

# Microbial respiration - a biomineral perspective

Lucian C. Staicu<sup>1,2,\*</sup>, Julie Cosmidis<sup>3</sup>, Muammar Mansor<sup>4</sup>, Catarina M. Paquete<sup>5</sup>, Andreas Kappler<sup>4</sup>

<sup>1</sup>Department of Bacterial Genetics, Faculty of Biology, University of Warsaw, 02-096 Warsaw, Poland

<sup>2</sup>Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282, United States

<sup>3</sup>Department of Earth Sciences, University of Oxford, OX1 3AN Oxford, United Kingdom

<sup>4</sup>Department of Geosciences, University of Tübingen, 72070 Tübingen, Germany

<sup>5</sup>Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal

\*Corresponding author. Lucian C. Staicu, Department of Biological Sciences, Mellon Hall, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15282, United States. E-mail: [staiculucian@gmail.com](mailto:staiculucian@gmail.com)

Editor: Marcus Horn

## Abstract

Microbial biomineralization is a key process in natural and anthropogenic environments. Certain bacteria and archaea produce cellular energy via anaerobic respiration using metals and metalloids as terminal electron acceptors, producing intra- and extracellular biominerals. This article explores the biomineralization of arsenic (As), iron (Fe), sulfur (S) and selenium (Se), in relation with microbial respiratory processes. Ferric iron (Fe<sup>III</sup>) and the oxyanions of As, S and Se are used as terminal electron acceptors by specialized bacteria and archaea, providing significant amounts of energy under anoxic and nutrient-limiting conditions. These transformations result in the formation of various types of arsenic sulfides, iron (oxyhydr)oxides and sulfides, elemental S/S<sup>0</sup> and elemental Se/Se<sup>0</sup> biominerals, which will be the focus of this review. Certain biominerals (e.g. S<sup>0</sup>) function as storage compounds; others, like Se<sup>0</sup>, may increase the density and the buoyancy of bacteria harboring them or are by-products of this process. Arsenic sulfides and iron (oxyhydr)oxides and sulfides appear to be by-product biominerals or have a yet unknown function. The use of these biominerals as biosignatures is an open topic and an ongoing debate. Further exploration of the reviewed biominerals is needed from both fundamental and applied viewpoints, aspects which will be covered in this review.

**Keywords:** anaerobic respiration; biogenic particles; biomineralization; microbial biominerals; respiratory enzymes

## Introduction

Microbial biomineralization is the process by which microorganisms produce crystalline or amorphous minerals. This process can be genetically controlled and tightly regulated (e.g. magnetotactic bacteria produce intracellular magnetite and greigite minerals arranged in a linear structure—Uebe and Schüler 2021) or can occur as a by-product of microbial metabolism (extracellular iron minerals) (Staicu and Stolz 2021). From this perspective, two types of biomineralization can be identified: biologically controlled mineralization (BCM) and biologically induced mineralization (BIM) (Konhauser and Riding 2012). BCM implies a set of genes involved in the biomineralization, growth and localization of the biomineral, which is usually intracellular. BIM occurs as a result of the metabolic activity of the microbial cells which induce local chemical changes (e.g. pH rise), the minerals precipitating extracellularly (Cosmidis and Benzerara 2022). Some authors identify a third type, microbial influenced biomineralization, which, in fact, can be considered a subtype of BIM. This biomineralization entails that certain organic structures (e.g. Extracellular Polymeric Substances / EPS) act as nucleation surfaces for mineral precipitation in supersaturated solutions and it does not require cells to be alive or metabolically active (Cosmidis and Benzerara 2022). In contrast to BCM, in BIM and biologically influenced biomineralization there is no known genetic control on crystal nucleation or growth. Biomineralization in eukaryotes entail a function (e.g. apatite in bones has a structural role, aragonite or silica in shells provide protection against preda-

tors), whereas in prokaryotes this function may be apparent or unknown.

Biomineralization can be associated with microbial respiratory processes. In this process, microorganisms couple the oxidation of organic or inorganic electron donors (e-donor) with the reduction of organic or inorganic electron acceptors (e-acceptor), resulting in a flow of electrons that generates chemical energy (ATP). In certain cases, microbial respiration using metals and metalloids as terminal electron acceptors leads directly or indirectly to solid products such as metal sulfides (AsS, FeS), elemental selenium (Se<sup>0</sup>), elemental sulfur (S<sup>0</sup>), and iron (oxyhydr)oxides. Since it involves various chemical elements, biomineralization serves as a chemical hub in both the oxidative and reductive pathways of several biogeochemical cycles (Staicu and Barton 2024). Therefore, understanding these processes is relevant from fundamental (identifying potential biological functions impacted by certain biominerals in prokaryotes) and applied perspectives (e.g. attractive for industrial applications such as biohydrometallurgy and bioelectrochemical systems).

In this manuscript, we focus on biominerals associated with microbial respiratory processes, including iron oxides, FeS<sub>x</sub>, S<sup>0</sup>, AsS and Se<sup>0</sup>. The main focus of this review is neutrophilic, pure (axenic) microbial cultures involved in the production of the above-listed biominerals. For each biomineral, the article explores thermodynamics, enzymology, and mineralogy, as well as some aspects related to their characterization, environmental distribution and application. These sections are preceded by a brief in-

Received 19 April 2025; revised 13 September 2025; accepted 15 September 2025

© The Author(s) 2025. Published by Oxford University Press on behalf of FEMS. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

roduction to anaerobic respiration in prokaryotes aimed at facilitating the reader with a better understanding of the biomineralization process. This article aims to link anaerobic respiration and microbial biomineralization in a framework that integrates the latest updates with the perspectives resulting from this multidisciplinary topic.

## A brief overview of microbial respiration

Microbial respiration is a process in which microbes transform organic and inorganic matter while generating reducing power to produce ATP, the essential energy molecule for all living organisms. In this process, electron acceptors play a crucial role in facilitating energy generation. The reduced cofactors, such as NADH and FADH<sub>2</sub> generated during catabolic processes, are oxidized through electron transfer pathways to suitable electron acceptors. These can be membrane enzymes or protein complexes present at the inner membrane. This electron flow is linked to the translocation of charge (e.g. electrons or ions), forming an electrochemical gradient across the inner membrane. This gradient creates a proton motive force that drives ATP synthesis by the membrane-bound ATP synthase (Schäfer 2013). This process is in contrast with fermentation, which proceeds in the absence of an external electron acceptor, with cells maintaining redox reactions by transferring electrons to organic intermediates, thereby allowing the production of ATP.

While oxygen serves as the primary electron acceptor in aerobic respiration, in the absence of oxygen, microbial respiration relies on alternative electron acceptors that can be utilized intracellularly or extracellularly. These include nitrate (NO<sub>3</sub><sup>-</sup>), sulfate (SO<sub>4</sub><sup>2-</sup>), arsenate (AsO<sub>4</sub><sup>3-</sup>), selenate (SeO<sub>4</sub><sup>2-</sup>), selenite (SeO<sub>3</sub><sup>2-</sup>) and metal ions (e.g. Fe(III), Mn(IV)), among others. These inorganic compounds, besides being used in dissimilatory metabolisms to generate energy, can also participate in assimilatory processes, where they are reduced and incorporated into biomass. The ATP yield in anaerobic respiration is less efficient than in aerobic respiration since the terminal electron acceptors used in anaerobic respiration have a lower reduction potential than oxygen (Fig. 1). ATP synthase efficiency is directly related to the electron donor and the terminal electron acceptor, in which the greater difference in reduction potential between the two results in higher energy generation.

Dissolved electron acceptors can enter microbial cells and undergo reduction intracellularly, while solid electron acceptors, such as metal oxides, are reduced extracellularly. For soluble electron acceptors, the initial step of anaerobic respiration is the transport of dissolved electron acceptors across the cell wall, as their terminal reductases are located within the cell. However, the specific transport mechanisms vary depending on the type of electron acceptor and the organism. Although small molecules, such as O<sub>2</sub> or CO<sub>2</sub>, can easily diffuse across the cell membrane, other compounds require specific cellular machinery to enter the cell. ATP-binding cassette (ABC)-type transporters are the most well-known and well-characterized transporters. These protein complexes couple the energy released from ATP hydrolysis to the translocation of a wide variety of substances in and out of cells (Locher 2009). Once inside the cell, the electrons reduce the electron acceptors, which may involve a single step, or several events catalyzed by different enzymes.

When the electron acceptor is insoluble and cannot permeate the membrane, specific electron transfer pathways are required to transport the electrons outside the cell. This is the case with metal oxides, which are often present as solid minerals (Gralnick

and Newman 2007). To overcome this challenge, microorganisms have evolved specialized mechanisms, such as extracellular electron transfer, that facilitate electron exchange between intracellular redox reactions and extracellular electron acceptors (Shi et al. 2016, Kappler et al. 2021). This process can occur through a direct mechanism, where electrons derived from catabolic reactions are transported to cell-surface exposed proteins that directly contact and transfer the electrons to the solid electron acceptor, or through an indirect process using soluble electron shuttles (Costa et al. 2018, Liu et al. 2018). Several electron shuttles have been identified in nature, and microorganisms either produce their own electron shuttles or utilize those available in the environment (Glasser et al. 2017). These electron shuttles (e.g. flavins, quinones, humic substances) facilitate the reduction of solid electron acceptors that cannot easily enter the cell interior (Gralnick and Newman 2007) and, for that reason, they mediate electron transfer from exposed redox proteins to insoluble (and poorly soluble) electron acceptors (Paquete et al. 2014).

## Iron biominerals

### Fe(III) (oxyhydr)oxides

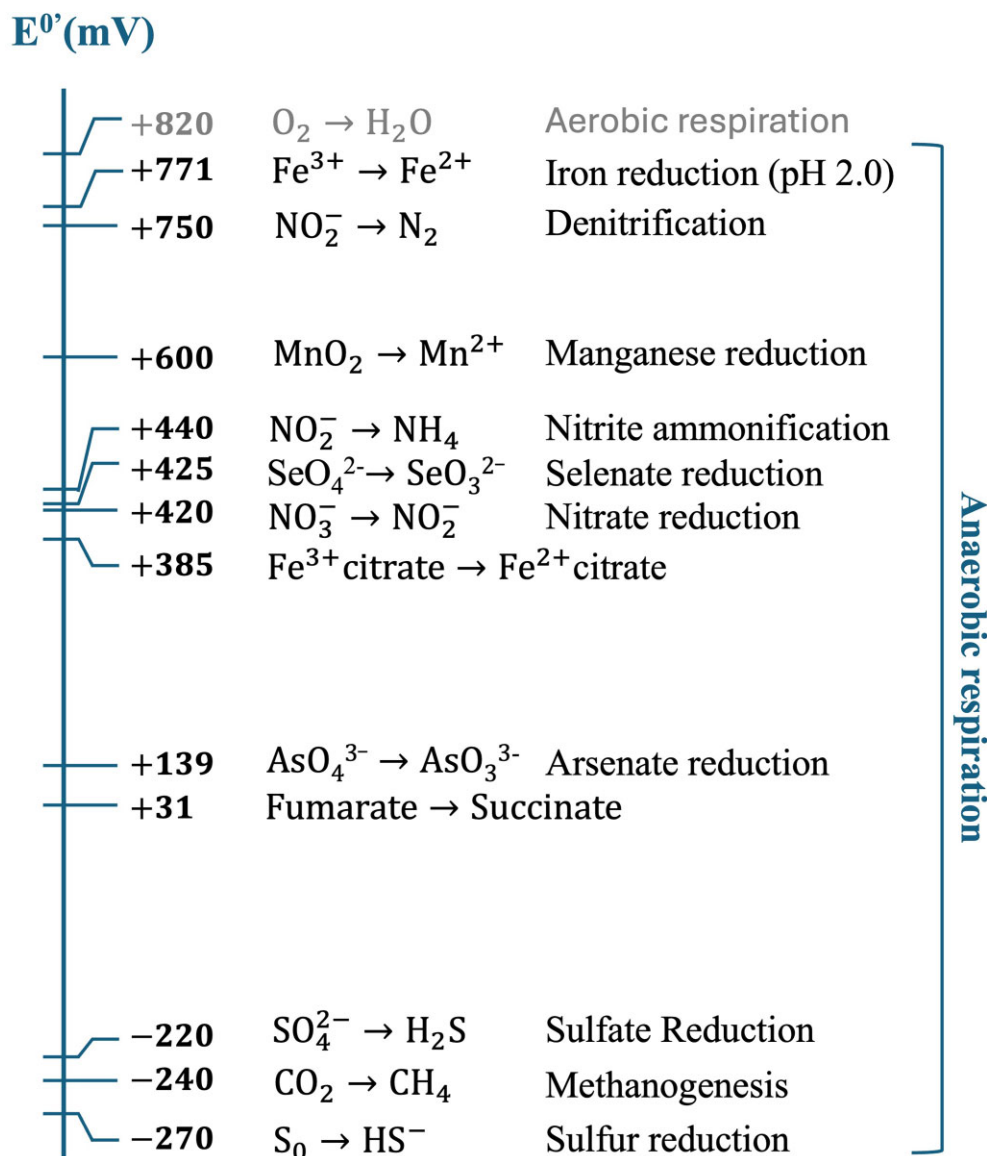
#### *Fe minerals in nature and their importance*

Fe(III) (oxyhydr)oxides minerals can be both a product and a substrate of microbial respiration (Kappler et al. 2021). Ferrihydrite (FeOOH), lepidocrocite (gamma-FeOOH), goethite (alpha-FeOOH), hematite (alpha-Fe<sub>2</sub>O<sub>3</sub>), and magnetite (Fe<sub>3</sub>O<sub>4</sub>) are abundant Fe mineral phases in the environment. They play a key role in many environmental processes due to their high reactivity, small particle size and the resulting high surface area. This includes the sorption of nutrients (e.g. phosphate and trace metals such as Ni) and toxic metals (e.g. As), the sorption of organic carbon which protects it from biodegradation, and the redox transformation of organic and inorganic pollutants (Borch et al. 2010).

#### *Abiotic and biotic Fe(II) oxidation*

Fe(III)-bearing minerals can be formed at neutral pH abiotically via oxidation of Fe(II) by oxidants such as dioxygen (O<sub>2</sub>) or nitrite (NO<sub>2</sub><sup>-</sup>), photochemically, i.e. catalyzed by light of different wavelengths, and also enzymatically by phototrophic, nitrate-reducing and microaerophilic microorganisms (Kappler et al. 2021). While the first metabolic group uses photosynthesis to couple Fe(II) oxidation to CO<sub>2</sub> reduction and biomass formation (i.e. photoferrotrophy), the latter two use respiration with either nitrate or oxygen as electron acceptors for their metabolism, i.e. for energy generation.

The oxidation of Fe(II) by microorganisms at neutral pH by respiration with either nitrate or molecular oxygen as electron acceptor poses several challenges for the microorganisms compared to respiratory oxidation of organic compounds or gases such as hydrogen (H<sub>2</sub>) or methane (CH<sub>4</sub>). While the oxidation of electron donors that are typically dissolved in water can be performed after the uptake of the substrates into the cells, followed by a release of soluble or gaseous compounds (including CO<sub>2</sub>), the respiratory Fe(II)-oxidizers face various physiological challenges. On the one hand, many Fe(II)-bearing substrates are present as (in)soluble minerals (e.g. siderite (FeCO<sub>3</sub>), vivianite (Fe<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>), magnetite (Fe<sub>3</sub>O<sub>4</sub>), as well as Fe(II)-bearing clays) (Kappler et al. 2021). On the other hand, the oxidation of Fe(II) at neutral pH leads to poorly soluble Fe(III). This oxidized form of Fe precipitates readily as one of the typical Fe(III) (oxyhydr)oxide minerals—the identity mostly depending on the environmental



**Figure 1.** Reduction potential ( $E^{\circ}$  vs SHE) of electron acceptors used in microbial respiration, emphasizing those used under standard anoxic conditions (Aghababaie et al. 2015). Note that these are reduction potentials under standard conditions (1 M concentration for all reactants and substrates at pH = 0, 25°C and 1 bar). The reaction feasibility under biologically relevant conditions can be determined by the methods outlined in Amend and LaRowe (2019).

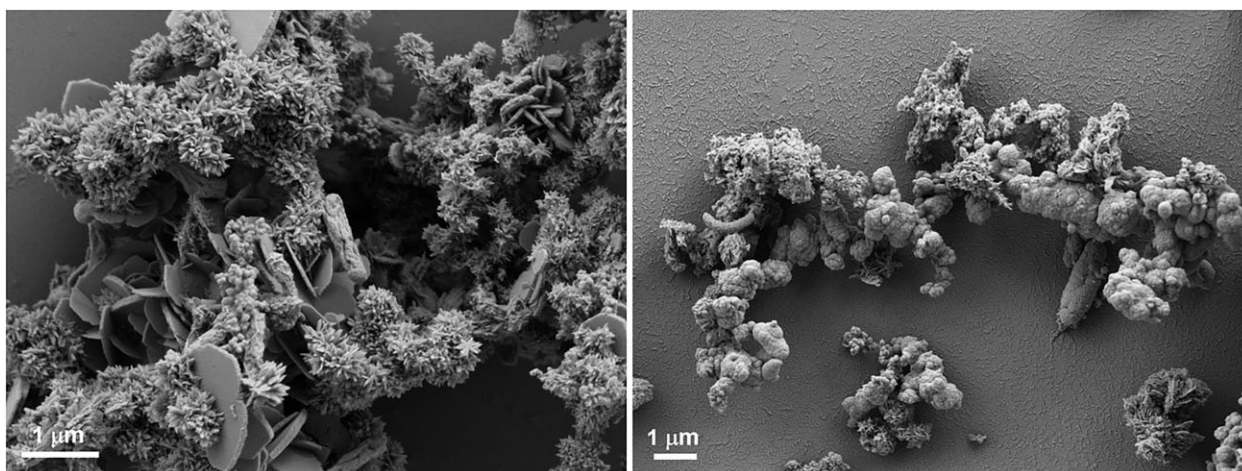
conditions—potentially harming the cellular metabolic activity by precipitating within the cells or at the cell surface (Kappler et al. 2005, Miot et al. 2009a). Different Fe(II)-oxidizers have been shown to deal with these issues in different ways: from extracellular oxidation of Fe(II) minerals, uptake and oxidation of only dissolved  $Fe^{2+}$  or organic-complexed Fe(II) (Miot et al. 2009b, Byrne et al. 2016, Peng et al. 2018, Han et al. 2020, Jakus et al. 2021, Zhou et al. 2022) to changes in cell-surface charge or cell surface pH to prevent cell encrustation (Hegler et al. 2010, Saini and Chan 2013). Finally, the group of microaerophilic Fe(II)-oxidizers produces extracellular organic structures (twisted stalks, sheaths) to deposit the Fe(III) minerals away from the cell itself and thus to maintain the cell metabolic activities (Krepski et al. 2013, Laufer et al. 2017, Vigliaturo et al. 2020).

Finally, it should also be mentioned, that the formation of zero-valent iron (Fe(0)) has also been described in iron(III)-reducing cultures of the methanogenic microorganism *Methanosarcina barkeri* al-

though it remains unclear how widespread this activity is and whether it plays a role under more environmentally relevant conditions (Shang et al. 2020).

### Properties of Fe biominerals

Biotic Fe(III) (oxyhydr)oxides, formed by microbial Fe(II) oxidation, show distinct properties that distinguish them from their abiotic counterparts (Posth et al. 2014) (Fig. 2). During Fe(II) oxidation, biomolecules, including proteins and carbohydrates, and likely also nucleic acids become associated with the minerals. The positive surface charges of Fe(III) (oxyhydr)oxide minerals at neutral pH facilitate the interactions with the biomolecules that contain negatively charged functional groups or functional groups with free electron pairs, including carboxyl, phosphoryl, and hydroxyl groups. The association of the minerals with these biomolecules influences the properties of the formed minerals. On the one hand, already during the formation of the Fe(III) (oxyhydr)oxides,



**Figure 2.** Electron micrographs showing biominerals formed by the phototrophic Fe(II)-oxidizer *Rhodobacter ferrooxidans* SW2 (mainly flower-like goethite and flat lepidocrocite structures, left) and the autotrophic nitrate-reducing enrichment culture KS (flower-cluster-like Fe(III) phosphates and Fe(III) oxyhydroxides including ferrihydrite, right). The minerals were identified by X-ray diffraction and Mössbauer spectroscopy. Images were taken by Dr. B. Wan and Dr. J. Shuster.

the organic compounds influence the crystallization of the minerals. Generally, crystal growth is impeded by the competitive sorption of the organic compounds, leading to less crystalline and smaller-sized mineral particles (Posth et al. 2014, Schulz et al. 2022). On the other hand, the surface charge of the minerals can shift from positive to negative affecting interactions with other ions. While the repulsion of silicate has been observed for some iron biominerals (Schad et al. 2022), silicate is generally known to sorb strongly to abiotic Fe(III) (oxyhydr)oxides and to other ferric iron biominerals (Senn et al. 2015, Zhou et al. 2024). Trace metals (such as Ni, and many other rare earth elements) also show different sorption behavior to abiotic and biogenic iron minerals (Eickhoff et al. 2014, Heim et al. 2015, Schmid et al. 2016, Kovalick et al. 2024). Therefore, a deeper investigation into the reactivity of biominerals is required for understanding the environmental behavior of trace metals, nutrients and organic carbon; in particular the reactivity of iron biominerals needs to be studied in more detail. It would be of particular interest to study how the transformation of the iron biominerals under fluctuating redox conditions, both via redox-induced solid-state transformation and dissolution-precipitation processes, changes the reactivity and properties of the minerals with regard to their interactions with trace metals, nutrients and organic carbon. Particularly, the high reactivity of mixed-valent ferrous-ferric mineral phases such as green rust or magnetite could be of relevance under such conditions.

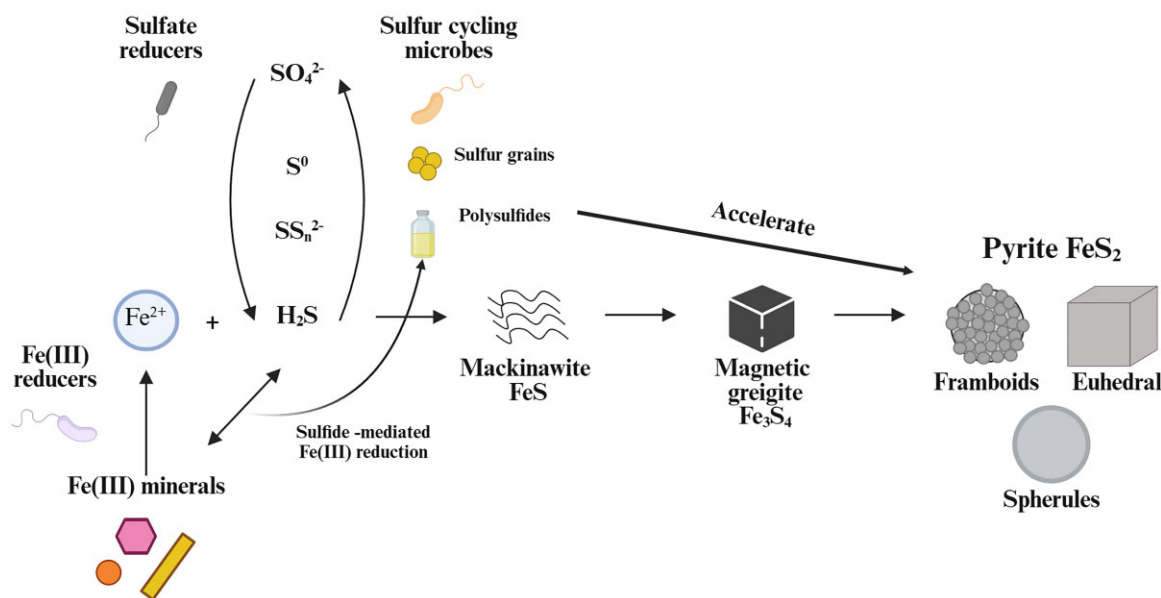
The association of the minerals with biomolecules, or even with whole, intact cells (Fig. 2), also affects the density of Fe minerals. While abiotic minerals such as ferrihydrite or goethite have densities of 3.6–4.0 g/cm<sup>3</sup>, a study using different Fe(II)-oxidizing bacteria showed that biotic Fe(III) (oxyhydr)oxide minerals have much lower densities, in the range of 1.6–2.4 g/cm<sup>3</sup> (Posth et al. 2010). This, in turn, may influence sedimentation behavior of the mineral particles and cell-mineral aggregates, meaning that Fe(III) (oxyhydr)oxide minerals that are formed in the water column of a lake or ocean would have a much longer residence time in the water column and be available for prolonged Fe redox cycling at the chemocline (Schad et al. 2022, Dreher et al. 2025) than their abiotic counterparts (e.g. detrital mineral phases), with potential consequences for their interactions with nutrients, pollutants, and other organisms.

## Application and relevance of iron biominerals

Minerals produced by microorganisms, including iron biominerals, can be used for many interesting future practical applications (summarized recently in Cosmidis 2023). This includes the use of magnetic nanoparticles produced by magnetotactic bacteria for biomedical applications such as magnetically targeted drug delivery, pathogen detection, magnetic hyperthermia for the treatment of tumors, or as contrast agents in magnetic imaging techniques as well as for remediation of toxic metals such as Cd or As from groundwater (see references in Caraballo et al. 2022, Cosmidis 2023 and Li et al. 2025). Additionally, very recently a potential role of redox-active iron biominerals in electron storage, i.e. the use as biogebatteries, has been suggested (Peiffer et al. 2021). In addition, iron-metabolizing microbes play a vital role in recovering valuable metals (such as rare earth elements or even gold or platinum), extracting toxic metals (such as arsenic): (Hohmann et al. 2010, Omoregie et al. 2013, Sowers et al. 2017), and supporting bioremediation (Gadd 2010). Furthermore, iron biominerals have been studied in the context of life on other planets (Price et al. 2022) and the search for evidence for life or certain microbial processes on early Earth (Lin et al. 2017).

## Iron sulfide biominerals

Microbial respiration of sulfur (S) species and ferric iron (Fe(III)) produces reduced sulfide (H<sub>2</sub>S) and ferrous iron (Fe(II)), which precipitate together to form a variety of iron sulfides such as mackinawite (FeS), greigite (Fe<sub>3</sub>S<sub>4</sub>) and pyrite (FeS<sub>2</sub>) via BIM (Fig. 3). The production of sulfide is driven by various sulfide producing microorganisms (SPM), which catalyze the reduction of sulfate (SO<sub>4</sub><sup>2-</sup>) or disproportionation of sulfur species such as elemental sulfur (S<sup>0</sup>) and thiosulfate (S<sub>2</sub>O<sub>3</sub><sup>2-</sup>) (Jørgensen et al. 2019). Among these, sulfate reduction is the most important, with sulfate reducing microorganisms thought to constitute >90% of the sulfide source at low temperatures globally (Rickard et al. 2017). Meanwhile, Fe(III) reducing microorganisms (FeRM) couple the reduction of Fe(III) with the oxidation of organic carbon, dihydrogen (H<sub>2</sub>) or methane (CH<sub>4</sub>) (Kappler et al. 2021). SPM and FeRM co-exist in a variety of anoxic habitats, such as in sediments and stratified water columns. The formation of biogenic iron sulfides via BIM by these microorganisms is tied to the fate of carbon, oxygen, nitro-



**Figure 3.** Summary of formation pathways for iron sulfides and the microorganisms involved. Fe(III)-reducers reduce various Fe(III) minerals to dissolved  $\text{Fe}^{2+}$ . The reaction between dissolved  $\text{Fe}^{2+}$  and  $\text{H}_2\text{S}$  released from sulfur-cycling microorganisms results in mackinawite precipitation. Its transformation to greigite and pyrite is accelerated in the presence of various intermediate sulfur species ( $\text{S}^0$ ,  $\text{SS}_n^{2-}$ ) produced by sulfur-cycling microorganisms and through the abiotic reaction between  $\text{H}_2\text{S}$  and Fe(III) minerals.

gen, phosphorus and various trace metals at both short-term and geological time scales (Mansor et al. 2025).

Enzymes involved in the production of sulfide and Fe(II) can be easily detected from (meta)omic datasets using functional annotations via hidden Markov models (Garber et al. 2020). For SPM, these include (1) the set of sulfate adenylyltransferase (Sat), APS reductase (Apr) and dissimilatory sulfate reductases (DsrAB and DsrMKJOP) for sulfate reduction, (2) sulfur reductase (Sre) or NAD(P)H sulfur oxidoreductase (Nsr) for  $\text{S}^0$  reduction, and (3) sulfur oxygenase reductase (Sor), thiosulfate-thiol sulfurtransferase (RDL2), cytochrome  $c_3$  or membrane-bound quinone-dependent thiosulfate reductase (Phs) for the disproportionation of  $\text{S}^0$  or thiosulfate (Zhou et al. 2025). For FeRM, enzymes such as (1) cytoplasmic membrane-associated tetrahaem c-type cytochrome (CymA), (2) metal-reducing associated genes (MtrCAB) and (3) outer membrane cytochromes (OmcF, OmcS and OmcZ) are indicators of Fe(III) reduction (Garber et al. 2020).

Mackinawite (FeS) is the first product to form upon the reaction between iron and sulfide (Rickard and Luther 2007). Individual crystallites of mackinawite are typically less than 20 nm in size, but it can aggregate in solution to form visible black clumps (Picard et al. 2018). Several studies have investigated mackinawite formation by sulfate reducers in the context of pollutant remediation, carbon sequestration and biosignatures (Picard et al. 2016, 2019, 2021, Ikogou et al. 2017, Stanley and Southam 2018, Mansor et al. 2019a,b, Nabeh et al. 2022). Biogenic mackinawite is typically larger, displays higher aggregation extent, and is associated with more organic matter compared to abiogenic mackinawite precipitated in the absence of microorganisms (Picard et al. 2018, 2019, 2021, Mansor et al. 2019a, Nabeh et al. 2022). The order of addition—iron first or sulfide first—affects the association between cells and mackinawite. When Fe is added first to the culture medium, positively charged  $\text{Fe}^{2+}$  binds to the negatively charged cell wall. Sulfide produced by the sulfate reducers then precipitates with  $\text{Fe}^{2+}$  on the cell wall, leading to encrustation and likely decreased cell viability. When sulfide is already present,

$\text{Fe}^{2+}$  added later immediately reacts with sulfide, causing precipitation in the extracellular space (Picard et al. 2018, 2021); Nabeh et al. 2022. Both scenarios could be envisioned in the environment, with impacts on microbial ecology and biosignature detection.

Besides sulfate reducers, recent studies have started to investigate the role of other microorganisms in precipitating mackinawite. For example, *Geobacter sulfurreducens* is a well-known Fe(III)-reducer that can also reduce  $\text{S}^0$  to sulfide. In the co-presence of Fe(III) and sulfide, mackinawite is formed with implications for phosphorous bioavailability (Bronner et al. 2023) and for sulfur and iron-based bioremediation technology (Liu et al. 2023). Similarly, *Shewanella* species produce mackinawite when grown in the co-presence of thiosulfate and Fe(III), with a noticeable enhancement on mineral-mediated extracellular electron transfer that is being considered for application in microbial fuel cells (Nakamura et al. 2010, Jiang et al. 2014, Kondo et al. 2015). The hyperthermophilic archaeon *Thermococcus kodakarensis* produces mackinawite when grown on  $\text{S}^0$ , which is of importance for iron sulfide formation and life detection near hydrothermal vents (Gorlas et al. 2018, 2022, Truong et al. 2023, 2024).

Greigite ( $\text{Fe}_3\text{S}_4$ ) is a mixed-valent Fe(II)-Fe(III) sulfide mineral that can form upon mackinawite aging. Intracellular greigite is known to be formed by magnetotactic bacteria together with magnetite ( $\text{Fe}_3\text{O}_4$ ) via BCM, where these magnetic nanominerals are used by the microorganisms to aid in navigating the Earth's magnetic field (Amor et al. 2020). Reports of extracellular greigite formation via BIM in microbial cultures are relatively rare (Picard et al. 2018, Mansor et al. 2019a, Gorlas et al. 2022, Bronner et al. 2023, Sekerci et al. 2025). The transformation of mackinawite to greigite requires an oxidant, which could be low amounts of oxygen, protons or polysulfides (Rickard and Luther 2007, Mansor et al. 2025). This transformation is faster in the presence of sulfate reducers, with the mineral being detectable within 6 months, in comparison to abiotic controls that showed no transformation of mackinawite to greigite (Picard et al. 2018, Mansor et al. 2019a). In cultures of *G. sulfurreducens*, greigite is detected as early as 21 days (Bron-

ner et al. 2023). Extracellular greigite formation is even faster in the presence of the hyperthermophilic *T. kodakarensis*, becoming detectable within a few days. These greigite nanoparticles occur in close proximity to cells and  $S^0$ -containing vesicles (Gorlas et al. 2018, 2022, Truong et al. 2023). Overall, differences between extracellular biogenic and abiogenic greigite are relatively understudied.

Over time, and under the right environmental conditions, mackinawite and greigite can transform to pyrite ( $FeS_2$ ). Pyrite is the most abundant iron (di)sulfide mineral on Earth, where it is present in the form of striking framboids in addition to euhedral and spherical morphology (Mansor et al. 2025). The burial of pyrite is one of the most important factors affecting the oxygen balance in the ocean-atmosphere system (Berner 1989, Canfield 2005, Canfield and Farquhar 2009). Strictly, pyrite is an iron disulfide mineral, with sulfur having  $-1$  redox state instead of  $-2$ . Hence, pyrite formation is driven by the availability of polysulfides ( $SS_n^{2-}$ ; where  $n$  is the number of sulfur atoms in a chain). Polysulfides are formed when  $H_2S$  is oxidized by the following chemical species, listed in the order of the reaction kinetics:  $MnO_2 > O_2 > Fe(III) > S^0$  (Avetisyan et al. 2021).

Pyrite formation in cultures of sulfate reducers is rare (Picard et al. 2016), which can be explained mostly by cultivation conditions employing only  $Fe^{2+}$  and sulfide, thus excluding any oxidants that can generate polysulfides. Another interesting possibility is that any formed pyrite is immediately reductively dissolved by sulfate reducers, thus evading detection (Boyd and Payne 2025). In cultures containing  $Fe(III)$  minerals,  $S^0$  or mixtures of microbial species capable of cycling Fe and S between redox states (compiled in Mansor et al. 2025), pyrite can be formed at a rate of 0.01–5 mmol/L/day, which is comparable to the rates observed in nature (Mansor et al. 2025). Intriguingly, biogenic pyrite formed in cultures adopts either a spherical (Berg et al. 2020, Duverger et al. 2020, Truong et al. 2023) or euhedral morphology (Thiel et al. 2019, Allen et al. 2021). Natural spherical pyrite has rarely been reported from a microbial mat in the hypersaline Dead Sea (Thomas et al. 2016) and more recently near a hydrothermal vent. Given the lack of pyrite framboids in laboratory cultures, observations of natural framboids cannot be interpreted as a biosignature, especially since many abiotic mechanisms are known (Ohfuji and Rickard 2005).

## Elemental sulfur ( $S^0$ )

### Sulfur occurrence in the environment

Elemental or zero-valent sulfur ( $S^0$ ) is formed in the environment through the re-oxidation of reduced sulfur species (e.g. sulfide) produced by dissimilatory sulfate reduction (Fig. 4A), being considered an indirect product of anaerobic microbial respiration. Once formed, microbial  $S^0$  does not always accumulate in the environment. Whether it is a transient or the final product of microbial sulfide oxidation varies depending on the composition of the microbial community and local environmental conditions. In marine sediments,  $S^0$  is mainly found in the first few centimeters below the sediment surface, and at relatively low concentrations ( $<6.5 \mu\text{mol}/\text{cm}^3$  in euxinic sediments, but typically closer to  $1 \mu\text{mol}/\text{cm}^3$ ) (Jørgensen 2021). Elemental sulfur is most abundant in marine environments with intense sulfate reduction rates (Zopf et al. 2004), where the microbial oxidation of reduced sulfur compounds to  $S^0$  is likely to outpace  $S^0$  consumption by different metabolic processes. Other environments where biogenic  $S^0$  may accumulate include salt marshes, hydrothermal sites, sul-

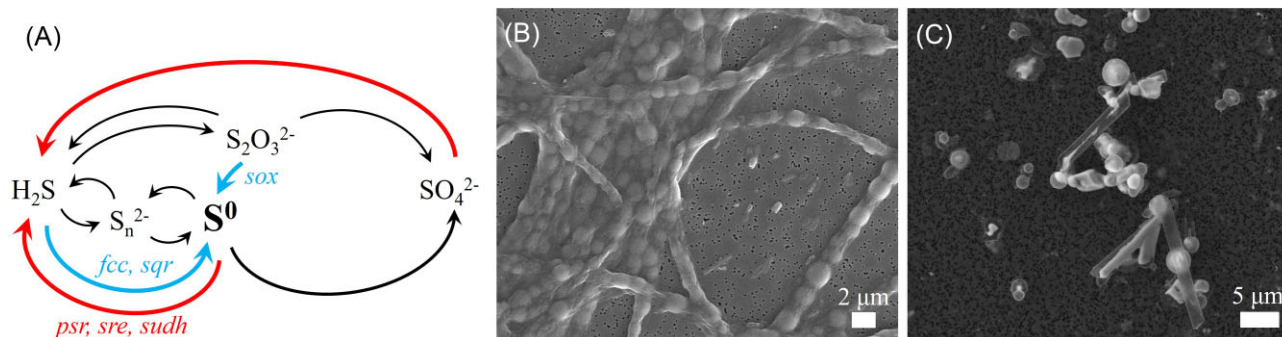
fidic springs, or caves (Taylor et al. 1999, Kamyshny and Ferdelman 2010, Hamilton et al. 2015, Koeksoy et al. 2018). In some rare cases,  $S^0$  can form conspicuous and widespread deposits, for instance at the surface of an Arctic glacier fed by a supraglacial sulfidic spring system (Borup Fiord Pass, Ellesmere Island, Canada; Gleeson et al. 2010, Trivedi et al. 2020), or  $S^0$  plumes forming in surface waters above oceanic oxygen minimum zones (Ohde et al. 2007, Lavik et al. 2009).

### Sulfur biomineralization

Sulfide can be oxidized to  $S^0$  abiotically through chemical oxidation by oxygen or oxidized metals ( $Fe(III)$  and  $Mn(III, IV)$ ), or biologically through different dissimilatory metabolic pathways. These include prokaryotes performing anoxygenic photosynthesis, using reduced sulfur compounds as electron donors for  $CO_2$  fixation in the presence of light (e.g. green and purple sulfur bacteria). On the other hand, chemotrophic S-oxidizing bacteria derive energy for  $CO_2$  fixation from the oxidation of reduced sulfur using either oxygen, nitrate or  $Mn(IV)$  oxides (they are classically called “colourless” sulfur bacteria due to their lack of photosynthetic pigments) (Dahl 2020a,b). Under low-temperature conditions, biological sulfide oxidation rates are typically more than three orders of magnitude faster than abiotic ones (Luther et al. 2011), suggesting that most low-temperature (non hydrothermal)  $S^0$  in nature has a biological origin. The main enzymes involved in sulfide oxidation are the FAD-containing flavocytochrome c (FccAB) and sulfide: quinone oxidoreductases (SQR).  $S^0$  can also be formed through microbial oxidation of thiosulfate, catalyzed by enzymes of the SOX system (Dahl 2020b).

Biogenic sulfur is formed as a biomineral either intracellularly or extracellularly (Kleinjan et al. 2003). Intracellularly,  $S^0$  takes the form of spherical globules (Dahl 2020b) (Fig. 4B), where sulfur may be present as nano-crystalline or amorphous cyclooctasulfur ( $S_8$ ), occasionally associated with linear forms of polymeric sulfur such as polysulfides or polythionates (Pasteris et al. 2001, Prange et al. 2002, Prange 2008, Nims et al. 2019). Depending on microbial species, intracellular  $S^0$  may be located within invaginations of the cytoplasmic membrane, or form inclusions in the cytoplasm with no connection to the cytoplasmic membrane (Dahl 2020b). In some cases (e.g. *Beggiatoa*, *Thiothrix*, or *Thiovulum*), proteins forming an envelope around the  $S^0$  biominerals have been observed. The best studied of these structural proteins are the strongly hydrophobic proteins SgpA, SgpB, SgpC present in the purple sulfur bacterium *Allochromatium vinosum*, which have been shown to play essential roles in intracellular  $S^0$  accumulation (Brune 1995, Prange et al. 2004). A fourth sulfur globule protein, SgpD, has been discovered more recently and plays a yet unclear role in the formation of  $S^0$  inclusions (Kümpel et al. 2023).

Extracellular  $S^0$  biominerals can form at the cell surface or away from the cells and adopt spherical or more irregular morphologies (Fig. 4C). It may be composed of different cyclooctasulfur polymorphs, namely the thermodynamically stable orthorhombic  $\alpha$ - $S_8$  (sometimes in a nanocrystalline form), or the metastable monoclinic  $\beta$ - and  $\gamma$ - $S_8$  (Cron et al. 2019, Marnocha et al. 2019). Metastable forms of  $S^0$  are allowed to form and persist in the environment through stabilizing interactions with microbially derived organics. Organic envelopes have indeed been observed at the surface of extracellular  $S^0$  biominerals formed in laboratory cultures (Kleinjan et al. 2005, Cron et al. 2019, Marnocha et al. 2019) as well as in natural biofilms (Cron et al. 2021). These organic coatings are usually composed of polysaccharides and pro-



**Figure 4.** (A) Simplified biogeochemical sulfur cycle, illustrating microbial respiration processes (in red) as well other microbially mediated processes conducting to the formation (in blue) and consumption of  $S^0$ . Abbreviations of genes mentioned in the text are shown in italics. Note that sulfur intermediates such as sulfite or tetrathionate have been omitted. (B) Intracellular  $S^0$  globules within *Thiothrix* sp. filamentous cells (SEM image by Chrissie Nims). (C) Extracellular  $S^0$  globules and rods produced by *Sulfuricurvum kujiense* (SEM image by Wang Shuo).

teins, while in at least some species they are lipidic, being derived from outer membrane vesicles (Li et al. 2020).

### Sulfur utilisation

Most organisms that form  $S^0$  biominerals have the metabolic capacity to complete the oxidation to sulfate for energy generation. In those cases,  $S^0$  usually serves an energy storage function (Cosmidis and Benzerara 2022), being produced and stored under favorable environmental conditions and then used as an electron donor when conditions change. This process is observed in *S*-oxidizers that produce  $S^0$  both intra- and extracellularly. For instance, *Beggiatoa* oxidizes sulfide to intracellular  $S^0$  under high-sulfide conditions, while under low-sulfide conditions this stored  $S^0$  is oxidized to sulfate (Berg et al. 2014). Similarly, the green sulfur bacterium *Chlorobaculum tepidum* forms extracellular  $S^0$  globules during sulfide oxidation, and then uses this biogenic  $S^0$  as an electron donor for photosynthesis when sulfide is exhausted (Hanson et al. 2016). The utilization of extracellular  $S^0$  may require cell attachment to the particles, via the involvement of thiol groups interacting with  $S^0$  at the cell surface. It can also occur at distance, through pili-assisted extracellular electron transport, or extracellular conversion of  $S^0$  to soluble polysulfides or thiosulfate. The complex molecular systems involved in intracellular sulfur trafficking and oxidation have been described elsewhere (Jia et al. 2024) and are outside the scope of this review.

Under certain conditions,  $S^0$  formed by purple sulfur bacteria can serve as an electron acceptor for anaerobic respiration, leading to its reduction back to sulfide (Fig. 4A). For instance, *Allochrochromatium vinosum* uses intracellular  $S^0$  as a source of reducing power for anoxygenic photosynthesis in the absence of sulfide, releasing sulfate, while in the dark,  $S^0$  is used as an electron acceptor, releasing sulfide (Mas and Gemerden 1995).  $S^0$  reduction to sulfide has also been proposed for non-photosynthetic sulfur bacteria. *Thiovulum*, which stores intracellular  $S^0$  under aerobic conditions, can anaerobically reduce its stored  $S^0$  by respiration, possibly with formate as the electron donor or via sulfur disproportionation (Marshall et al. 2012).  $S^0$  respiration to sulfide was also observed in the anaerobic growth of *Beggiatoa* in the presence of acetate (Nelson and Castenholz 1981).

The ability to respire  $S^0$  (or more rarely, disulfide) using  $H_2$  or organic substrates as electron donors is also widespread among non  $S^0$ -biomineralizing bacteria and archaea, most of which are hyperthermophilic (Hedderich et al. 1998). The molecular mechanisms involved in  $S^0$  respiration remain to be elucidated, but three main enzymes have been identified: polysulfide reductase (Psr), sulfur

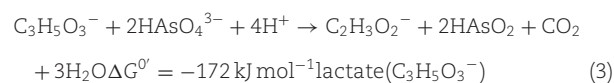
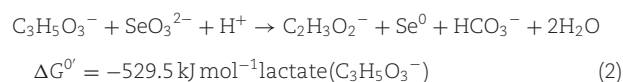
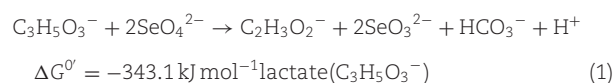
reductase (Sre) and sulfide dehydrogenase (Sudh) (Ma and Adams 1994, Hedderich et al. 1998, Florentino et al. 2017, Wang et al. 2023). Beyond its role in respiration, sulfur is used in some fermentative archaea in a process known as sulfhydrogenesis, whereby  $S^0$  or polysulfides act as a sink for excess electrons to reoxidize reduced ferredoxin accumulated during fermentation, thereby balancing the cell's redox state (Ma and Adams 1994).

### Arsenic and selenium

The presence of arsenic and selenium in the environment is on the rise due to various anthropogenic activities such as fossil fuel burning for energy production, metal and crude oil refining, agriculture etc., exerting a significant negative impact on terrestrial and aquatic ecosystems as well as on human communities (Ganie et al. 2024, Vinceti et al. 2024).

### The thermodynamics of As and Se respiration

The use of selenium (Se) and arsenic (As) oxyanions: selenate/ $Se^{6+}$  ( $SeO_4^{2-}$ ), selenite/ $Se^{4+}$  ( $SeO_3^{2-}$ ) and arsenate/ $As^{5+}$  ( $AsO_4^{3-}$ ) as terminal electron acceptors in microbial anaerobic respiration began to be documented by the end of the 1980s and early 1990s (Stolz et al. 2006). Interestingly, both elements were historically known as potent toxicants for bacteria, so their role in anaerobic respiration was surprising. All oxyanions provide significant amounts of cellular energy for bacteria capable of utilizing it (Fig. 1; Eqs. 1-3) (Staicu and Barton 2021).



It is noteworthy to point out that both  $SeO_4^{2-}$  (reduced to  $SeO_3^{2-}$ ) and  $SeO_3^{2-}$  (reduced to solid  $Se^0$ ) accept electrons in this process, whereas only  $AsO_4^{3-}$  (reduced to arsenite/ $As^{3+}$ ,  $AsO_3^{3-}$ ) fulfills this function. This means that Se not only provides more cellular energy compared to As, but it is also more chemically versatile in anaerobic respiration (Eqs. 1-3). Energetically speaking, with lactate as electron donor,  $SeO_4^{2-}$  provides twice more energy than  $AsO_4^{3-}$ , while the reduction of  $SeO_3^{2-}$  to solid  $Se^0$  gen-

erates over three times more. Gibbs Free Energy ( $\Delta G^{\circ}$ ) is the free energy change determined under standard conditions. While the optimum growth of most arsenic- and selenium-respiring bacteria cultivated in the laboratory occurs at non-standard conditions (e.g. 28–30°C, pH ~ 7–7.5), the standard free energy change values are regularly employed in scientific literature of bacterial energetics.

## Biogenic Se<sup>0</sup>

Se<sup>0</sup> biomineralization in bacteria results from detoxification and respiratory processes, and it generally produces amorphous minerals (Ruiz-Fresneda et al. 2023). In the case of detoxification, Se<sup>0</sup> particles form intracellularly, following the uncontrolled cellular uptake of toxic Se oxyanions (Ni et al. 2015). On the other hand, the respiratory-related biomineralization of Se is more complex and raises a number of unresolved questions. A major question is the fate of SeO<sub>3</sub><sup>2-</sup> produced via the respiration of SeO<sub>4</sub><sup>2-</sup> in the periplasmic space. Since SeO<sub>3</sub><sup>2-</sup> is toxic, the best option for bacteria would be to export it away from the cell.

*Thauera selenatis* ( $\beta$ -proteobacteria; Gram-negative/G-) was the first bacterium described to perform anaerobic respiration using selenate as an e-acceptor (Fig. 5B). It was isolated by Joan Macy and coworkers in 1989 from a bioreactor setting where Se-laden effluents were treated biologically. The reduction of selenate to selenite is coupled with the oxidation of acetate (e-donor) to CO<sub>2</sub> and intracellular polyhydroxybutyrate (PHB) granules (Macy et al. 1993). In *Thauera selenatis*, the respiration of selenate is driven by the periplasmic selenate respiratory enzyme SerABC (Schröder et al. 1997). SerABC is a trimeric molybdoenzyme with three homologous subunits: Ser A, which is the catalytic subunit and coordinates a molybdopterin cofactor; Ser B, an iron-sulfur protein rich in cysteine residues, with [3Fe-4S] and [4Fe-4S] clusters, and Ser C which contains a b-type cytochrome with a standard reduction potential of +234 mV (Schröder et al. 1997).

The complex SrdBCA was identified as the main player in selenate reduction in Gram-positive bacteria. Because Gram-positive/G+ bacteria lack an outer membrane and a periplasmic space, it is assumed that SrdBCA is membrane-bound (Fig. 5B) (Staicu and Barton 2021). This enzyme, isolated from *Bacillus selenatarsenatis* SF-1, is anchored in the membrane by its SrdC subunit, while SrdA (the catalytic subunit containing a MoCo cofactor) and SrdB are located outside the cell. Due to this structure, the Se<sup>0</sup> biomineralization process is likely to occur in the extracellular environment/vicinity of the cell wall. Similar to *Thauera selenatis*, an in-depth electron microscopy study is also much needed for Gram-positive bacteria capable of respiring selenate.

Selenite respiratory reductase has so far only been identified in *Bacillus selenitireducens* MLS10. This is encoded by an operon containing six genes: *srrA* (catalytic subunit of 80 kDa, with a TAT leader sequence and one putative 4Fe-4S binding site), *srrB* (small subunit of 17 kDa with 4 putative 4Fe-4S binding sites), *srrC* (43 kDa, anchoring subunit), *srrD* (24 kDa, chaperone protein), *srrE* (38 kDa) and *srrF* (45 kDa, rhodanese-domain containing proteins) (Wells et al. 2019).

A special case is extracellular respiration, where the electrons are routed in the periplasm to the cell wall anchoring the reductase (e.g. Mtr pathway from *Shewanella oneidensis* MR-1). The Mtr pathway contains five primary protein components: MtrC, STC, MtrA, MtrB, and CymA (Shi et al. 2007), MtrC and OmcA being located outside/in the vicinity of the cell wall (Fig. 5C). It has been demonstrated that the fumarate reductase FccA can replace STC in this pathway, being also involved in extracellular electron

transfer (Fonseca et al. 2013). While the mechanisms of extracellular respiration is well documented for dissimilatory iron reduction in the model organisms *Shewanella oneidensis* and *Geobacter sulfurreducens*, as well as in other organisms (Shi et al. 2016, Paquete et al. 2022), their involvement in the reduction of other solid compounds is less understood. Extracellular respiration has been well documented in the *Shewanella* and *Geobacter* genera (Gralnick and Newman 2007). An interesting strain of *Shewanella* that can respire As and Se oxyanions is *Shewanella* sp. O23S (related to *Shewanella baltica*). This strain was isolated from a former gold mine in Poland (Drewniak et al. 2015) and its genome was sequenced and analyzed (Uhrynowski et al. 2019). Notably, *Shewanella* sp. O23S can reduce selenite and selenate to Se<sup>0</sup> under both oxic and anoxic conditions. Anaerobically, the biomineralization process produces polydisperse, globular Se<sup>0</sup> particles attached to the surface of the bacterial cells, indicative of extracellular respiration (Fig. 6) (Staicu et al. 2022a). For an in-depth presentation of the molecular aspects of selenium respiration, the reader is referred to Wells and Stolz (2020) and Staicu and Barton (2021).

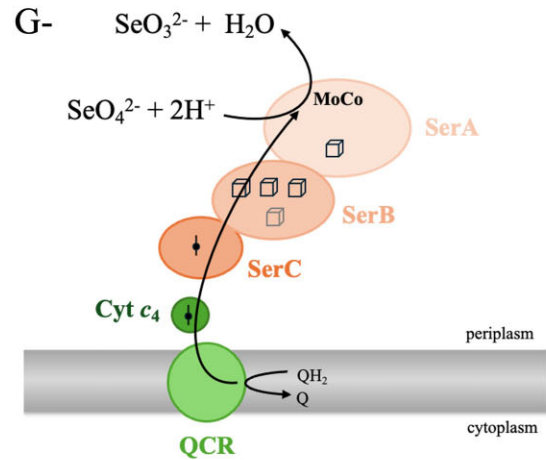
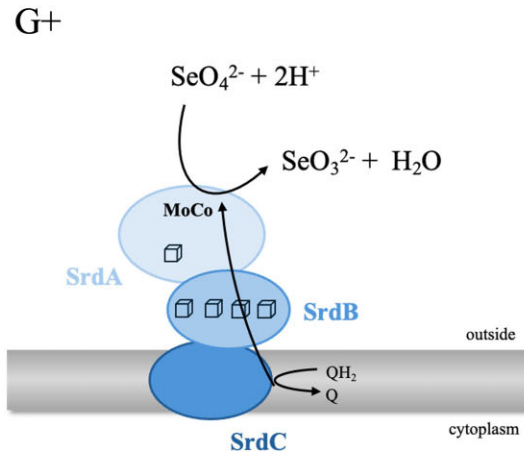
Transmission electron microscopy (TEM) coupled with Energy-dispersive X-ray spectroscopy (EDS) identified Se and S co-occurring in various ratios. This may indicate a potential contribution of S to the stability of biogenic Se<sup>0</sup>. X-ray Diffraction (XRD) and Raman analysis identified amorphous Se<sup>0</sup> biominerals (Staicu et al. 2022a). When the surface charge (zeta potential) of the control incubation (nitrate was used as e-acceptor) is compared with that of solutions containing biogenic Se<sup>0</sup>, a marked difference is observed. The zeta potential value of the control is around 0 mV, while the values of Se<sup>0</sup> biomineral solutions drop to -20 to -30 mV. This may indicate an active accumulation of proteins during the biomineralization process (Staicu et al. 2017). *Shewanella* sp. O23S has also been tested in a bioremediation approach using real metal-rich industrial effluents (Staicu et al. 2015), showing promising results for the removal of As, Se and various metals (Staicu et al. 2022b).

## Biogenic AsS

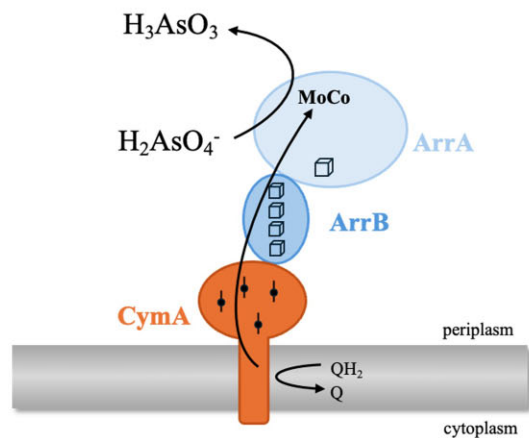
Despite its toxicity, arsenic can be used by certain bacteria to sustain growth. Microbial respiration using AsO<sub>4</sub><sup>3-</sup> as an e-acceptor was discovered around 1994, when strain MIT-13 (Ahmann et al. 1994), later classified as *Sulfurospirillum arsenophilum* (Stolz et al. 1999), was isolated from Aberjona watershed (Boston, Massachusetts), a heavily urbanized river historically polluted with metals and arsenic. Later, it was demonstrated that arsenate reduction is accomplished by an operon whose structure varies across bacteria, featuring different anchoring sub-units (e.g. ArrC, CymA), chaperone proteins (ArrD) and regulatory elements (Fig. 5D). The core enzyme (Arr) is highly conserved, containing the large catalytic subunit (ArrA) and the smaller iron-sulfur cluster subunit (ArrB) (Krafft and Macy 1998). The arsenate reductase from *Shewanella* sp. ANA-3 is a heterodimer (131 kDa): ArrA (95 kDa) and ArrB (27 kDa). It contains one molybdenum (Mo) atom, four sulfur (S) atoms associated with a bis-molybdopterin guanine dinucleotide cofactor, and several [4Fe-4S] clusters (Glasser et al. 2018). It shows no activity in the presence of alternative electron acceptors such as antimonite, nitrate, selenate, and sulfate (Malasarn et al. 2008).

*Sulfurospirillum arsenophilum* uses lactate as e-donor and the respiratory enzyme is located in the periplasmic space. A model of arsenate respiration in Gram-negative bacteria is presented in Fig. 6D. The respiration of arsenate does not lead to the formation of arsenic biominerals directly, unlike in the case of sele-

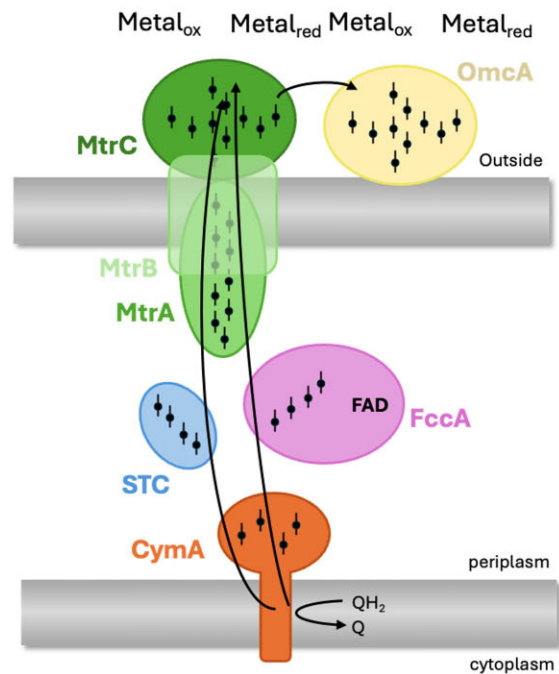
## (A) Selenate respiration



## (B) Arsenate respiration



## (C) External electron transfer



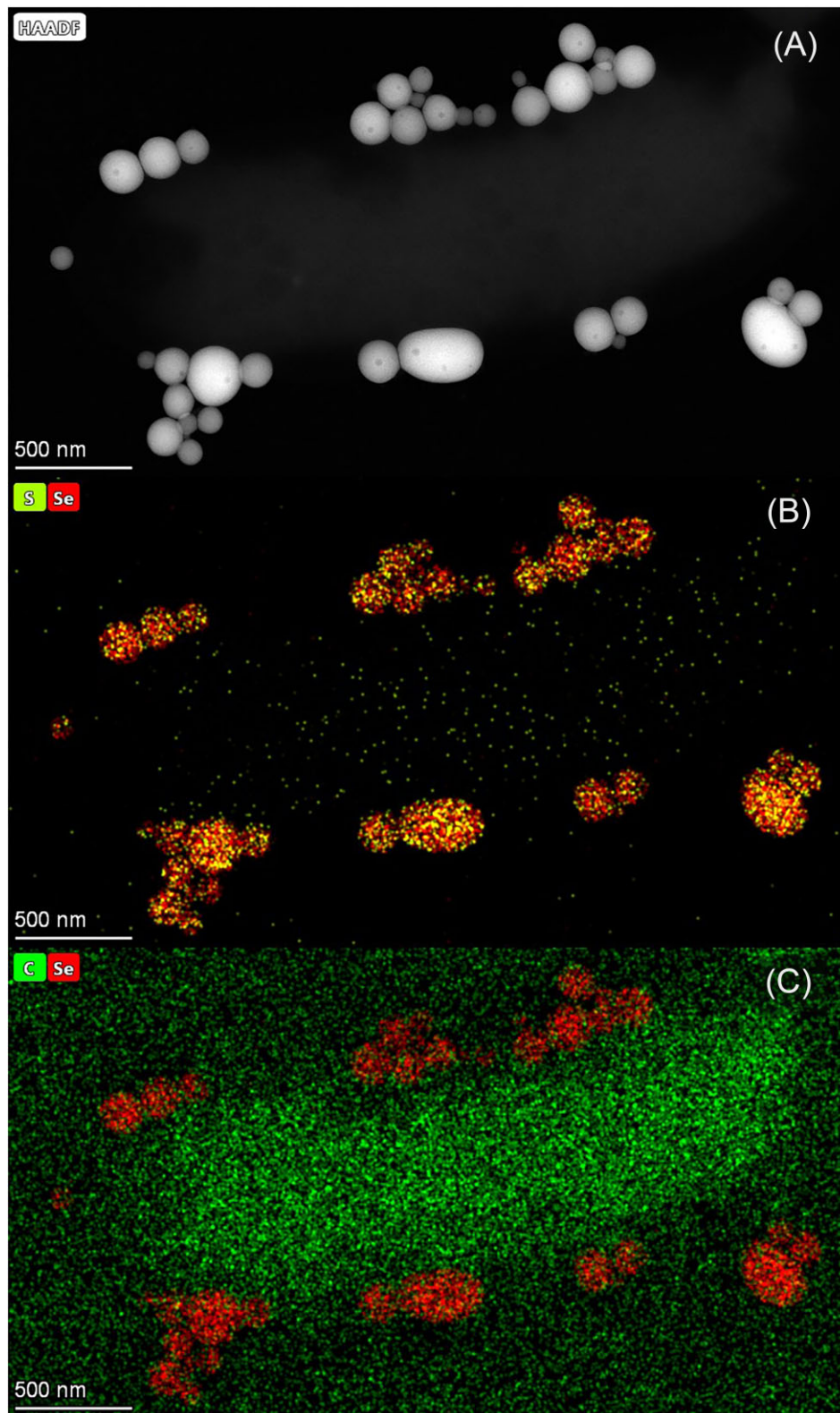
**Figure 5.** Reductive pathways of various electron acceptors, with the proteins involved: (A) Selenate respiration (Kuroda et al. 2011); (B) Arsenate respiration (Glasser et al. 2018); (C) External electron transfer (Lemaire et al. 2020). **Acronyms:** ArrAB: Arsenate reductase; CymA: Tetraheme c-type cytochrome; Cyt c<sub>4</sub>: Cytochrome c<sub>4</sub>; FAD: Flavin Adenine Dinucleotide (electron carrier); FccA: Fumarate reductase; G+: Gram positive; G-: Gram negative; MoCo: Molybdenum cofactor; MtrA: Periplasmic decaheme c-type cytochrome; MtrB: Multiheme cytochrome; MtrC: Outer membrane cytochrome; OmcA: Outer membrane cytochrome; Q: Ubiquinone; QH<sub>2</sub>: Ubiquinol; QCR: Quinol-cytochrome c reductase; STC: Small tetraheme cytochrome; SerABC: Selenate respiratory reductase from *Thauera selenatis*; SrdABC: Selenate respiratory reductase from *Bacillus selenatarsenatis* SF-1.

nate and selenite respiration. Both arsenate and arsenite are water soluble, but the generation of As(III) via anaerobic respiration can be coupled with highly reactive hydrogen sulfides (H<sub>2</sub>S), yielding AsS biominerals. H<sub>2</sub>S is a byproduct of various metabolic processes, such as sulfate respiration or cysteine desulfurization, and is widely present in nature. For a more comprehensive presentation of this process, the reader is referred to Stolz et al. (2006) and Glasser et al. (2018).

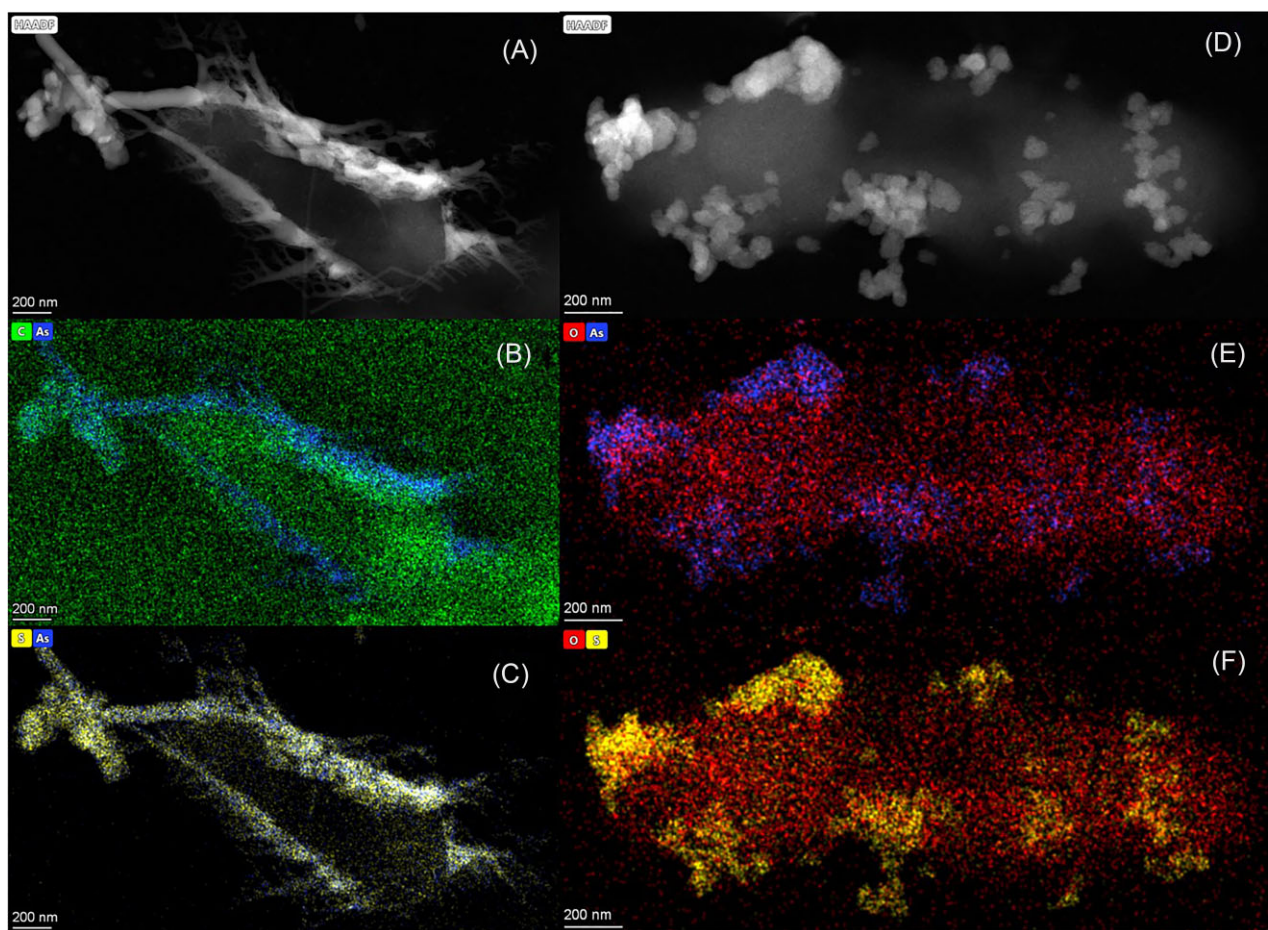
*Shewanella* sp. O23S can also respire arsenate (the *arr* operon is located on the pSheB plasmid) (Uhrynowski et al. 2019). When respiring arsenate to arsenite and degrading cysteine to H<sub>2</sub>S, the

strain was shown to produce extracellular AsS biominerals of different morphologies: nanorod AsS (realgar) and granular (orpiment) As<sub>2</sub>S<sub>3</sub> (Fig. 7).

Similar to biogenic Se<sup>0</sup>, AsS biominerals produced by this strain are amorphous, and the solutions exhibit a negative surface charge (-20 to -30 mV). Interestingly, when Se oxyanions and arsenate are mixed in the same system, the strain produces AsS and Se<sup>0</sup> minerals in parallel (Staicu et al. 2022a). This suggests that the strain may utilize different biochemical pathways to transform As and Se. Indeed, the combination of the two elements results in both inhibitory and stimulatory outcomes in terms of reduction



**Figure 6.** Biogenic  $\text{Se}^0$  produced by *Shewanella* sp. O23S. Micrographs with the corresponding EDS elemental maps obtained from bacterial cells and biomineralization products: A) Scanning transmission electron microscopy (STEM mode); B) Se elemental mapping; C) Sulfur elemental mapping (Staicu et al. 2022a).



**Figure 7.** Biogenic AsS produced by *Shewanella* sp. O23S. Micrographs with the corresponding EDS elemental maps obtained from bacterial cells and biomineralization products: (A) Scanning transmission electron microscopy (STEM mode) of nanorod (AsS) structures; (B) Arsenic elemental mapping; (C) Sulfur elemental mapping; (D) Scanning transmission electron microscopy (STEM mode) of granular ( $\text{As}_2\text{S}_3$ ) structures; (E) Arsenic elemental mapping; (F) Sulfur elemental mapping (Staicu et al. 2022a).

kinetics, depending on the oxyanion couple (arsenate-selenite and arsenate-selenate, respectively). It may be that *Shewanella* sp. O23S employs a multi-substrate respiratory enzyme. However, this does not exclude the presence of detoxification reactions in conjunction with the respiratory process. The strain possesses the detoxification operon (*ars*) on the same plasmid that harbors the *arr* operon (Uhrynowski et al. 2019), and it is widely established that selenium detoxification occurs through a number of strategies (e.g. glutathione reductase).

## Perspectives

The microbial formation of iron minerals, in particular by Fe(II)-oxidizing microorganisms but also by Fe(III)-reducers, has been studied intensively in the last two to three decades and the geochemical parameters controlling the identity and properties of iron biominerals are well understood. However, the potential for applying iron biominerals in bioremediation (removing pollutants such as certain toxic metals), for urban mining and biomining (harvesting precious resources such as phosphates and rare earth elements), as catalysts in biotechnological processes (e.g. biofuel production), and for energy/electron storage (biogeobatteries) has been underexplored so far and should be the focus of future studies.

Research on iron sulfide biomineralization has advanced considerably in the past few years. Nonetheless, intriguing questions remain, particularly regarding how microorganisms regulate the formation of extracellular greigite and pyrite framboids. Future cultivation studies that incorporate mixed microbial communities, fluctuating redox conditions, and natural sediment components (e.g. clays, calcite, quartz) may help to answer these questions.

Regarding  $\text{S}^0$ , an open question remains to determine how  $\text{S}^0$ -biomineralizing microbes control the properties of their biominerals, which differ significantly from their abiotic counterparts in terms of size, morphology, surface charge, or crystal structure. While experiments in abiotic (Cosmidis et al. 2019) and microbial systems (Cron et al. 2019) have revealed a strong influence of organics on  $\text{S}^0$  mineralogical properties, the specific biomolecules deployed by microbes to control their biomineralization products remain to be identified. A deeper understanding of these molecular controls would unlock the possibility to engineer  $\text{S}^0$  biominerals for different technological applications.  $\text{S}^0$  can, for instance, be applied in water treatment, where it can feed autotrophic denitrification. In addition,  $\text{S}^0$  reduction can be associated with organic matter remineralization or metal pollutant remediation, applications for which  $\text{S}^0$  properties such as bioavailability, small particle size, and hydrophilicity are critical (Zhang et al. 2021). There is

also growing interest for using microbially derived components in battery technologies (Li et al. 2024), and it has been suggested that microbial S<sup>0</sup> biominerals are interesting candidates to be used as cathode materials in high-capacity Li-S batteries, provided that we can gain better control of their yield and properties.

An open question related to biogenic AsS is whether the two observed morphologies (granules and wires/rods) are produced at the same time or if one morphology evolves from the other as a function of incubation time (e.g. AsS evolving from As<sub>2</sub>S<sub>3</sub>). The size of biogenic Se<sup>0</sup> is puzzling, requiring clarification with regard to its intracellular formation and its potential export to the extracellular milieu without affecting the bacterial cell. In general, the intra- or extracellular localization of the minerals can pose various challenges to cellular integrity and viability. A major research topic pertains to the existence of biological functions in the genetically unregulated production of microbial biominerals—a fertile domain in need of future exploration (Cosmidis and Benzerara 2022).

## Acknowledgements

L.S. acknowledges Romania—U.S. Fulbright Commission for the Fulbright Scholar Visiting Award 2024–2024 (Grant # 869) and Prof. John Stolz from Duquesne University, Pittsburgh, USA. M.M. acknowledges the support by the German Research Foundation (DFG), project ID 494840258 and 503493769. C.M.P. acknowledges MOSTMICRO-ITQB base funding with references UIDB/04612/2020 and UIDP/04612/2020 and LS4FUTURE Associated Laboratory (LA/P/0087/2020). J.C. is supported by the European Union's Horizon H2020 research and innovation programme ERC (BioFacts, grant agreement 101076666).

## Author contributions

Lucian C. Staicu (Conceptualization, Writing – original draft), Julie Cosmidis (Conceptualization, Writing – original draft), Muammar Mansor (Conceptualization, Writing – original draft), Catarina M. Paquete (Conceptualization, Writing – original draft), and Andreas Kappler (Conceptualization, Writing – original draft)

Conflict of interest: None declared.

## References

- Aghababae M, Farhadian M, Jeyhanipour A et al. Effective factors on the performance of microbial fuel cells in wastewater treatment—a review. *Environ Technol Rev* 2015;**4**: 71–89. <https://doi.org/10.1080/09593330.2015.1077896>.
- Ahmann D, Roberts AL, Krumholz LR et al. Microbe grows by reducing arsenic. *Nature* 1994;**371**: 750. <https://doi.org/10.1038/371750a0>.
- Allen KD, Wegener G, Sublett DM et al. Biogenic formation of amorphous carbon by anaerobic methanotrophs and select methanogens. *Sci Adv* 2021;**7**: eabg9739. <https://doi.org/10.1126/sciadv.abg9739>.
- Amend JP, LaRowe DE. Minireview: demystifying microbial reaction energetics. *Environ Microbiol* 2019;**2**: 3539–47. <https://doi.org/10.1111/1462-2920.14778>.
- Amor M, Mathon FP, Monteil CL et al. Iron-biomineralizing organelle in magnetotactic bacteria: function, synthesis and preservation in ancient rock samples. *Environ Microbiol* 2020;**22**: 3611–32. <https://doi.org/10.1111/1462-2920.15098>.
- Avetisyan K, Zweig I, Luther GW et al. Kinetics and mechanism of polysulfides and elemental sulfur formation by a reaction between hydrogen sulfide and δ-MnO<sub>2</sub>. *Geochim Cosmochim Acta* 2021;**313**: 21–37. <https://doi.org/10.1016/j.gca.2021.08.022>.
- Berg JS, Duverger A, Cordier L et al. Rapid pyritization in the presence of a sulfur/sulfate-reducing bacterial consortium. *Sci Rep* 2020;**10**: 1–13. <https://doi.org/10.1038/s41598-020-64990-6>.
- Berg JS, Schwedt A, Kreuzmann A-C et al. Polysulfides as intermediates in the oxidation of sulfide to sulfate by *Beggiatoa* spp. *Appl Environ Microb* 2014;**80**: 629–36. <https://doi.org/10.1128/AEM.02852-13>.
- Berner RA. Biogeochemical cycles of carbon and sulfur and their effect on atmospheric oxygen over phanerozoic time. *Glob Planet Change* 1989;**1**: 97–122. [https://doi.org/10.1016/0921-8181\(89\)90018-0](https://doi.org/10.1016/0921-8181(89)90018-0).
- Borch T, Kretzschmar R, Kappler A et al. Biogeochemical redox processes and their impact on contaminant dynamics. *Environ Sci Technol* 2010;**44**: 15–23. <https://doi.org/10.1021/es9026248>.
- Boyd ES, Payne D. Expanded diversity of microbial groups capable of anaerobic pyrite reduction and assimilation of dissolution products. *Environ Microbiol* 2025;**27**: e70125. <https://doi.org/10.1111/1462-2920.70125>.
- Bronner R, Thompson K, Dreher C et al. Co-reduction of Fe(III) and S<sup>0</sup> drives Fe-S biomineral formation and phosphate mobilisation. *Geochim Perspect Lett* 2023;**24**: 27–32. <https://doi.org/10.7185/geochemlet.2301>.
- Bruno DC. Isolation and characterization of sulfur globule proteins from *Chromatium vinosum* and *Thiocapsa roseopersicina*. *Arch Microbiol* 1995;**163**: 391–9. <https://doi.org/10.1007/BF00272127>.
- Byrne JM, van der Laan G, Figueroa AI et al. Size dependent microbial oxidation and reduction of magnetite nano- and micro-particles. *Sci Rep* 2016;**6**: 30969. <https://doi.org/10.1038/srep30969>.
- Canfield DE, Farquhar J. Animal evolution, bioturbation, and the sulfate concentration of the oceans. *Proc Natl Acad Sci* 2009;**106**: 8123–7. <https://doi.org/10.1073/pnas.0902037106>.
- Canfield DE. The early history of atmospheric oxygen: homage to Robert M. Garrels. *Annu Rev Earth Planet Sci* 2005;**33**: 1–36. <https://doi.org/10.1146/annurev.earth.33.092203.122711>.
- Caraballo MA, Asta MP, Perez JPH et al. Past, present and future global influence and technological applications of iron-bearing metastable nanominerals. *Gondwana Res* 2022;**110**: 283–304. <https://doi.org/10.1016/j.gr.2021.11.009>.
- Cosmidis J, Benzerara K. Why do microbes make minerals? *CR Geosci* 2022;**354**: 1–39. <https://doi.org/10.5802/crgeos.107>.
- Cosmidis J, Nims CW, Diercks D et al. Formation and stabilization of elemental sulfur through organomineralization. *Geochim Cosmochim Acta* 2019;**247**: 59–82. <https://doi.org/10.1016/j.gca.2018.12.025>.
- Cosmidis J. Will tomorrow's mineral materials be grown? *Microb Biotechnol* 2023;**16**: 1713–22. <https://doi.org/10.1111/1751-7915.14298>.
- Costa NL, Clarke TA, Philipp L-A et al. Electron transfer process in microbial electrochemical technologies: the role of cell-surface exposed conductive proteins. *Bioresour Technol* 2018;**255**: 308–17. <https://doi.org/10.1016/j.biortech.2018.01.133>.
- Cron B, Henri P, Chan CS et al. Elemental sulfur formation by *Sulfuricum kujiense* is mediated by extracellular organic compounds. *Front Microbiol* 2019;**10**: 2710. <https://doi.org/10.3389/fmicb.2019.02710>.
- Cron B, Macalady JL, Cosmidis J. Organic stabilization of extracellular elemental sulfur in a sulfurovum-rich biofilm: a new role for EPS?

- Front Microbiol 2021;**12**: 720101. <https://doi.org/10.3389/fmicb.2021.720101>.
- Dahl C. A biochemical view on the biological sulfur cycle. In: Lens PNL (ed.), *Environmental Technologies to Treat Sulfur Pollution: principles and Engineering*. London, UK: IWA Publishing, 2020a,55–96. <https://doi.org/10.2166/9781789060966>.
- Dahl C. Bacterial intracellular sulphur globules. In: Jendrossek D (ed.), *Bacterial Organelles and Organelle-like Inclusions*. Cham: Springer International Publishing, 2020b, 19–51.
- Dreher CL, Schad M, Duda JP et al. Impact of silica on the identity of minerals formed in Archean oceans during Fe cycling by cyanobacteria and iron(III)-reducing bacteria. *Geochim Cosmochim Acta* 2025;**404**: 202–22. (in press). <https://doi.org/10.1016/j.gca.2025.07.023>.
- Drewniak L, Stasiuk R, Uhrynowski W et al. *Shewanella* sp. O23S as a driving agent of a system utilizing dissimilatory arsenate-reducing bacteria responsible for self-cleaning of water contaminated with arsenic. *Int J Mol Sci* 2015;**16**: 14409–27. <https://doi.org/10.3390/ijms160714409>.
- Duverger A, Berg JS, Busigny V et al. Mechanisms of pyrite formation promoted by sulfate-reducing bacteria in pure culture. *Front Earth Sci (Lausanne)* 2020;**8**: 1–15.
- Eickhoff M, Obst M, Schröder C et al. Nickel partitioning in biogenic and abiogenic ferrihydrite: the influence of silica and implications for ancient environments. *Geochim Cosmochim Acta* 2014;**14**: 65–79. <https://doi.org/10.1016/j.gca.2014.05.021>.
- Florentino AP, Stams AJM, Sánchez-Andrea I. Genome sequence of *desulfurella amilii* strain TR1 and comparative genomics of desulfurellaceae family. *Front Microbiol* 2017;**8**: 222. <https://doi.org/10.3389/fmicb.2017.00222>.
- Fonseca BM, Paquete CM, Neto SE et al. Mind the gap: cytochrome interactions reveal electron pathways across the periplasm of *Shewanella oneidensis* MR-1. *Biochem J* 2013;**449**: 101–8. <https://doi.org/10.1042/BJ20121467>.
- Gadd GM. Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology* 2010;**156**: 609–43. <https://doi.org/10.1099/mic.0.037143-0>.
- Ganie SY, Javaid D, Hajam YA et al. Arsenic toxicity: sources, pathophysiology and mechanism. *Toxicol Res* 2024;**13**: tfad111. <https://doi.org/10.1093/toxres/tfad111>.
- Garber AI, Nealson KH, Okamoto A et al. FeGenie: a comprehensive tool for the identification of iron genes and iron gene neighborhoods in genome and metagenome assemblies. *Front Microbiol* 2020;**11**: 37. <https://doi.org/10.3389/fmicb.2020.00037>.
- Glasser NR, Oyala PH, Osborne TH et al. Structural and mechanistic analysis of the arsenate respiratory reductase provides insight into environmental arsenic transformations. *Proc Natl Acad Sci* 2018;**115**:E8614–23. <https://doi.org/10.1073/pnas.1807984115>.
- Glasser NR, Saunders SH, Newman DK. The colorful world of extracellular electron shuttles. *Annu Rev Microbiol* 2017;**71**: 731–51. <https://doi.org/10.1146/annurev-micro-090816-093913>.
- Gleeson DF, Pappalardo RT, Grasby SE et al. Characterization of a sulfur-rich Arctic spring site and field analog to Europa using hyperspectral data. *Remote Sens Environ* 2010;**114**: 1297–311. <https://doi.org/10.1016/j.rse.2010.01.011>.
- Gorlas A, Jacquemot P, Guigner JM et al. Greigite nanocrystals produced by hyperthermophilic archaea of the thermococcales order. *PLoS One* 2018;**13**: 1–10. <https://doi.org/10.1371/journal.pone.0201549>.
- Gorlas A, Mariotte T, Morey L et al. Precipitation of greigite and pyrite induced by Thermococcales: an advantage to live in Fe- and S-rich environments? *Environ Microbiol* 2022;**24**: 626–42. <https://doi.org/10.1111/1462-2920.15915>.
- Gralnick JA, Newman DK. Extracellular respiration. *Mol Microbiol* 2007;**65**: 1–11. <https://doi.org/10.1111/j.1365-2958.2007.05778.x>.
- Hamilton TL, Jones DS, Schaperdoth I et al. Metagenomic insights into S(0) precipitation in a terrestrial subsurface lithoautotrophic ecosystem. *Front Microbiol* 2015;**5**: 756. <https://doi.org/10.3389/fmicb.2014.00756>.
- Han X, Tomaszewski EJ, Sorwat J et al. Oxidation of green rust by anoxygenic phototrophic Fe(II)-oxidizing bacteria. *Geochem Perspect Lett* 2020;**12**: 52–7. <https://doi.org/10.7185/geochemlet.2004>.
- Hanson TE, Bonsu E, Tuerk A et al. *Chlorobaculum tepidum* growth on biogenic S(0) as the sole photosynthetic electron donor. *Environ Microbiol* 2016;**18**: 2856–67. <https://doi.org/10.1111/1462-2920.12995>.
- Hedderich R, Klimmek O, Kröger A et al. Anaerobic respiration with elemental sulfur and with disulfides. *FEMS Microbiol Rev* 1998;**22**: 353–81. <https://doi.org/10.1111/j.1574-6976.1998.tb00376.x>.
- Hegler F, Schmidt C, Schwarz H et al. Does a low pH-microenvironment around phototrophic Fe<sup>II</sup>-oxidizing bacteria prevent cell encrustation by Fe<sup>III</sup> minerals? *FEMS Microbiol Ecol* 2010;**74**: 592–600. <https://doi.org/10.1111/j.1574-6941.2010.00975.x>.
- Heim C, Simon K, Ionescu D et al. Assessing the utility of trace and rare earth elements as biosignatures in microbial iron oxyhydroxides. *Front Earth Sci* 2015;**3**: 6. <https://doi.org/10.3389/feart.2015.00006>.
- Hohmann C, Winkler E, Morin G et al. Anaerobic Fe(II)-oxidizing bacteria show As resistance and co-precipitate As during Fe(III) mineral precipitation. *Environ Sci Technol* 2010;**44**: 94–101. <https://doi.org/10.1021/es900708s>.
- Ikogou M, Ona-Nguema G, Juillot F et al. Long-term sequestration of nickel in mackinawite formed by *Desulfovibrio capillatus* upon Fe(III)-citrate reduction in the presence of thiosulfate. *Appl Geochem* 2017;**80**: 143–54. <https://doi.org/10.1016/j.apgeochem.2017.02.019>.
- Jakus N, Mellage A, Hoeschen C et al. Anaerobic neutrophilic pyrite oxidation by a chemolithoautotrophic nitrate-reducing iron(II)-oxidizing culture enriched from a fractured-aquifer. *Environ Sci Technol* 2021;**55**: 9876–84. <https://doi.org/10.1021/acs.est.1c02049>.
- Jia T, Niu L, Qi Z et al. The biosynthesis, storage and utilization of elemental sulfur: enzymatic pathways, molecular mechanisms, and future perspectives. *Crit Rev Environ Sci Technol* 2024;**55**: 483–506. <https://doi.org/10.1080/10643389.2024.2421087>.
- Jiang X, Hu J, Lieber AM et al. Nanoparticle facilitated extracellular electron transfer in microbial fuel cells. *Nano Lett* 2014;**14**: 6737–42. <https://doi.org/10.1021/nl503668q>.
- Jørgensen BB, Findlay AJ, Pellerin A. The biogeochemical sulfur cycle of marine sediments. *Front Microbiol* 2019;**10**: 1–27.
- Jørgensen BB. Sulfur biogeochemical cycle of marine sediments. *Geochem Perspect* 2021;**10**: 145–6. <https://doi.org/10.7185/geochempersp.10.2>.
- Kamyschny A, Ferdelman TG. Dynamics of zero-valent sulfur species including polysulfides at seep sites on intertidal sand flats (Wadden Sea, North Sea). *Mar Chem* 2010;**121**: 17–26. <https://doi.org/10.1016/j.marchem.2010.03.001>.
- Kappler A, Bryce C, Mansor M et al. An evolving view on biogeochemical cycling of iron. *Nat Rev Micro* 2021;**19**: 360–74. <https://doi.org/10.1038/s41579-020-00502-7>.
- Kappler A, Schink B, Newman DK. Fe(III)-mineral formation and cell encrustation by the nitrate-dependent Fe(II)-oxidizer strain BoFeN1. *Geobiology* 2005;**3**: 235–45. <https://doi.org/10.1111/j.1472-4669.2006.00056.x>.

- Kleinjan WE, de Keizer A, Janssen AJH. Equilibrium of the reaction between dissolved sodium sulfide and biologically produced sulfur. *Colloids Surf B Biointerfaces* 2005;**43**: 228–37. <https://doi.org/10.1016/j.colsurfb.2005.05.004>.
- Kleinjan WE, Keizer A, Janssen AJH. Biologically produced sulfur. In: Steudel R (ed.), *Elemental Sulfur and Sulfur-rich Compounds*. Berlin, Heidelberg: Springer, 2003, 167–88.
- Koeksoy E, Halama M, Hagemann N et al. A case study for late archaean and proterozoic biogeochemical iron- and sulphur cycling in a modern habitat-the Arvadi Spring. *Geobiology* 2018;**16**: 353–68. <https://doi.org/10.1111/gbi.12293>.
- Kondo K, Okamoto A, Hashimoto K et al. Sulfur-mediated electron shuttling sustains microbial long-distance extracellular electron transfer with the aid of metallic iron sulfides. *Langmuir* 2015;**31**: 7427–34. <https://doi.org/10.1021/acs.langmuir.5b01033>.
- Konhauser K, Riding R. Bacterial biomineralization. In: Knoll AH, Canfield DE, Konhauser KO (eds.), *Fundamentals of Geobiology*. Hoboken, NJ: John Wiley and Sons, 2012, 105–30. <https://doi.org/10.1002/9781118280874>.
- Kovalick A, Heard AW, Johnson AC et al. Living in their heyday: iron-oxidizing bacteria bloomed in shallow-marine, subtidal environments at ca. 1.88 Ga. *Geobiology* 2024;**22**: e70003. <https://doi.org/10.1111/gbi.70003>.
- Krafft T, Macy JM. Purification and characterization of the respiratory arsenate reductase of *Chrysiogenes arsenatis*. *Eur J Biochem* 1998;**255**: 647–53. <https://doi.org/10.1046/j.1432-1327.1998.2550647.x>.
- Krepski ST, Emerson D, Hredzak-Showalter PL et al. Morphology of biogenic iron oxides records microbial physiology and environmental conditions: toward interpreting iron microfossils. *Geobiology* 2013;**11**: 457–71. <https://doi.org/10.1111/gbi.12043>.
- Kümpel C, Grein F, Dahl C. Fluorescence microscopy study of the intracellular sulfur globule protein SgpD in the purple sulfur bacterium *allochromatium vinosum*. *Microorganisms* 2023;**11**: 1792.
- Kuroda M, Yamashita M, Miwa E et al. Molecular cloning and characterization of the *srdBCA* operon, encoding the respiratory selenate reductase complex, from the selenate-reducing bacterium *Bacillus selenatarsenatis* SF-1. *J Bacteriol* 2011;**193**: 2141–8. <https://doi.org/10.1128/JB.01197-10>.
- Laufer K, Nordhoff M, Halama M et al. Microaerophilic Fe(II)-oxidizing zetaproteobacteria isolated from low-Fe marine coastal sediments: physiology and composition of their twisted stalks. *Appl Environ Microb* 2017;**83**: e03118–16. <https://doi.org/10.1128/AEM.03118-16>.
- Lavik G, Stührmann T, Brüchert V et al. Detoxification of sulphidic African shelf waters by blooming chemolithotrophs. *Nature* 2009;**457**: 581–4. <https://doi.org/10.1038/nature07588>.
- Lemaire ON, Méjean V, Iobbi-Nivol C. The *Shewanella* genus: ubiquitous organisms sustaining and preserving aquatic ecosystems. *FEMS Microbiol Rev* 2020;**44**: 155–70. <https://doi.org/10.1093/femsre/fuz031>.
- Li F, Cai J, Zhao X et al. Research progress in the remediation of arsenic- and cadmium-contaminated groundwater mediated by iron and manganese biomineralization. *Catalysts* 2025;**15**: 570. <https://doi.org/10.3390/catal15060570>.
- Li S, Lin X, Wang S et al. Bacteria derived nanomaterials for lithium-based batteries. *Carbon* 2024;**216**: 118564. <https://doi.org/10.1016/j.carbon.2023.118564>.
- Li W, Zhang M, Kang D et al. Mechanisms of sulfur selection and sulfur secretion in a biological sulfide removal (BISURE) system. *Env iron Int* 2020;**137**: 105549. <https://doi.org/10.1016/j.envint.2020.105549>.
- Lin W, Paterson GA, Zhu Q et al. Origin of microbial biomineralization and magnetotaxis during the Archean. *Proc Natl Acad Sci* 2017;**114**: 2171–6. <https://doi.org/10.1073/pnas.1614654114>.
- Liu X, Shi L, Gu JD. Microbial electrocatalysis: redox mediators responsible for extracellular electron transfer. *Biotechnol Adv* 2018;**36**: 1815–27. <https://doi.org/10.1016/j.biotechadv.2018.07.001>.
- Liu Y, Zhao Q, Liao C et al. Anaerobic bioreduction of elemental sulfur improves bioavailability of Fe (III) oxides for bioremediation. *Sci Total Environ* 2023;**858**: 159794. <https://doi.org/10.1016/j.scitotenv.2022.159794>.
- Locher KP. Structure and mechanism of ATP-binding cassette transporters. *Philos Trans R Soc B, Biol Sci* 2009;**364**: 239–45. <https://doi.org/10.1098/rstb.2008.0125>.
- Luther GW, Findlay AJ, MacDonald DJ et al. Thermodynamics and kinetics of sulfide oxidation by oxygen: a look at inorganically controlled reactions and biologically mediated processes in the environment. *Front Microbiol* 2011;**2**: 62. <https://doi.org/10.3389/fmicb.2011.00062>.
- Ma K, Adams MW. Sulfide dehydrogenase from the hyperthermophilic archaeon *pyrococcus furiosus*: a new multifunctional enzyme involved in the reduction of elemental sulfur. *J Bacteriol* 1994;**176**: 6509–17. <https://doi.org/10.1128/jb.176.21.6509-6517.1994>.
- Macy JM, Rech S, Auling G et al. *Thauera selenatis* gen. nov., sp. nov., a member of the beta subclass of Proteobacteria with a novel type of anaerobic respiration. *Int J Syst Bacteriol* 1993;**43**: 135–42. <https://doi.org/10.1099/00207713-43-1-135>.
- Malasarn D, Keeffe JR, Newman DK. Characterization of the arsenate respiratory reductase from *Shewanella* sp. strain ANA-3. *J Bacteriol* 2008;**190**: 135–42. <https://doi.org/10.1128/JB.01110-07>.
- Mansor M, Berti D, Hochella MF et al. Phase, morphology, elemental composition and formation mechanisms of biogenic and abiogenic Fe-Cu-sulfide nanoparticles: a comparative study on their occurrences under anoxic conditions. *Am Min* 2019a;**104**: 703–17. <https://doi.org/10.2138/am-2019-6848>.
- Mansor M, Winkler C, Hochella MF et al. Nanoparticulate nickel-hosting phases in sulfidic environments: effects of ferrous iron and bacterial presence on mineral formation mechanism and solid-phase nickel distribution. *Front Earth Sci—Earth and Planetary Materials* 2019b;**7**: 151.
- Mansor M, Duverger A, Pasquier V et al. Biogenic pyrite and metastable iron sulfides: emerging formation pathways and geological and societal relevance. *Geo-Bio Interface* 2025;**2**: 1–31. <https://doi.org/10.1180/gbi.2024.9>.
- Marnocha CL, Sabanayagam CR, Modla S et al. Insights into the mineralogy and surface chemistry of extracellular biogenic S<sup>0</sup> globules produced by *chlorobaculum tepidum*. *Front Microbiol* 2019;**10**: 271. <https://doi.org/10.3389/fmicb.2019.00271>.
- Marshall IPG, Blainey PC, Spormann AM et al. A single-cell genome for *thiovulum* sp. *Appl Environ Microbiol* 2012;**78**: 8555–63. <https://doi.org/10.1128/AEM.02314-12>.
- Mas J, Gernerden H. Storage products in purple and green sulfur bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds.) *Anoxygenic Photosynthetic Bacteria*. Advances in Photosynthesis and Respiration. Dordrecht: Springer, 1995, 973–90.
- Miot J, Benzerara K, Morin M et al. Iron biomineralization by neutrophilic iron-oxidizing bacteria. *Geochim Cosmochim Acta* 2009a;**73**: 696–711. <https://doi.org/10.1016/j.gca.2008.10.033>.

- Miot J, Benzerara K, Morin M et al. Transformation of vivianite by anaerobic nitrate-reducing iron-oxidizing bacteria. *Geobiology* 2009b;**7**: 373–84. <https://doi.org/10.1111/j.1472-4669.2009.00203.x>.
- Nabeh N, Brokaw C, Picard A. Quantification of organic carbon sequestered by biogenic iron sulfide minerals in long-term anoxic laboratory incubations. *Front Microbiol* 2022;**13**: 662219. <https://doi.org/10.3389/fmicb.2022.662219>.
- Nakamura R, Okamoto A, Tajima N et al. Biological iron-monosulfide production for efficient electricity harvesting from a deep-sea metal-reducing bacterium. *ChemBioChem* 2010;**11**: 643–5. <https://doi.org/10.1002/cbic.200900775>.
- Nelson DC, Castenholz RW. Use of reduced sulfur compounds by *beggiatoa* sp. *J Bacteriol* 1981;**147**: 140–54. <https://doi.org/10.1128/jb.147.1.140-154.1981>.
- Ni TW, Staicu LC, Nemeth RS et al. Progress toward clonable inorganic nanoparticles. *Nanoscale* 2015;**7**: 17320–7. <https://doi.org/10.1039/C5NR04097C>.
- Nims C, Cron B, Wetherington M et al. Low frequency raman spectroscopy for micron-scale and in vivo characterization of elemental sulfur in microbial samples. *Sci Rep* 2019;**9**: 7971. <https://doi.org/10.1038/s41598-019-44353-6>.
- Ohde T, Siegel H, Reißmann J et al. Identification and investigation of sulphur plumes along the Namibian coast using the MERIS sensor. *Cont Shelf Res* 2007;**27**: 744–56. <https://doi.org/10.1016/j.csr.2006.11.016>.
- Ohfuji H, Rickard D. Experimental syntheses of frambooids—a review. *Earth Sci Rev* 2005;**71**: 147–70. <https://doi.org/10.1016/j.earscirev.2005.02.001>.
- Omeregic EO, Couture RM, Van Cappellen P et al. Arsenic bioremediation by biogenic iron oxides and sulfides. *Appl Environ Microb* 2013;**79**: 4325–35. <https://doi.org/10.1128/AEM.00683-13>.
- Paquete CM, Fonseca BM, Cruz DR et al. Exploring the molecular mechanisms of electron shuttling across the microbe/metal space. *Front Microbiol* 2014;**5**: 318. <https://doi.org/10.3389/fmicb.2014.00318>.
- Paquete CM, Morgado L, Salgueiro CA et al. Molecular mechanisms of microbial extracellular electron transfer: the importance of multiheme cytochromes. *Front Biosci* 2022;**27**: 174. <https://doi.org/10.31083/j.fbl2706174>.
- Pasteris JD, Freeman JJ, Goffredi SK et al. Raman spectroscopic and laser scanning confocal microscopic analysis of sulfur in living sulfur-precipitating marine bacteria. *Chem Geol* 2001;**180**: 3–18. [https://doi.org/10.1016/S0009-2541\(01\)00302-3](https://doi.org/10.1016/S0009-2541(01)00302-3).
- Peiffer S, Kappler A, Haderlein SB et al. A biogeochemical-hydrological framework for the role of redox active compounds in aquatic systems. *Nat Geosci* 2021;**14**: 264–72. <https://doi.org/10.1038/s41561-021-00742-z>.
- Peng C, Sundman A, Catrouillet C et al. Oxidation of Fe(II)-organic-matter complexes in the presence of the mixotrophic nitrate-reducing Fe(II)-oxidizing bacterium *acidovorax* sp. BoFeN1. *Environ Sci Technol* 2018;**52**: 5753–63. <https://doi.org/10.1021/acs.est.8b00953>.
- Picard A, Gartman A, Clarke DR et al. Sulfate-reducing bacteria influence the nucleation and growth of mackinawite and greigite. *Geochim Cosmochim Acta* 2018;**220**: 367–84. <https://doi.org/10.1016/j.gca.2017.10.006>.
- Picard A, Gartman A, Cosmidis J et al. Authigenic metastable iron sulfide minerals preserve microbial organic carbon in anoxic environments. *Chem Geol* 2019;**530**: 119343. <https://doi.org/10.1016/j.chemgeo.2019.119343>.
- Picard A, Gartman A, Girguis PR. What do we really know about the role of microorganisms in iron sulfide mineral formation? *Front Earth Sci (Lausanne)* 2016;**4**: 1–10.
- Picard A, Gartman A, Girguis PR. Interactions between iron sulfide minerals and organic carbon: implications for biosignature preservation and detection. *Astrobiology* 2021;**21**: 587–604. <https://doi.org/10.1089/ast.2020.2276>.
- Posth N, Canfield DE, Kappler A. Biogenic Fe(III) minerals: from formation to diagenesis and preservation in the rock record. *Earth-Sci Rev* 2014;**135**: 103–21. <https://doi.org/10.1016/j.earscirev.2014.03.012>.
- Posth N, Huelin S, Konhauser KO et al. Size, density and mineralogy of cell-mineral aggregates formed during anoxygenic phototrophic Fe(II) oxidation. *Geochim Cosmochim Acta* 2010;**74**: 3476–93. <https://doi.org/10.1016/j.gca.2010.02.036>.
- Prange A, Chauvistré R, Modrow H et al. Quantitative speciation of sulfur in bacterial sulfur globules: x-ray absorption spectroscopy reveals at least three different species of sulfur. *Microbiology (Reading, Engl)* 2002;**148**: 267–76. <https://doi.org/10.1099/00221287-148-1-267>.
- Prange A, Engelhardt H, Trüper HG et al. The role of the sulfur globule proteins of *allochromatium vinosum*: mutagenesis of the sulfur globule protein genes and expression studies by real-time RT-PCR. *Arch Microbiol* 2004;**182**: 165–74. <https://doi.org/10.1007/s00203-004-0683-3>.
- Prange A. Speciation analysis of microbiologically produced sulfur by X-ray absorption near edge structure spectroscopy. In: Dahl C, Friedrich CG (eds.), *Microbial Sulfur Metabolism*. Berlin: Springer, 2008, 259–72. <https://doi.org/10.1007/978-3-540-72682-1>.
- Price A, Macey MC, Pearson VK et al. Oligotrophic growth of nitrate-dependent Fe<sup>2+</sup>-oxidising microorganisms under simulated early martian conditions. *Front Microbiol* 2022;**13**: 800219. <https://doi.org/10.3389/fmicb.2022.800219>.
- Rickard D, Luther GW. Chemistry of iron sulfides. *Chem Rev* 2007;**107**: 514–62. <https://doi.org/10.1021/cr0503658>.
- Rickard D, Mussmann M, Steadman JA. Sedimentary sulfides. *Elements* 2017;**13**: 117–22. <https://doi.org/10.2113/gselements.13.2.117>.
- Ruiz-Fresneda MA, Staicu LC, Lazuén-López G et al. Allotropy of selenium nanoparticles: colourful transition, synthesis, and biotechnological applications. *Microb Biotechnol* 2023;**16**: 877–92. <https://doi.org/10.1111/1751-7915.14209>.
- Saini G, Chan CS. Near-neutral surface charge and hydrophilicity prevent mineral encrustation of Fe-oxidizing micro-organisms. *Geobiology* 2013;**11**: 191–200. <https://doi.org/10.1111/gbi.12021>.
- Schad M, Byrne JM, ThomasArrigo L et al. Microbial Fe cycling in a simulated Precambrian ocean environment: implications for secondary mineral (trans)formation and deposition during BIF genesis. *Geochim Cosmochim Acta* 2022;**331**: 165–91. <https://doi.org/10.1016/j.gca.2022.05.016>.
- Schäfer G. Membrane-associated energy transduction in bacteria and archaea. In: Lennarz WJ, Lane MD (eds.), *Encyclopedia of Biological Chemistry II*. Amsterdam: Elsevier, 2013, 38–5.
- Schmid G, Zeitvogel F, Hao L et al. Submicron-scale heterogeneities in nickel sorption of various cell-mineral aggregates formed by Fe(II)-oxidizing bacteria. *Environ Sci Technol* 2016;**50**: 114–25. <https://doi.org/10.1021/acs.est.5b02955>.
- Schröder I, Rech S, Krafft T et al. Purification and characterization of the selenate reductase from *Thauera selenatis*. *J Biol Chem* 1997;**272**: 23765–8. <https://doi.org/10.1074/jbc.272.38.23765>.
- Schulz K, ThomasArrigo LK, Kaegi R et al. Stabilization of ferrihydrite and lepidocrocite by silicate during Fe(II)-catalyzed mineral

- transformation: impact on particle morphology and silicate distribution. *Environ Sci Technol* 2022;**56**: 5929–38. <https://doi.org/10.1021/acs.est.1c08789>.
- Sekerci F, Fischer S, Joshi P et al. Sulfur microenvironments as hotspots for biogenic pyrite formation. *Sci Rep* 2025;**15**: 20148. <https://doi.org/10.1038/s41598-025-05178-8>.
- Senn A-C, Kaegi R, Hug SJ et al. Composition and structure of Fe(III)-precipitates formed by Fe(II) oxidation in water at near-neutral pH: interdependent effects of phosphate, silicate and Ca. *Geochim Cosmochim Acta* 2015;**162**: 220–46. <https://doi.org/10.1016/j.gca.2015.04.032>.
- Shang H, Daye M, Sivan O et al. Formation of zerovalent iron in iron-reducing cultures of *Methanosarcina barkeri*. *Environ Sci Technol* 2020;**54**: 7354–65. <https://doi.org/10.1021/acs.est.0c01595>.
- Shi L, Dong H, Reguera G et al. Extracellular electron transfer mechanisms between microorganisms and minerals. *Nature Rev Microbiol* 2016;**14**: 651–62. <https://doi.org/10.1038/nrmicro.2016.93>.
- Shi L, Squier TC, Zachara JM et al. Respiration of metal (hydr)oxides by *Shewanella* and *Geobacter*: a key role for multiheme c-type cytochromes. *Mol Microbiol* 2007;**65**: 12–20. <https://doi.org/10.1111/j.1365-2958.2007.05783.x>.
- Sowers TD, Harrington JM, Polizzotto ML et al. Sorption of arsenic to biogenic iron (oxyhydr)oxides produced in circumneutral environments. *Geochim Cosmochim Acta* 2017;**198**: 194–207. <https://doi.org/10.1016/j.gca.2016.10.049>.
- Staicu LC, Barton LL. Selenium respiration in anaerobic bacteria: does energy generation pay off? *J Inorg Biochem* 2021;**222**: 111509. <https://doi.org/10.1016/j.jinorgbio.2021.111509>.
- Staicu LC, Barton LL. *Geomicrobiology: natural and Anthropogenic Settings*. Springer, Cham. 2024. <https://doi.org/10.1007/978-3-031-54306-7>.
- Staicu LC, Stolz JF. Microbes vs. metals: harvest and recycle. *FEMS Microbiol Ecol* 2021;**97**: fiab056. <https://doi.org/10.1093/femsec/fiab056>.
- Staicu LC, van Hullebusch ED, Lens PNL. Industrial selenium pollution: wastewaters and physical-chemical treatment technologies. In: van Hullebusch ED (ed.), *Bioremediation of Selenium Contaminated Wastewaters*, Cham: Springer, 2017, 103–30. <https://doi.org/10.1007/978-3-319-57831-6>.
- Staicu LC, van Hullebusch ED, Oturan MA et al. Removal of colloidal biogenic selenium from wastewater. *Chemosphere* 2015;**125**: 130–8. <https://doi.org/10.1016/j.chemosphere.2014.12.018>.
- Staicu LC, Wójtowicz PJ, Molnár Z et al. Interplay between arsenic and selenium biomineralization in *Shewanella* sp. O23S. *Environ Pollut* 2022a;**306**: 119451. <https://doi.org/10.1016/j.envpol.2022.119451>.
- Staicu LC, Wójtowicz PJ, Baragaño D et al. Bioremediation of a poly-metallic, arsenic-dominated reverse osmosis reject stream. *Lett Appl Microbiol* 2022b;**75**: 1084–92. <https://doi.org/10.1111/lam.13578>.
- Stanley W, Southam G. The effect of gram-positive (*Desulfosporosinus orientis*) and gram-negative (*Desulfovibrio desulfuricans*) sulfate-reducing bacteria on iron sulfide mineral precipitation. *Can J Microbiol* 2018;**64**: 629–37. <https://doi.org/10.1139/cjm-2017-0545>.
- Stolz JF, Basu P, Santini JM et al. Arsenic and selenium in microbial metabolism. *Annu Rev Microbiol* 2006;**60**: 107–30. <https://doi.org/10.1146/annurev.micro.60.080805.142053>.
- Stolz JF, Ellis DJ, Blum JS et al. *Sulfurospirillum barnesii* sp. nov. and *Sulfurospirillum arsenophilum* sp. nov., new members of the *Sulfurospirillum* clade of the epsilon Proteobacteria. *Int J Syst Bacteriol* 1999;**49**: 1177–80. <https://doi.org/10.1099/00207713-49-3-1177>.
- Taylor CD, Wirsén CO, Gaill F. Rapid microbial production of filamentous sulfur mats at hydrothermal vents. *Appl Environ Microbiol* 1999;**65**: 2253–5. <https://doi.org/10.1128/AEM.65.5.2253-2255.1999>.
- Thiel J, Byrne JM, Kappler A et al. Pyrite formation from FeS and H<sub>2</sub>S is mediated through microbial redox activity. *Proc Natl Acad Sci* 2019;**116**: 6897–902. <https://doi.org/10.1073/pnas.1814412116>.
- Thomas C, Ebert Y, Kiro Y et al. Microbial sedimentary imprint on the deep Dead Sea sediment. *Depos Rec* 2016;**2**: 118–38. <https://doi.org/10.1002/dep2.16>.
- Trivedi CB, Stamps BW, Lau GE et al. Microbial metabolic redundancy is a key mechanism in a sulfur-rich glacial ecosystem. *Msystems* 2020;**5**: e00504–20. <https://doi.org/10.1128/mSystems.00504-20>.
- Truong C, Bernard S, Le Pape P et al. Production of carbon-containing pyrite spherules induced by hyperthermophilic thermococcales: a biosignature? *Front Microbiol* 2023;**14**: 1145781. <https://doi.org/10.3389/fmicb.2023.1145781>.
- Truong C, Bernard S, Baudin F et al. Carbon-containing pyrite spherules: mineral biosignatures in black smokers? *Eur J Mineral* 2024;**36**: 813–30. <https://doi.org/10.5194/ejm-36-813-2024>.
- Uebe R, Schüler D. The formation of iron biominerals in magnetotactic bacteria. In: A. Sigel A, Freisinger E, Sigel RKO (eds.), *Metal Ions in Life Sciences, Vol. 21: metals, Microbes, and Minerals—The Biogeochemical Side of Life*. De Gruyter, Berlin, 2021, 159–84. <https://doi.org/10.1515/9783110589771>.
- Uhrynowski W, Radlinska M, Drewniak L. Genomic analysis of *Shewanella* sp. O23S—the natural host of the pSheB plasmid carrying genes for arsenic resistance and dissimilatory reduction. *Int J Mol Sci* 2019;**20**: 1018. <https://doi.org/10.3390/ijms20051018>.
- Vigliaturo R, Marengo A, Bittarello E et al. Micro- and nano-scale mineralogical characterization of Fe(II)-oxidizing bacterial stalks. *Geobiology* 2020;**18**: 606–18. <https://doi.org/10.1111/gbi.12398>.
- Vinceti M, Filippini T, Biswas A et al. Selenium: a global contaminant of significant concern to environment and human health. In: Naidu R (ed.), *Inorganic Contaminants and Radionuclides*, Amsterdam: Elsevier, 2024, 427–80.
- Wang S, Jiang L, Cui L et al. Transcriptome analysis of cyclooctasulfur oxidation and reduction by the neutrophilic chemolithoautotrophic *Sulfurovum indicum* from deep-sea hydrothermal ecosystems. *Antioxidants* 2023;**12**: 627. <https://doi.org/10.3390/antiox12030627>.
- Wells M, McGarry J, Gaye MM et al. Respiratory selenite reductase from *Bacillus selenitireducens* strain MLS10. *J Bacteriol* 2019;**201**: e00614–18. <https://doi.org/10.1128/JB.00614-18>.
- Wells M, Stolz JF. Microbial selenium metabolism: a brief history, biogeochemistry and ecophysiology. *FEMS Microbiol Ecol* 2020;**96**: fiab209. <https://doi.org/10.1093/femsec/fiab209>.
- Zhang L, Qiu Y-Y, Zhou Y et al. Elemental sulfur as electron donor and/or acceptor: mechanisms, applications and perspectives for biological water and wastewater treatment. *Water Res* 2021;**202**: 117373. <https://doi.org/10.1016/j.watres.2021.117373>.
- Zhou A, Templeton AS, Johnson JE. Dissolved silica affects the bulk iron redox state and recrystallization of minerals generated by photoreduction in a simulated Archean ocean. *Geobiology* 2024;**22**: e12587. <https://doi.org/10.1111/gbi.12587>.
- Zhou N, Kupper RJ, Catalano JG et al. Biological oxidation of Fe(II)-bearing smectite by microaerophilic iron oxidizer *Sideroxydans lithotrophicus* using dual mto and Cyc2 iron oxidation pathways. *Environ Sci Technol* 2022;**56**: 17443–453. <https://doi.org/10.1021/acs.est.2c05142>.

Zhou Z, Tran PQ, Cowley ES et al. Diversity and ecology of microbial sulfur metabolism. *Nat Rev Micro* 2025;**23**: 122–40. <https://doi.org/10.1038/s41579-024-01104-3>.

Zopfi J, Ferdelman TG, Fossing H. Distribution and fate of sulfur intermediates—sulfite, tetrathionate, thiosulfate, and elemental sulfur—in marine sediments.

In: Amend JP, Edwards KJ, Lyons TW (eds.), *Sulfur Biogeochemistry—Past and Present*. Boulder: Geological Society of America, 2004, 97–116. <https://doi.org/10.1130/SPE379>.