systems [8,20••]. *Thioalkalivibrio sulfidiphilus* was the most dominant sulfide-oxidizing bacterium (SOB) in the full-scale reactors [8]. The pilot-scale Thiopaq Ultra, however, was dominated by *Tv. sulfidiphilus* (5–54%) and *Alkalilimnicola ehrlichii* (8–63%) [20••]. While *Thioalkalivibrio* is a well-known SOB, the prevalence of *Alkalilimnicola* is noteworthy as it has not been previously associated with sulfide oxidation under (micro)oxic conditions.

Moreover, SOMs in haloalkaline BD systems were electroactive in a bioelectrochemical (BES)-based BD system [21,22]. As such, they generated current while converting sulfide to sulfur (44%) and sulfate (56%). A combined BES-haloalkaline BD system could significantly enhance energy efficiency by eliminating the need for aeration of the oxic part while recovering energy from sulfide oxidation. Furthermore, the sulfur uptake by bacteria can also decrease the risk of electrode passivation due to sulfur deposition [22]. To date, BES systems for resource recovery from wastewater are under investigation to be used on a larger scale [23].

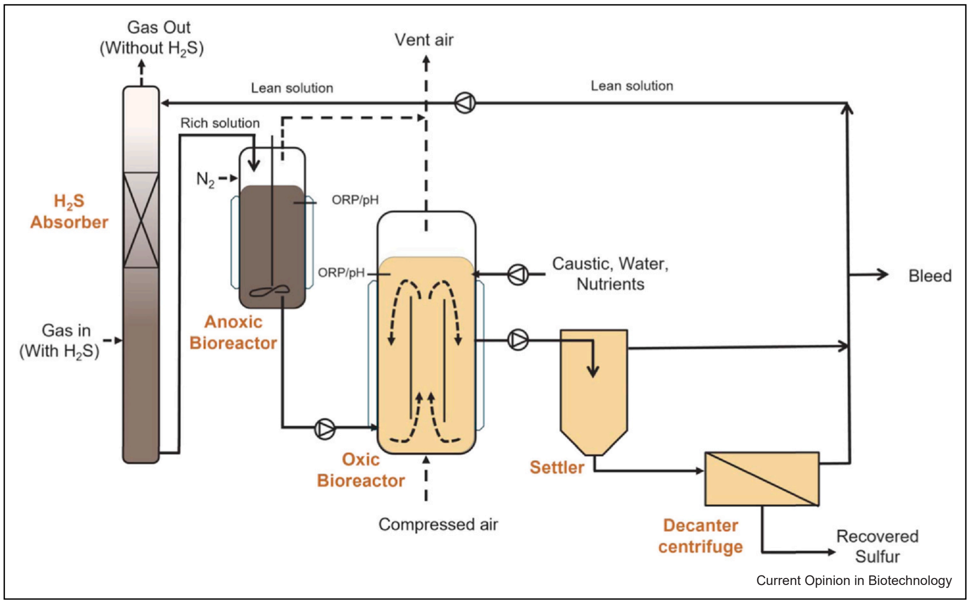
Significant progress has been made in the field of anoxic BD, which employs nitrate or nitrite as electron acceptors (as oxygen substitutes) to oxidize sulfide to sulfur or sulfate. One such advancement involves the replacement of traditional biotrickling filters (BTF) with continuous stirred tank bioreactors (CST-BRs) [24••]. In BTFs, the sulfur accumulates on the filters, thereby reducing their effectiveness. On the other hand, CST-BRs have shown promise, achieving a removal efficiency of 98.6% and recovering 88% of elemental sulfur [25]. A drawback is the increased operational costs due to the use of commercial chemicals as electron acceptors. However, this issue can be mitigated by using effluents from nitrification processes. For instance, in one anoxic BD system, ammonium-rich water is first nitrified to nitrate/nitrite, which is then used as an electron acceptor to oxidize sulfide into N_2 and elemental sulfur [26].

The composition of microbial communities in anoxic BD systems is largely unexplored. Current research primarily focuses on process development and stability, with only a handful of studies delving into the biodiversity of these systems. Dominant SOMs are affiliated to the genera *Thiobacillus*, *Sedimenticola*, *Sulfurimonas*, and *Rhodanobacter* [24••]. A more comprehensive understanding of the presence and activity of the microbial communities in the anoxic BD systems is needed, warranting further research in this area.

Example 2: biomining — mining of metals with microbes

Traditional mining, which generates a significant amount of waste in tailings (fine ground rock particles formed after ore processing), poses a severe challenge to land use and is energy intensive [27]. Biomining, on the

Figure 1



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Schematic representation of the improved haloalkaline BD process (Thiopaq Ultra). Dashed lines indicate the flow of gas streams; solid lines indicate liquid circulation.

other hand, offers a less harmful alternative. This method involves the direct use of microbes or microbially produced lixiviants (liquid media) to leach or extract metals, such as iron, copper, zinc, gold, and uranium, reducing waste generation [28••].

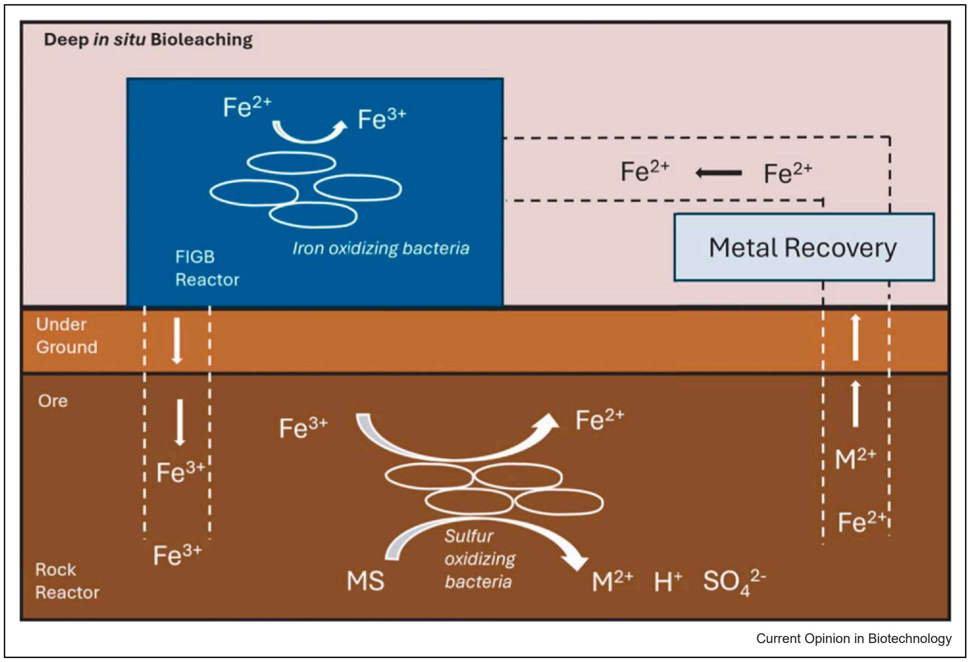
Bioleaching (BL) is primarily employed to extract metals from metal sulfides, utilizing chemolithotrophic iron/sulfur (Fe/S)-oxidizing microbes such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus caldus*, *Leptospirillum*, *Sulfolobus*, and *Ferroplasma* [29•–31]. These acidophilic micro-organisms thrive in highly acidic ($pH < 3$) and metal-rich environments, and by oxidizing ferrous iron (Fe^{2+}), they produce a BL lixiviant containing high concentrations of ferric iron (Fe^{3+}). The Fe^{3+} is involved in the conversion of sulfide to sulfate, not merely mediating but actively participating in the reaction via the formation of either thiosulfate (for acid-insoluble minerals) or polysulfides (for acid-soluble minerals) [29•,30,32]. The key reactions involved are shown in Box 1.

Despite its energy efficiency and a 70% lower carbon footprint than traditional methods, biomining may not be the most cost-effective option for high-quality ores, given the rate of BL is very slow. However, it presents an appealing approach for extracting metals from lower-grade ores, residual ores postmining (tailings), historical tailings, and electronic waste [27,28••].

Recently, *in situ* BL has been tested at a pilot scale for copper extraction from chalcopyrite ($CuFeS_2$) ores located more than 1 km deep via a process known as Deep *In Situ* Bioleaching (DISB; Figure 2). In this concept, an Fe^{3+}-rich bio-lixiviant is generated above ground in ferric iron–generating bioreactors (FIGB) and pumped into the underground chalcopyrite deposits via drilled channels. Acting as an electron acceptor, Fe^{3+} mediates the biotic/abiotic dissolution of Cu^{2+} in the acidic lixiviant, which is then pumped up from the other side of the ore and collected above ground in a recovery unit. Pure copper is extracted using solvent extraction and/or electrowinning, while the metal-depleted stream is recirculated back to the FIGB [28,33•]. In one DISB study, the FIGB was dominated by bacteria such as *Thiobacillus*, *Thiovirga*, *Alishewanella*, and a native fungal species [33•]. Even though these bacteria are not typically described as acidophiles, the overall consortium could maintain the desirable acidic conditions for leaching. Furthermore, iron-oxidizing bacteria might have been protected within biofilms and, therefore, not present in the sampled stream [33•].

BL has also been applied and studied in CST-BRs and heap-leaching reactors, extracting metals from tailings [34,35]. However, the extraction of chalcopyrite faces challenges, such as slow kinetics, passivation of ore due to the formation of a layer of sulfur compounds or decreased dissolution because of its inherent n-type electronic structure [36–39]. Ríos et al. [10•] proposed using moderate thermophilic acidophiles and controlling the

Figure 2



Schematic representation of DISB of metal sulfides using ferric iron ($Fe3^+$)-generating bioreactors (FIGB) [28,72]. This is an example of indirect BL, which occurs differently for acid-soluble and nonacid-soluble metal sulfides. The sulfur conversions can occur both chemically and mediated by bacteria.

oxidation–reduction potential to improve BL. Their study revealed that consortia comprising *Sulfobacillus thermotolerans*, *Sulfobacillus acidophilus*, and *Ferroplasma acidiphilum* could maintain a desired redox potential below 680 mV (SHE), yielding maximum copper recovery. The study also highlighted synergistic interactions in consortia with weak iron oxidizers and *At. caldus* yielding more Fe^{3+}, thereby increasing the redox potential above 680 mV (SHE) and forming an undesired sulfur layer. Therefore, careful design of an effective synthetic community is necessary [10•]. Another study explored enhancing chalcopyrite dissolution by decoupling the growth and BL activity of the acidophiles by increasing the temperature of the consortium at the end of the growth phase with only 4°C [40].

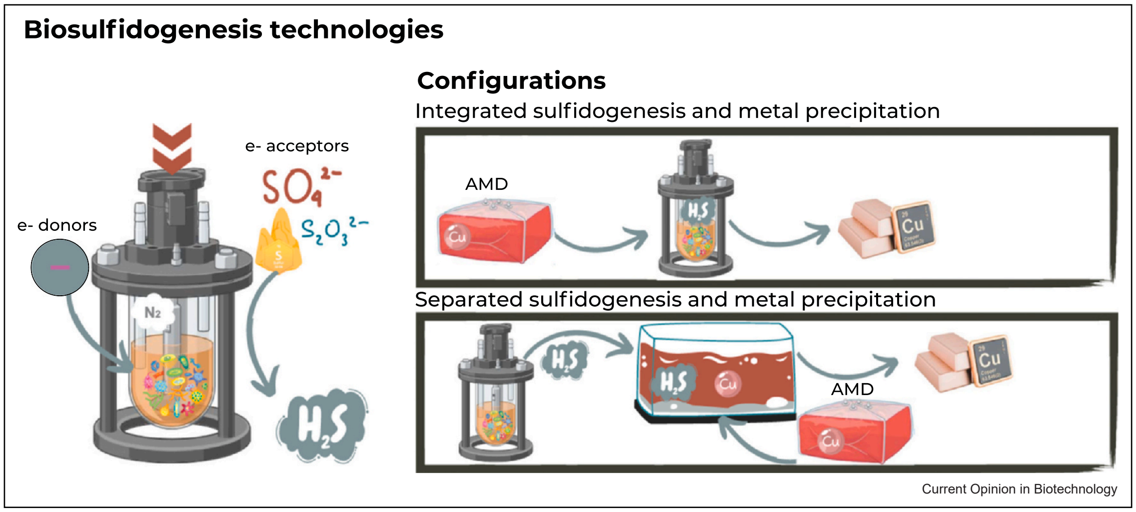
Example 3: biosulfidogenesis for metal removal from metalliferous waters

The aforementioned metalliferous waters require treatment, which has traditionally involved indiscriminate neutralization with gypsum, followed by storage in tailings ponds. Unfortunately, this approach squanders valuable metals, relegating them to a toxic waste status. A more sustainable strategy, however, emerges in removing metals as metal sulfides via hydrogen sulfide [41,42]. While off-site hydrogen sulfide production and transport pose safety and economic challenges, onsite sulfide generation offers a preferable alternative. Biosulfidogenesis (BS), the dissimilatory reduction of oxidized sulfur molecules to sulfide as an end product, has garnered significant attention [43–45]. It enables onsite sulfide production, facilitating the selective recovery of metals (e.g. Cu, Zn, Ni, Co) as high-purity metal sulfides suitable for recycling. Achieving this selectivity involves adjusting sulfide concentrations, metal content, and pH based on the corresponding pK values of desired metal sulfides [46]. For instance, copper sulfides will precipitate at lower pH values, while other metals, such as zinc or iron, will precipitate at increasing values [47]. Figure 3 shows different configurations of BS.

Sulfide production through biological sulfate reduction has been so far the focus of most investigations for several reasons: (1) sulfate is a readily available electron acceptor in acid mine drainage (AMD) being at concentrations up to molar amounts in some cases and, therefore, a cost-effective electron acceptor; (2) sulfate stability and solubility at low pH is high; (3) sulfate reduction at low pH consumes acidity and increases pH [48].

However, BS can be achieved from several sulfur-based electron acceptors such as sulfite, tetrathionate or elemental sulfur. Johnson and Sánchez-Andrea [34] high-

Figure 3



BS reactor and different configurations for metal precipitation from AMD.

light elemental sulfur reduction as the most promising technology, combining criteria such as economic efficiency per sulfide produced, solubility and stability. Elemental sulfur's efficiency requiring four times less electron donor for equivalent sulfide production significantly impacts process economics because adding electron donors is a large portion of the overall operating costs. Moreover, elemental sulfur, often a waste product, proves more cost-effective than traditional electron donors [49••,50]. Finally, a yet technologically underexplored metabolism for metal precipitation is elemental sulfur disproportionation, eliminating the need for external electron donors [51].

Biosulfidogenic technology, while promising, faces limitations due to the toxicity of metal-containing waters and their low pH under actual AMD conditions. Existing systems necessitate neutral conditions for sulfide production, followed by stripping in separate reactors. Recent investigations, however, have unveiled acidophilic sulfidogenic micro-organisms capable of resiliently precipitating metals. Notable species of SRMs include *Archaeoglobus profundus* and *Vulcanisaeta moutnovskia* (*Archaea*), alongside members of the bacterial phylum *Bacillota* (formerly *Firmicutes*) such as *Thermodesulfobium*, *Desulfosporosinus*, and *Acididesulfobacillus* [52–60••]. Additionally, members of the *Desulfobacterota* (formerly known as the *Deltaproteobacteria*), such as *Desulfothermobacter acidiphilus*, contribute to BS. Beyond sulfate reducers, sulfur reducers, such as *Desulfurella amilsii* or sulfur disproportionators such as *Acidianus* strain AS80 offer alternative pathways [61,62] for sulfide generation at low pH. The application of micro-organisms naturally adapted to these extremely acidic conditions will broaden the application range and robustness of biosulfidogenic processes for metal precipitation and recovery.

Challenges and prospects

The microbial sulfur cycle plays a crucial role in many biotechnological applications. However, its complexity, given by the redox activity, presents significant engineering challenges and opportunities. Understanding the microbial communities and underlying mechanisms is essential for improving bioprocesses.

While most studies rely on 16S rRNA gene amplicon sequencing to assess microbial community composition, a deeper understanding of the genes, transcript, proteins, and metabolic levels is vital. This knowledge unravels functionality and mechanisms, enabling targeted bioprocess enhancements.

Direct application of pure and mixed cultures with genetically engineered strains offers promising avenues. For instance, CRISPR-Cas modifications have boosted sulfur formation by *Thioalkalivibrio versutus* by 24.7% while reducing sulfate formation by 15.2% compared to the wild type. Genetic modifications also shed light on sulfur metabolism [63]. However, using mixed or pure cultures requires careful consideration: (i) containment of genetically modified organisms, (ii) maintaining process robustness despite contaminants in the feed, and (iii) ensuring reactor sterility.

Physiology-based modeling has proven valuable in predicting bioprocess performance [64,65]. However, in recent times, data-driven technologies, such as artificial intelligence (AI), machine learning, and 'digital twins' have been widely adopted across industries, including biomanufacturing and mining. These technologies hold significant promise for bioprocesses, particularly in design, control, and scalability [66,67].

Scaling up remains a significant hurdle in biotechnology. Maintaining consistent process conditions across diverse environmental contexts is far from trivial. Therefore, achieving a robust, functionally redundant, and reproducible bioprocess becomes paramount.

In situ processes, such as DISB, present their own challenges. One critical issue is how to effectively halt the process once the desired ore has been sufficiently leached [33•]. If left unchecked, the ongoing process may lead to AMD, contaminating the surrounding area and groundwater. Consequently, it is essential to incorporate a BL cessation strategy into the design and operation of such *in situ* processes from the outset.

Biotechnological processes have evolved beyond product recovery, aiming for multifaceted goals and value-added outcomes. Energy efficiency, cost-effectiveness, and self-sufficiency are now the driving forces. One example is a microbial electrosynthesis system in which methane production from CO_2 is directly coupled with sulfide oxidation to sulfate, creating an eco-friendly system [68]. Another example is microbial protein formation from sulfide-rich biogas by methane- and sulfide-oxidizing bacteria [69]. A growing trend involves leveraging sulfur oxidation and reduction for extracting rare Earth metals from e-waste and lithium batteries [27,70]. In addition to new products, there is immense value in exploring creative ways to utilize abundant end products such as sulfur [16]. Likewise, BS can yield sulfide nanoparticles and contribute to a 'metal sulfides-microbe' biohybrid system. These systems find applications in environmental contexts, bridging the gap between biology and materials science [71].

Conclusion

Micro-organisms within the sulfur cycle present remarkable opportunities for tackling sulfur pollution while recovering valuable metals and elemental sulfur. However, while harnessing these bacteria is very attractive, managing toxic sulfur compounds under extreme conditions poses safety and operational efficiency challenges. Researchers are exploring to obtain a deeper understanding of the crucial micro-organisms involved in this process and seeking better consortia, substrates, and operational conditions to enhance process performance. Looking ahead, AI-based technologies could simulate process/application conditions for predicting and improving bioprocess efficiency. Overall, where environmental challenges meet biotechnological innovation, sulfur cycle–based micro-organisms continue to shape biotechnological applications for a more resilient future.

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CRediT authorship contribution statement

Suyash Gupta wrote the draft version of the manuscript. All authors contributed to the final version of the manuscript.

Data Availability

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

Declaration of Competing Interest

There is no conflict of interest.

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- • of special interest
- •• of outstanding interest

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