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# **The complex interplay of sulfur and arsenic bioenergetic metabolisms in the arsenic geochemical cycle**

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## **Abstract**

Geochemical cycles of arsenic and sulfur are largely impacted by the activity of prokaryotes as microorganisms use these two elements for assimilatory and dissimilatory purposes. Knowledge of the enzymatic systems responsible for these processes is thus essential for understanding the geochemical cycles of both elements. But arsenic and sulfur commonly co-exist in the environment, leading to the formation of thiolated arsenic species with one to four arsenic-bonded oxygen atoms substituted by sulfur. Considering these chemical species and the biological processes at the heart of their formation and transformations is therefore crucial for comprehending the complexity of the global geochemical cycles of arsenic and sulfur. This chapter offers a comprehensive overview of the well-known prokaryotic arsenic and sulfur enzyme systems responsible for use of these two elements in bioenergetics, as well as the ever growing-group of prokaryotic enzymes revealed to be able to convert thioarsenic species or to convert both arsenic and sulfur.

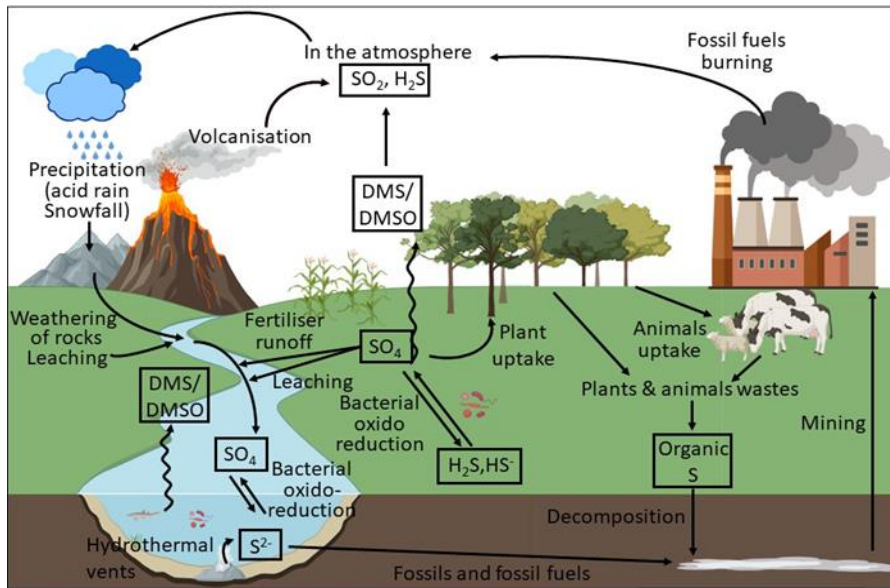
**Keywords:** sulfur; arsenic; thioarsenic; bioenergetics; molybdenum enzymes.

## Introduction

The Law of Conservation of Mass states that in a closed system mass is not created nor destroyed but always transformed. This notion has been integrated into the concept of the biogeochemical cycles of elements that, in fact, represent the way in which an element is recycled in the Earth's ecosystems through biotic and abiotic transformations, as well as through geological and anthropogenic processes. These cycles are often interconnected and, in general, microorganisms play a predominant role in them, influencing the oxidation state of the elements and, possibly, the bioavailability of compounds. This chapter focuses on the biological incidence in the cycle of two elements, sulfur (S) and arsenic (As), with an emphasis on the role of the energy metabolisms involved and on the interconnections between them. The two cycles attracted the attention of researchers for decades due to the fact that 1) S is one of the most abundant and reactive elements on Earth, 2) As is the most abundant toxic metalloid in the environment, and 3) S and As often coexist in the environment, the chemical reactions between them being studied for long-time. The role of S, for example, in the As biogeochemical cycle in groundwater has been recognized for several years, although still unclear (Fisher et al. 2008). Deciphering this role is therefore crucial for a better control of As contamination in the environment (Qing et al. 2023). This is however particularly difficult due to the multitude of enzymatic systems involved in the S and As cycling and particularly the bioenergetic ones, as it will be briefly exposed. The energy metabolism, also called “dissimilatory metabolism”, represents the ensemble of cellular catabolic and redox reactions leading to the production of chemical energy, in the form of ATP and reducing power (*i.e.*, NAD(P)H, FADH<sub>2</sub>), using either light or chemical compounds as energy sources. The world of microorganisms is characterized by a great variety of bioenergetic metabolic pathways, demonstrating an astonishing ability to adapt to all kinds of environments, which will be highlighted in this chapter describing the diverse enzyme systems known so far. The various interplays currently recognized between the two cycles will then be presented, highlighting the enzyme systems proposed to be involved.

### 1. The biogeochemical cycles of sulfur and arsenic

Sulfur can exist in a myriad of both organic and inorganic forms (see Steudel (2020) for an exhaustive overview). The S cycle is summarized in **Figure 1** and a detailed presentation can be found in Fike et al. (2015) and Chaudhary et al. (2023). This element is mainly found as a mineral in sediments and rocks or as sulfate in seawater, and it is mobilized by volcanic activities, mining and combustion of fossil fuels, or by microorganisms.



**Figure 1.** The sulfur biogeochemical cycle.

Moreover, since S is a cellular constituent, the combustion and degradation of biomass also represent a central part in its biogeochemical cycle. Although S presents a certain toxicity (*e.g.*, as sulfide, sulfite, sulfur dioxide; see (Komarnisky et al. 2003)), it is indeed essential for all living organisms as structural element for proteins, metabolites, cofactors (*e.g.*, Fe-S clusters, Molybdenum Cofactor (MoCo)), glucosides, vitamins. The assimilatory S metabolism, for the incorporation of S in cell constituents, mainly absorbed as sulfate, is therefore ubiquitous in all domains of life (Dahl 2017; Li et al. 2020; Dordevic et al. 2023). In addition, microorganisms have adapted to use inorganic and organic S compounds as energy substrates. In this dissimilatory metabolism, S can be involved either as final electron acceptor in anaerobic respiration or as electron/energy supply in both phototrophic and respiratory chemotrophic chains (Muyzer and Stams 2008; Dahl 2017; Chaudhary et al. 2023). It is worth to specify that S atoms are never free inside the cell and are always linked to- and transported by- carriers like S transferases or low molecular weight organic molecules (*e.g.*, glutathione). These systems connect different enzymatic complexes in the S metabolism preventing aspecific reactions of S inside the cell and creating the so-called S trafficking (see for example (Dahl 2015) for an overview). S is a very reactive element and this feature is given by the wide range of chemical forms and oxidation states it can assume: from -II, the most reduced valence state, to +VI, the most oxidized one. In **Table 1**, the oxidation states of the most common inorganic and organic

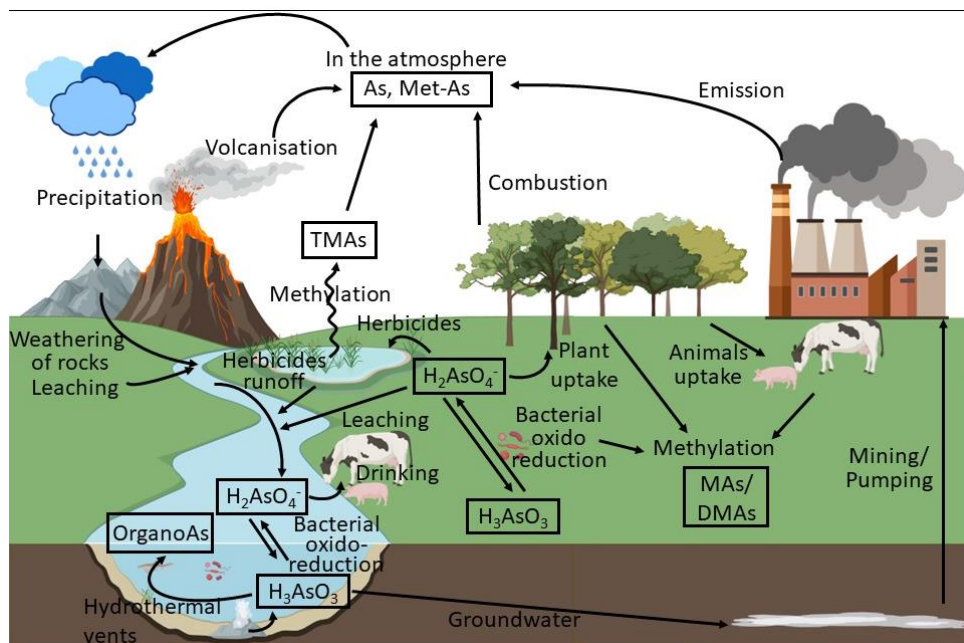
S compounds found in bioenergetics are listed, but a wider spectrum of biologically important S groups can be found (Iciek et al. 2019; Lau and Pluth 2019; Steudel 2020).

**Table 1.** Oxidation states of the most common inorganic and organic sulfur compounds (from Karen et al. 2014).

Compound	Oxidation state of S atom
Hydrogen sulfide (H <sub>2</sub> S)	-II
Polysulfide ( <sup>-</sup> S-S <sub>(n)</sub> -S <sup>-</sup> )	0 for internal S -I for external S
Elemental S (S <sup>0</sup> or S <sub>8</sub> )	0
Thiosulfate (S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> )	-I/0 for external S and V/IV for central S
Tetrathionate (S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> )	0 for internal S and V for external S
Sulfite (SO <sub>3</sub> <sup>2-</sup> )	IV
Sulfate (SO <sub>4</sub> <sup>2-</sup> )	VI
Dimethyl sulfoxide ((CH <sub>3</sub> ) <sub>2</sub> S=O)/Dimethyl sulfide ((CH <sub>3</sub> ) <sub>2</sub> S)	0/-II
Thiol/sulfhydryl group (R-SH)	-II
Persulfide (R-SSH)	-I
Disulfide (R-SS-R)	-I

Moreover, S atoms can form chains of different lengths and circularise to form rings of various sizes (S<sub>n</sub>), the most thermodynamically stable of which is composed by 8 S atoms (*i.e.*, orthorhombic  $\alpha$ -S<sub>8</sub>). These features make the chemistry and biology of S extremely varied and complex. Although this dissimilatory metabolism has been studied for a long time, it is so intricate and diverse in the microbial world that it has not been fully deciphered yet. The present contribution will only cover the main bioenergetic enzyme systems (**section 2**).

Arsenic is also a ubiquitous element, present on Earth in lower concentrations than S, but it is much more toxic. Most of the As on Earth is present in the lithosphere, with comparable amounts present in the ocean and soil. Geothermal and volcanic activities, weathering of rocks, mining and other anthropogenic activities cause the release of this element on the surface (Smedley 2002; Zhu et al. 2014; Raturi et al. 2023) (**Figure 2**).



**Figure 2.** The arsenic biogeochemical cycle.

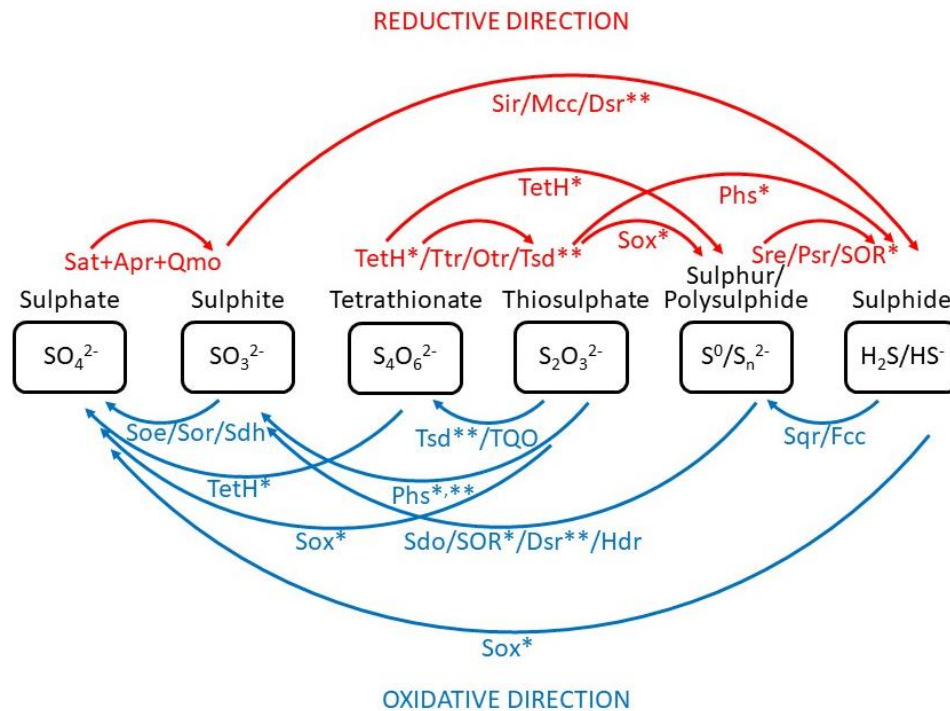
Arsenic has only 3 main different oxidation states (-III, III, V; the 0 state is rare), with As(III) and As(V) being the most common species in the environment. It can exist in both inorganic and organic forms, with a less complex chemistry than sulfur. The arsenite oxide (As(III),  $H_3AsO_3/ H_2AsO_3^-$ ) is more abundant in anoxic and reducing environments and is the most soluble and bioavailable form (and also considered the most toxic). On the other hand, arsenic, in the oxidation state V (arsenate As(V),  $H_2AsO_4^-/ HAsO_4^{2-}$ ), is more present in oxic environments, but readily reacts with other compounds forming, for example, insoluble ferric salts, being therefore less mobile and bioavailable. As is also part of a number of organic compounds (predominantly methylated forms and arsenosugars). The last and less common oxidation state of As, -III, is encountered in arsenides which are crystalline binary compounds formed with cations ( $Na^+$ ,  $Ga^+$ ,  $Cd^{2+}$ ,  $Zn^{2+}$ ...) and the gaseous arsine,  $AsH_3$ . Therefore, the redox state of the environment and the microbial metabolism influence the speciation and bioavailability of As and, consequently, the contamination level of water and soil (Zhu et al. 2014; Raturi et al. 2023).

The toxicity of As is due to its deleterious reactions with biological systems: As(III), in fact, reacts with the sulfhydryl groups (-SH) of proteins and other small molecules (e.g., glutathione) interfering with their activity; and As(V) is an analogue of phosphate and competes with it inside cells (Zhu et al. 2014; Raturi et al. 2023). Uptake of As into cells is generally made either by passive diffusion or by aspecific transporters naturally dedicated to other nutrients, such as phosphate transporters for As(V) and aquaglyceroporins for As(III). The resistance processes

evolved to fight against As toxicity will not be presented in this chapter but several comprehensive reviews are available (for example, Zhu et al. (2014); Sattar et al. (2016); Raturi et al. (2023)). Beside resistance, some microbes have evolved an energy metabolism in which As plays the role of electron donor or acceptor in photosynthetic or respiratory chains (van Lis et al. 2013; Oremland et al. 2017; Shi et al. 2020). This metabolism has been discovered in the last decades and is therefore less well understood than that of S. Only three enzymes involved in this metabolism are currently known and will be presented in **section 3**.

## **2. Enzymes of the sulfur bioenergetics cycle**

As depicted in **Figure 3**, the enzymes of the bioenergetic S metabolism, in prokaryotes, are often redundant (performing the same chemical conversion). They can be divided in two groups: those involved in the reductive reactions, in which S compounds act as electron acceptors in anaerobic respiratory chains; and those involved in oxidative reactions in which, instead, reduced S compounds represent the electron donors to respiratory or photosynthetic chains. These reactions are mainly restricted to inorganic S compounds with the notable exception of Dimethyl sulfide/Dimethyl sulfoxide (DMS/DMSO). It is to note that an important role in the S biogeochemical cycle is also played by S disproportionating enzymes, simultaneously reducing and oxidizing a compound thus forming two different products. The disproportionating enzymes are highlighted in **Figure 3**: thiosulfate-oxidizing incomplete Sox system, Sulfur Oxygenase Reductase SOR, thiosulfate reductase Phs and tetrathionate hydrolase TetH. It is worth mentioning that “dismutation”, sometimes equally used with “disproportionation”, characterizes a whole pathway rather than a single enzyme where reductive and oxidative reactions could be performed. These pathways are very diverse and widespread in both bacterial and archaeal domains, but they have not yet been fully unraveled because of the aforementioned complexity and richness of the chemistry and biology of S (Slobodkin and Slobodkina 2019).



\*: disproportionating enzyme; \*\*: reversible enzyme

**Figure 3.** The enzymes of the sulphur biogeochemical cycle. Several enzymes are involved in several reactions because they are either reversible or release several products.

### 2.1. Reduction of sulfur compounds

The dissimilatory reduction of S compounds is known since the late 1800 and an historical overview has been provided by (Rabus et al. 2013). The most studied metabolism is the reduction of sulfate ( $\text{SO}_4^{2-}$ ), the most oxidised form of S, but all other S compounds can be “respired” (e.g., sulfite, thiosulfate, elemental sulfur/polysulfide and tetrathionate). Microorganisms have often 1) several electron donors (among them molecular hydrogen ( $\text{H}_2$ ) and different organic carbon compounds), 2) multiple inorganic compounds as electron acceptors for anaerobic respiration, displaying an astonishing metabolic versatility (Muyzer and Stams 2008; Rabus et al. 2013). Given the diversity of S-reducing pathways, only the more relevant examples for this chapter will be presented.

#### 2.1.1. $\text{SO}_4^{2-}$ reduction

The dissimilatory  $\text{SO}_4^{2-}$  reduction is the best-known sulfur metabolism: it needs as a first step the substrate import and its successive ATP-dependent activation by the cytoplasmic enzyme ATP sulfurylase / sulfate adenylyl transferase (Sat) to form adenosine-phosphosulfate (APS). This intermediate is reduced to sulfite ( $\text{SO}_3^{2-}$ ) by the APS reductase (AprAB) that receives electrons from quinones (Q) through the quinone-interacting membrane-bound oxidoreductase



(QmoABC). Finally,  $\text{SO}_3^{2-}$  is reduced to sulfide ( $\text{HS}^-$ ) by the dissimilatory sulfite reductase system (Dsr). This last step is still the most controversial with a lot of putative components whose function is unknown. The current model has been proposed by (Santos et al. 2015) and involves the direct six-electron reduction of  $\text{SO}_3^{2-}$  to  $\text{HS}^-$  without passing through the intermediates trithionate ( $\text{S}_3\text{O}_6^{2-}$ ) and thiosulfate ( $\text{S}_2\text{O}_3^{2-}$ ), a proposed alternative pathway (Rabus et al. 2013). In this model, the soluble cytoplasmic complex DsrAB reduces  $\text{SO}_3^{2-}$  by oxidising the DsrC protein through the formation of a trisulfide group which is further re-reduced by the DsrMKJOP membrane complex that complementarily oxidises Q (Santos et al. 2015). This whole pathway can function in reverse to participate as well in the dissimilatory oxidation of elemental S, producing  $\text{SO}_3^{2-}$  (see next paragraph and **Figure 3**).

### 2.1.2. Reduction of other sulfur compounds

As mentioned above, all inorganic S compounds and DMSO can be used as final electron acceptors by some microorganisms. As described above, DsrAB/DsrC is responsible for the reduction of  $\text{SO}_3^{2-}$  to  $\text{HS}^-$  in the  $\text{SO}_4^{2-}$  reduction pathways, but other systems are known for the reduction of  $\text{SO}_3^{2-}$  in prokaryotes unable to respire  $\text{SO}_4^{2-}$ . This is the case of Mcc, a soluble periplasmic enzyme, whose structure is resolved (Hermann et al. 2015), containing three Cu(I) ions and twenty four heme *c*. Mcc allows prokaryotes, such as *Wolinella (W.) succinogenes* to respire  $\text{SO}_3^{2-}$  (see Eller et al. 2019) and produce  $\text{HS}^-$ , using quinol as electron donor.

Several other S-reducing enzymes belong to the Mo-*bis*PGD (Molybdenum *bis* Pyranopterin Guanosine Dinucleotide) superfamily (**Figure 3** and **Figure 4**, Panel A): sulfur reductase Sre, polysulfide reductase Psr, Phs, tetrathionate reductase Ttr and DMSO reductase. They harbour a MoCo in their catalytic subunit, coordinated by two pyranopterins that, in the majority of prokaryotes, is conjugated to a guanosine (**Figure 4**, Panel B). The large majority of the enzymes belonging to this family are phylogenetically closely related and have a very conserved organization with 3 subunits: a catalytic one (A), an electron transfer one (B) and a membranous one (C) reacting with Q (**Figure 4**, Panel C). To this structural similarity, a great diversity of reactions catalyzed and substrates used is opposed, but the features allowing the substrate specificity have not yet been unraveled. One aspect, studied for decades, is the specificity of these members for the type of Q. Some enzymes (for example the respiratory nitrate reductase Nar (Arias-Cartin et al. 2010)) accommodate a broad spectrum of Q, while others (for example Phs or Psr (Stoffels et al. 2012, Wilkens and Simon 2023)) a restricted one. One of the driving forces behind the specificity seems to be the redox potential of the Q relative to the one of the inorganic substrate (see **Table 2**).

**Table 2.** Redox couples of some arsenic and sulfur compounds and of quinones involved as electron donor or acceptor of the enzymatic systems. Values from (Thauer et al. 1977; Grimaldi et al. 2013) except when specified.

Redox couples	E <sub>0</sub> ' pH7
As(III)/As(V)	+60mV /+160mV <sup>a</sup>
HS <sup>-</sup> /SO <sub>4</sub> <sup>2-</sup>	-220mV
HS <sup>-</sup> /SO <sub>3</sub> <sup>2-</sup>	-116mV
HS <sup>-</sup> /S <sup>0</sup>	-270mV
S <sub>n</sub> <sup>2-</sup> /S <sub>n-1</sub> <sup>2-</sup> + HS <sup>-</sup>	-260mV
S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> /HS <sup>-</sup> + HSO <sub>3</sub> <sup>-</sup>	-400mV
S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> /S <sub>4</sub> O <sub>6</sub> <sup>2-</sup>	+24/+170mV <sup>b</sup> /+198mV <sup>b</sup>
SO <sub>3</sub> <sup>2-</sup> /SO <sub>4</sub> <sup>2-</sup>	-516mV
H <sup>+</sup> /H <sub>2</sub>	-414mV
O <sub>2</sub> /H <sub>2</sub> O	+820mV
<b>QUINONES</b>	<b>E<sub>m</sub>' at pH7 (average Q/SQ SQ/QH<sub>2</sub>)</b>
MMK/MMKH <sub>2</sub>	-220mV
MK/MKH <sub>2</sub>	-74mV
DMK/DMKH <sub>2</sub>	-9mV/+36
UQ/UQH <sub>2</sub>	+113mV

<sup>a</sup>(Markalova 2016)

<sup>b</sup>(Kaprarek 1972; Kurth et al. 2015)

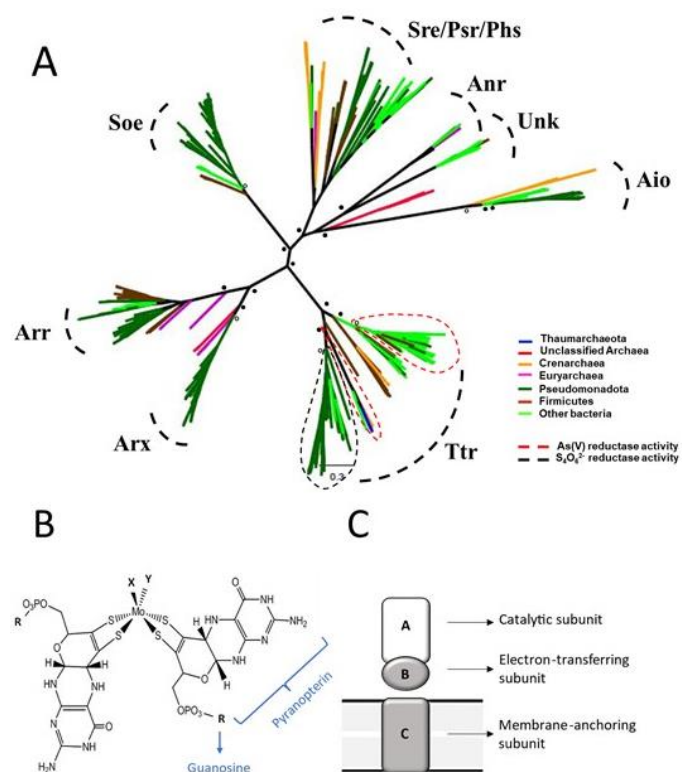
SQ: semi quinone; MMK: methyl-menaquinone; MK: menaquinone; DMK: demethyl-menaquinone; UQ: ubiquinone; QH<sub>2</sub>: quinol.

A summary of the known features characterizing these enzymes and the diversity within this family is provided by Rothery et al. (2008); Grimaldi et al. (2013) and Magalon et al. (2016).

Tetrathionate (S<sub>4</sub>O<sub>6</sub><sup>2-</sup>), the compound after SO<sub>3</sub><sup>2-</sup> in the oxidation state scale, can be reduced by multiple enzymes. The Ttr is a member of the Mo-*bis*PGD superfamily, present in *Salmonella* (*Sa.*) *enterica* and reducing S<sub>4</sub>O<sub>6</sub><sup>2-</sup> to S<sub>2</sub>O<sub>3</sub><sup>2-</sup> but also S<sub>3</sub>O<sub>6</sub><sup>2-</sup> to S<sub>2</sub>O<sub>3</sub><sup>2-</sup> and SO<sub>3</sub><sup>2-</sup> (Hinsley and Berks 2002). This periplasmic-sided membrane enzyme shows the typical organization of a Mo-*bis*PGD member, with a catalytic A subunit, harbouring the MoCo and a [4Fe-4S] cluster, an electron transfer B subunit with 4[4Fe-4S] clusters and a membrane-anchoring C subunit devoid of cofactors (Hensel et al. 1999). It has been proposed to react with menaquinones (MK) (Barrett and Clark 1987). Two other enzymatic periplasmic systems, whose 3D structure is solved and not belonging to the Mo-*bis*PGD family, have been proposed to reduce S<sub>4</sub>O<sub>6</sub><sup>2-</sup>: the octaheme tetrathionate reductase OTR and the thiosulfate dehydrogenase TsdA. OTR is a soluble cytochrome (Mowat et al. 2004) with a still unknown physiological function but preliminary studies suggested that it was able *in vitro* to reduce S<sub>4</sub>O<sub>6</sub><sup>2-</sup> to S<sub>2</sub>O<sub>3</sub><sup>2-</sup> (Atkinson et al. 2007). TsdA is a monomeric soluble diheme cytochrome, reversible *in vitro*, but *in vivo* either

reduces  $S_4O_6^{2-}$  or oxidises  $S_2O_3^{2-}$  depending on the bacteria and the metabolism in which it is involved. In *Campylobacter jejuni* for example, it reduces  $S_4O_6^{2-}$  (Liu et al. 2013).

The Psr, a Mo-*bis*PGD enzyme, reduces polysulfide ( $-S-S_{(n)}-S-$ ) to  $HS^-$  and has been first characterized in *W. succinogenes*, an anaerobic bacterium coupling polysulfide reduction to formate or  $H_2$  oxidation (Klimmek 1991; Krafft et al. 1992). This heterotrimer heading towards the periplasm shows the typical organization, with a membrane-anchoring C subunit devoid of cofactors linking the enzyme to the Methyl-menaquinones (MMK) for the electrons' supply (Wilkins and Simon 2023). The enzyme from *Thermus thermophilus* has been crystallized as a dimer of heterotrimer PsrABC (Jormakka et al. 2008). To underline the potential versatility of these enzymes, a Psr identified in *Shewanella (Sh.) oneidensis* MR-1 has been proposed able to convert both elemental sulfur ( $S^0$ ) and  $S_2O_3^{2-}$  (Burns and DiChristina 2009), while a closely related enzyme, Sre (see **Figure 4A**), present in *Allochromatium (Al.) vinosum* and *Acidianus (Ac.) ambivalens* is proposed to use  $S^0$  as substrate (Laska et al. 2003). A third enzyme with the catalytic subunit phylogenetically close enough to cluster together with Psr in a unique clade (**Figure 4A** and (Duval et al. 2008)) is the Phs, which uses  $S_2O_3^{2-}$  to release  $HS^-$  and  $SO_3^{2-}$ .



**Figure 4.** The enzymes of the Mo-*bis*PGD superfamily. A) Phylogenetic tree of some members of the Mo-*bis*PGD superfamily involved in sulfur and arsenic bioenergetic conversion; B) The

Mo-*bis*PGD cofactor; C) General architecture of the archetypal member of the Mo-*bis*PGD superfamily with A, B and C subunits.

It is required for both  $S_2O_3^{2-}$  and  $S^0$  respiration coupled to formate oxidation in *Sa. enterica* (Hinsley and Berks 2002). This enzyme can catalyse the reverse reaction as well (Stoffels et al. 2012). The general organization of the Phs is very similar to the one described for the Psr (Heinzinger et al. 1995) except that the membrane-anchoring C subunit, that is responsible for reaction with MK, harbors two *b* hemes (Stoffels et al. 2012).

As mentioned above, in addition to inorganic S compounds, DMSO/DMS are bioenergetic organic S compounds. DMSO can be used as electron acceptor in anaerobic respirations and reduced into DMS by various enzymes. The first one is the DMSO reductase Dor, that historically gave its name to the Mo-*bis*PGD superfamily (formerly DMSOred) but that represents an exception in several respects. Several crystal structures of this enzyme have been solved (see for a comprehensive review Kappler et al. 2019). Dor is a monomer, consisting of only the catalytic subunit devoid of Fe-S center and only carrying a MoCo. It is in fact a soluble enzyme that doesn't directly react with Q but with DorC functioning with MK/DMK (dimethylmenaquinone). Dms, another member of the Mo-*bis*PGD, is instead a heterotrimeric enzyme, catalyzing either DMSO reduction or DMS oxidation. It is a typical Mo-*bis*PGD enzyme reacting with MK and carrying a Fe-S center in the catalytic subunit. Finally, YedY/MsrP is a different enzyme demonstrated to reduce DMSO and also carrying a MoCo but a monopterin one (Kappler et al. 2019).

## 2.2. Oxidation of sulfur compounds

Each S compound can be energetically oxidized, usually up to  $SO_4^{2-}$ . S oxidizing bacteria are physiologically and phylogenetically very diverse which is reflected on the diversity of their metabolisms. Phototrophs gain energy from light using a myriad of inorganic compounds as electron donors, among them S ones. The most studied phototrophic bacteria are the green sulfur bacteria (GSB), especially the *Chlorobiaceae* family, with *Chlorobaculum (C.) tepidum* as the model organism, and the purple sulfur bacteria (PSB), in particular *Al. vinosum* belonging to the *Chromatiaceae* family (see Frigaard and Dahl 2009; Dahl 2017; Kushkevych et al. 2021 for comprehensive reviews). On the other hand, chemotrophs obtain energy from the oxidation of inorganic and, in some cases, organic S species, using  $O_2$  (*i.e.*, aerobes or microaerobes) or other elements (mostly nitrate but also As(V), Fe(III)) as final electron acceptors (see Ghosh and Dam 2009 for review).

### 2.2.1. HS<sup>-</sup> oxidation

HS<sup>-</sup>/H<sub>2</sub>S is a very reactive S compound that is toxic at elevated concentrations but is used as electron/energy source for photosynthetic and respiratory chains by a large number of prokaryotes. Its oxidation is catalyzed by two different enzymes (**Figure 3**) that can be found together and in multiple copies in certain microorganisms, but the reason of this redundancy is still not fully understood. The best-known bioenergetic system is the periplasmically oriented and membrane-bound Sulfide Quinone Oxidoreductase (Sqr) that uses a Q as electron acceptor and releases soluble S<sub>n</sub><sup>2-</sup> in the periplasm (Griesbeck et al. 2002). This enzyme, carrying a flavin as cofactor, has been purified from different microbes and biochemically characterized. It is to note that Sqr (in this case oriented towards the cytoplasm) has also been demonstrated to be involved in a detoxifying pathway, combined with the Persulfide Dioxygenase PDO (Xia et al. 2017; Koch and Dahl 2018). The Sqr is phylogenetically related to a second system oxidizing HS<sup>-</sup> to S<sub>n</sub><sup>2-</sup>, the flavocytochrome *c* sulfide dehydrogenase Fcc, that can be found together with the Sqr in a single organism, making complicated the understanding of their respective roles *in vivo*. The Fcc, instead, has been found only in dissimilatory S bacteria and hypothesized to participate mainly in energy S metabolisms (Dahl 2017). This enzyme is often found soluble in the periplasm but, in few cases, it has been found bound to the periplasmic side of the membrane, but it always uses a cytochrome *c* as electron acceptor rather than Q. Several crystallographic structures have been solved (see Osipov et al. 2018 for the most recent). It is a heterodimer composed by the catalytic flavo-subunit FccB and a smaller cytochrome *c* subunit FccA, coded in an operon. The taxonomic distribution and the classification of these phylogenetically related enzymes, as well as analyses of their conserved motifs, have been provided by Sousa et al. (2018). The Sqr, from which three structures are available, have been found in all domains of life forming several phylogenetic groups (Marcia et al. 2010; Sousa et al. 2018).

### 2.2.2. S<sub>n</sub><sup>2-</sup> and S<sup>0</sup> oxidation

S<sub>n</sub><sup>2-</sup> externally provided or S<sub>n</sub><sup>2-</sup>/persulfides occurring as an intermediate from HS<sup>-</sup> and, eventually, from the truncated Sox-mediated S<sub>2</sub>O<sub>3</sub><sup>2-</sup> oxidation, can be further oxidized to biogenic S<sup>0</sup> by a still unknown pathway. It is proposed that this reaction can be purely chemical (Dahl 2017; Kushkevych et al. 2021). Biogenic S<sup>0</sup> is thus often a metabolic intermediate during growth on S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, S<sub>4</sub>O<sub>6</sub><sup>2-</sup> and HS<sup>-</sup>, or even during growth on commercial S<sup>0</sup> supplied as external energy substrate (Franz et al. 2009; Marnocha et al. 2016; Liu et al. 2022). Biogenic S globules can be stored extracellularly or in the periplasm. They are made of cyclo-S<sub>8</sub>-sulfur, S chains or

polythionates chains, depending on the microorganism. They can be covered with an organic layer which can differ from one organism to another: polysaccharides and proteins in extracellular globules from *C. tepidum* and *Thioalkalivibrio versutus* (Marnocha et al. 2016; Liu et al. 2022); specific proteins (called Sgps) in the intracellular *Al. vinosum* ones (Kumpel et al. 2023). The process for the mobilization and metabolization of these globules, with  $\text{SO}_4^{2-}$  as the most common final product, as well as the process of excretion and further uptake, are still unclear (see Marnocha et al. 2016).

While the above-mentioned processes take place in the periplasm, the oxidation of  $\text{S}^0/\text{S}_n^{2-}$ /persulfide can also take place in the cytoplasm, under the action of multiple enzymatic systems (**Figure 3**). The best known is the cytoplasmic reverse Dissimilatory Sulfite Reductase Dsr, already described in the previous paragraph as it is involved in the reductive S metabolism as well; the other one has been more recently discovered and is the HeteroDisulfide Reductase (Hdr)-like or sHdr pathway. For both enzymes, models have been proposed but some aspects need to be confirmed and a lot of questions are still open, representing one of the most challenging steps to unravel in S metabolisms. *rhd*, *tusA* and *dsrE* genes, coding for a rhodanese and sulfur carrier proteins, are typically found in the close vicinity of *dsr* or *hdr* genetic clusters in a large number of S-oxidizing organisms. These S transferases harbor reactive cysteines and are involved in an essential cascade of S transfer, in the form of persulfide or thiosulfonate, in the cytoplasm. Sulfur is thus not free inside the cell and the sulfane sulfur substrate is supposed to be delivered to the enzymatic system (Dsr or sHdr) in a protein-bound form (Liu et al. 2014; Stockdreher et al. 2014; Dahl 2015). After this cascade of protein persulfides ( $\text{Cys-SS}^-$ ), the S arrives to the DsrC transporter that forms a trisulfide group with the help of the membrane-bound DsrMKJOP complex and that is then oxidised by DsrAB in cooperation with DsrL, releasing  $\text{SO}_3^{2-}$  and NADH (Löffler et al. 2020) in a reverse model to the one proposed by (Santos et al. 2015) and detailed in the previous paragraph. Microorganisms that do not possess the Dsr system, code instead for the sHdr one that has been demonstrated to be involved in the dissimilatory S oxidation metabolism from -omics and genetic analyses (Koch and Dahl 2018). This system is related to the heterodisulfide reductase complex of methanogenic archaea and likely oxidizes protein persulfide/thiosulfonate and releases  $\text{SO}_3^{2-}$  in the cytoplasm (Boughanemi et al. 2016; Dahl 2017). This complex, encoded in S oxidising microorganisms by the *hdrC1B1AHypC2B2* genes cluster, has been purified from the bacterium *Aquifex (Aq.) aeolicus* as a complex containing the five subunits HdrAB1C1B2C2 anchored to the cytoplasmic membrane (Boughanemi et al. 2016) and proposed to form in fact an asymmetric HdrAA'B1C1B2C2 hexamer in *Hyphomicrobium denitrificans* (Ernst et al. 2021). Another

component has been proposed to interact specifically with this system in the last years, the lipote-binding protein LbpA, whose gene is often in association with the *hdr* genes but not with the *dsr* ones. It has been proposed to play the role of electron carrier between the sHdr complex and a NAD<sup>+</sup>-reducing dihydrolipoyl dehydrogenase for production of reducing power (*i.e.*, NADH) (Cao et al. 2018).

A third enzyme, the PDO (also classified as SDO for Sulfur Dioxygenase), a soluble cytoplasmic protein particularly studied in *Acidithiobacillus* species but found in numerous bacteria, has been described to oxidase S<sup>0</sup> with O<sub>2</sub>, releasing SO<sub>3</sub><sup>2-</sup> but might rather use S in a persulfidic form as substrate (such as glutathione persulfide GSSH) (Wang et al. 2014). Two PDO were suggested to be involved in persulfide oxidation during growth on S<sub>4</sub>O<sub>6</sub><sup>2-</sup> and in HS<sup>-</sup> oxidation pathway in conjunction with the Sqr and a rhodanese. A fourth enzyme, the SOR, first characterised in *Ac. ambivalens*, performs instead the O<sub>2</sub>-dependent S<sup>0</sup> disproportionation releasing SO<sub>3</sub><sup>2-</sup> and HS<sup>-</sup> (S<sub>2</sub>O<sub>3</sub><sup>2-</sup> is chemically produced). Found in a number of S-oxidizing prokaryotes (Kletzin et al. 2004; Pelletier et al. 2008; Wang et al. 2018), this cytoplasmic enzyme, whose structure is resolved as a 24mer sphere (Urich et al. 2006), has been proposed to be responsible for the production of S substrates further used by other enzymes rather than directly producing ATP or an electron transfer.

### 2.2.3. S<sub>2</sub>O<sub>3</sub><sup>2-</sup> oxidation

S<sub>2</sub>O<sub>3</sub><sup>2-</sup> is a stable and abundant molecule in the environment and can be oxidized through different pathways. The first one consists in the complete oxidation to SO<sub>4</sub><sup>2-</sup> catalyzed by the bacterial periplasmic multi-enzyme Sox system, transferring the electrons to soluble electron carriers (for example, cytochromes or High Potential Iron Sulfur Proteins (HiPIPs)). This system, described mainly for S<sub>2</sub>O<sub>3</sub><sup>2-</sup> oxidation originally in *Paracoccus (P.) pantotrophus*, has been shown to accept HS<sup>-</sup>, S<sup>0</sup> and SO<sub>3</sub><sup>2-</sup> as substrate *in vitro* (Friedrich et al. 2000; Rother et al. 2001) but it is not known if these are physiologically relevant reactions. If many accessory proteins constitute the whole system, the minimal *in vitro* functional system involves the following proteins: the cytochrome *c* SoxAX (which consists, in many bacteria, of distinct SoxA and SoxX proteins) catalyzes the first oxidation step by binding the S<sub>2</sub>O<sub>3</sub><sup>2-</sup> molecule to a persulfurated cysteine of the SoxY-SoxZ tandem “shuttle” (Grabarczyk and Berks 2017). This covalently bound group is then hydrolyzed by SoxB releasing a SO<sub>4</sub><sup>2-</sup> molecule. Some bacteria, such as *P. pantotrophus*, code for another component, SoxCD, that oxidizes the S atom left producing a SoxYZ-Cys-S-SO<sub>3</sub><sup>2-</sup> intermediate that is then hydrolyzed again by SoxB releasing a second SO<sub>4</sub><sup>2-</sup> molecule. Bacteria that do not possess this SoxCD component and that thus

contain the so-called incomplete or truncated Sox system, such as *Al. vinosum*, likely transfer the S atom to S globules or  $S_n^{2-}$  through an unknown mechanism proposed to be mediated by SoxL (Weissgerber et al. 2014).

$S_2O_3^{2-}$  can be also oxidized to  $S_4O_6^{2-}$  by condensation in the periplasm. This pathway has been first described in the archaeon *Ac. ambivalens* where the enzyme involved is a membrane-bound thiosulfate:quinone oxidoreductase (TQO) (Muller et al. 2004). This enzyme is composed of two subunits (DoxA and DoxD) and is widely distributed among chemolithotrophic bacteria and archaea whereas it is never found in phototrophs. The  $S_4O_6^{2-}$  produced can be then hydrolyzed by the tetrathionate hydrolase TetH, in the so-called  $S_4$  intermediate ( $S_4I$ ) pathway. This latter enzyme, typical of acidophiles, is a homodimer whose final products and localization (periplasmic or bound to the outer membrane) are still under debate. The structure resolution of the enzyme from *Acidithiobacillus ferrooxidans* allowed to propose a mechanism for  $S^0$ ,  $S_2O_3^{2-}$  and  $SO_4^{2-}$  generation from the hydrolysis of  $S_4O_6^{2-}$  (Kanao et al. 2021). In phototrophic bacteria, instead, the soluble periplasmic cytochrome *c* thiosulfate dehydrogenase TsdA catalyses the  $S_2O_3^{2-}$  oxidation to  $S_4O_6^{2-}$ , transferring electrons either to a dedicated cytochrome *c* TsdB, encoded upstream, or to a unspecific cytochrome or an HiPIP (see Kurth et al. 2016). An elegant model, based on the resolved crystal structure and on the study of an inhibitor-bound enzyme, proposes a substrate-driven heme ligand release explaining the reversibility (Jenner et al. 2022).

#### 2.2.4. $SO_3^{2-}$ oxidation

The last step of S oxidation consists in the exergonic transformation of  $SO_3^{2-}$  to  $SO_4^{2-}$ . This reaction is carried out by many different microorganisms that can use  $SO_3^{2-}$  coming both from the previous oxidation steps and from the environment (Dahl 2017). Despite being an important intermediate for dissimilatory S pathways, high concentrations of  $SO_3^{2-}$  can also be toxic. In fact, it has antimicrobial properties and is used in some agronomic practices.  $SO_3^{2-}$  can be oxidized by bacteria directly or indirectly, in the periplasm or in the cytoplasm. The best characterized enzyme in the periplasm is the sulfite dehydrogenase Sor/SDH. This enzyme, from which a number of crystallographic structures have been solved, has homologues in both eukaryotes and prokaryotes. It is a Mo-enzyme carrying a mono-pterin rather than the bis-pterin described above. While responsible for detoxifying  $SO_3^{2-}$  by reducing  $O_2$  in plants (*i.e.*, a true  $SO_3^{2-}$  oxidase), the enzyme uses soluble cytochromes as electron acceptor in other organisms (*i.e.*, a  $SO_3^{2-}$  dehydrogenase in this case) and has a bioenergetic role in prokaryotes (see (Kappler and Schwarz 2017) for a comprehensive review). In the cytoplasm, two different



pathways are known so far for  $\text{SO}_3^{2-}$  oxidation to  $\text{SO}_4^{2-}$ : a direct one involving the  $\text{SO}_3^{2-}$  dehydrogenase *Soe* and an indirect one *via* the intermediate APS. The latter pathway corresponds to the reverse reaction of the  $\text{SO}_4^{2-}$  reduction described above, through the enzymes *AprAB* and *Sat*, forming therefore ATP and  $\text{SO}_4^{2-}$ . Alternatively, the enzyme adenylylsulfate:phosphate adenylyltransferase (APAT) generates ADP and  $\text{SO}_4^{2-}$  from APS and phosphate (see (Kappler and Dahl 2001) for review). The enzyme *Soe*, instead, belongs to the *Mo-bisPGD* family, has the canonical heterotrimeric structural organization (*i.e.*, *SoeABC*), is anchored to the cytoplasmic membrane and reacts with Q *via* *SoeC* (Dahl et al. 2013; Boughanemi et al. 2020). The *soe* gene cluster often co-localises with the *dsr* genes and the function of these two enzymes is likely also related. This enzyme, purified from *Aq. aeolicus*, is also capable of reducing  $\text{S}_4\text{O}_6^{2-}$  and  $\text{S}^0$  *in vitro* (Boughanemi et al. 2020).

### 3. Enzymes of the arsenic bioenergetics cycle

Arsenic metabolism is considered to be an ancient metabolism. As the primordial Earth was an anoxic environment, As(III) was likely the predominant As species. It is therefore accepted that primordial resistance/energy metabolisms evolved under the selective pressure of As(III) (Kulp et al. 2008; van Lis et al. 2013; Zhu et al. 2014). The biotransformations of As have been known for a long time but the dissimilatory enzymes involved in these transformations have long been overlooked. To date, many efforts are made to fill the gaps in our understanding of the diversity and expanse of this metabolism, to then try to control As contamination and develop bioremediation strategies (Kabiraj et al. 2022; Raturi et al. 2023). Well characterized dissimilatory metabolisms of As are based on oxidoreduction reactions between oxyanions of As(III) and As(V). Microorganisms (both Archaea and Bacteria) with these metabolisms are often very versatile, having the capability of using different electron donors or acceptors for their energetic chains. The three enzymes identified to date to be involved in the dissimilatory As metabolism, *Aio*, *Arx* and *Arr*, are all members of the aforementioned *Mo-bisPGD* superfamily. The debate on which one is responsible of the primordial As(III) oxidation remains open (see Szyttenholm et al. 2020; Wells et al. 2020).

#### 3.1. As(III) oxidation

As(III)-oxidising bacteria have been known since the early 1900, but it was not until the 1990s that the first enzyme, *Aio* (formerly called *Aox*, *Aso*, *Aro*; see (Lett et al. 2012) for unification of the nomenclature), was purified and characterized (Anderson et al. 1992). Since then, the *Aio* has been detected in a large number of microorganisms, but its activity is not always

coupled to energy production, but also to just resistance mechanisms (see Santini et al. 2000). This periplasmic membrane enzyme is often involved in aerobic metabolisms but is also found in other bioenergetic chains, such as anoxygenic photosynthesis (Wu et al. 2023) or anaerobic respiration of nitrate or chlorate (van Lis et al. 2013; Wu et al. 2023). It has been purified and characterized from different bacteria and two crystallographic structures have been solved (from *Pseudorhizobium banfieldiae* (formerly *Rhizobium* sp. NT-26) and *Alcaligenes faecalis* (Ellis et al. 2001; Warelou et al. 2013)). Among the Mo-*bis*PGD enzymes, Aio represents a special case with a catalytic subunit harbouring a [3Fe-4S] centre rather than the more usual [4Fe-4S] one and with a Rieske-like [2Fe-2S] cluster instead of a typical 4[4Fe-4S] protein as electron-transferring B-subunit (**Figure 4C**). This last subunit carries the TAT signal addressing the enzyme to the periplasm and possibly anchors it to the membrane (Lieutaud et al. 2010) but it does not exchange electrons with Q. Other genetic modules can be present in some *aioBA* clusters, such as, *aioRS* coding for the two-component regulation system (AioS: sensor kinase; AioR: transcriptional regulator) and a gene coding for a cytochrome *c* that can play the role of electron acceptor in some but not all bacteria carrying the Aio enzyme (see van Lis et al. 2013; Kumari and Jagadevan 2016 for reviews).

The other As(III)-oxidising enzyme is the alternative As(III) oxidase Arx (**Figure 4A**), a membranous periplasmic enzyme that has been only recently discovered and that is involved in both anaerobic photosynthesis and chemolithoautotrophy (Hoeft et al. 2007; Kulp et al. 2008). To date, *arx* genes are recognized as widely distributed in different As rich environments (Oremland et al. 2017). The enzyme is proposed as a heterotrimer with ArxA as the catalytic subunit, containing the MoCo, the [4Fe-4S] cluster and the TAT signal peptide, ArxB as the electron transfer subunit, carrying four Fe-S clusters, and ArxC as the membrane-anchoring subunit, devoid of any cofactors and reacting with Q. In all the gene clusters known to date, a gene for a second ArxB subunit (ArxB') is present but the two subunits share a relatively low sequence similarity, and their respective function is still debated since ArxB' has been observed produced (Hernandez-Maldonado et al. 2017), but it is absent from the isolated enzyme (Richey et al. 2009). Optional additional genes can be found in bacterial genomes such as *arxD*, coding for a cytoplasmic chaperon needed for the Mo-subunit maturation, *arxSR* and *arxX* coding for regulators (see van Lis et al. 2013; Kumari and Jagadevan 2016 for reviews). Contrary to the Aio, that has been demonstrated to be unidirectional, Arx has been shown to be able to work in both directions (As(III) oxidation or As(V) reduction), both *in vitro* and *in vivo*, consuming in the latter case the *pmf* (Richey et al. 2009; Szyttenholm et al. 2020). The physiological direction of Arx has been proposed to be correlated to (if not imposed by) the thermodynamics of the

reaction: UQ reduction is thermodynamically favored from the oxidation of As(III), **Table 2**. This is in line with the UQ and Arx co-occurrence in bacteria established by a genomes analysis (no archaeon has been found yet to carry a *bona fide* Arx and to oxidise As(III)) (Szyttenholm et al. 2020).

### 3.2. As(V) reduction

Microbes growing by As(V) respiration were described later than the As(III) oxidizers (Ahmann et al. 1994). It was not until around ten years later that the genes encoding an As(V) reductase involved in this metabolism, the Arr, were sequenced in *Shewanella* sp. ANA-3 (Saltikov and Newman 2003), while the resolution of the crystallographic structure took additional fifteen years (Glasser et al. 2018). Up to date, no archaeon has been demonstrated to reduce As(V) by using a *bona fide* Arr (see next paragraph). This periplasmic enzyme allows the hosts to respire As(V) mostly from the oxidation of organic compounds (but H<sub>2</sub> can be the electron donor in some cases) (see van Lis et al. 2013; Kumari and Jagadevan 2016). The Arr is structurally very similar to the Arx, with the ArrA and ArrB subunits sharing the same cofactors and folding as the ArxA/ArxB counterparts, and it is also bidirectional *in vitro* (Richey et al. 2009, Szyttenholm et al. 2020). But unlike Arx, the Arr does not always contain the membrane-anchoring ArrC subunit that accepts electrons from quinones. In this case, the membrane tetraheme cytochrome CymA plays this role (Murphy and Saltikov 2007). Given the co-occurrence of Arr and MK, established from a genomic analysis, and symmetrically to what is proposed for Arx (see above), it is suggested that the physiological direction of the reaction could be linked *in vivo* to the low redox potential of the MK (**Table 2**) (Szyttenholm et al. 2020). It is worth noting that despite their similar structural organization and their bi-directionality, Arr and Arx form two distinct phylogenetic clades (**Figure 4A**), demonstrating that the sequences of these two enzymes are distinct and that they are truly two different enzymes. Multiple sequence alignment revealed a conserved sequence motif in Arr located, according to the crystal structure, in the active site, that is absent in Arx. In addition to the redox potential of Q, this motif was put forward to rationalise the directional bias (Glasser et al. 2018). As for Arx, the *arrD*, *arrRS* and *arrX* genes can be found in the gene cluster of some but not all bacteria (see van Lis et al. 2013; Kumari and Jagadevan 2016 for reviews).

In addition to the proper bioenergetic As enzymes just described, three known Mo-*bis*PGD enzymes of the bioenergetic cycle of S (*i.e.*, Soe-type, Ttr-type and Psr-type) have been proposed or even established as being capable of As conversion (either by As(III) oxidation or by As(V) reduction) (see in the next section).

#### 4. Interplay between sulfur and arsenic biogeochemistry

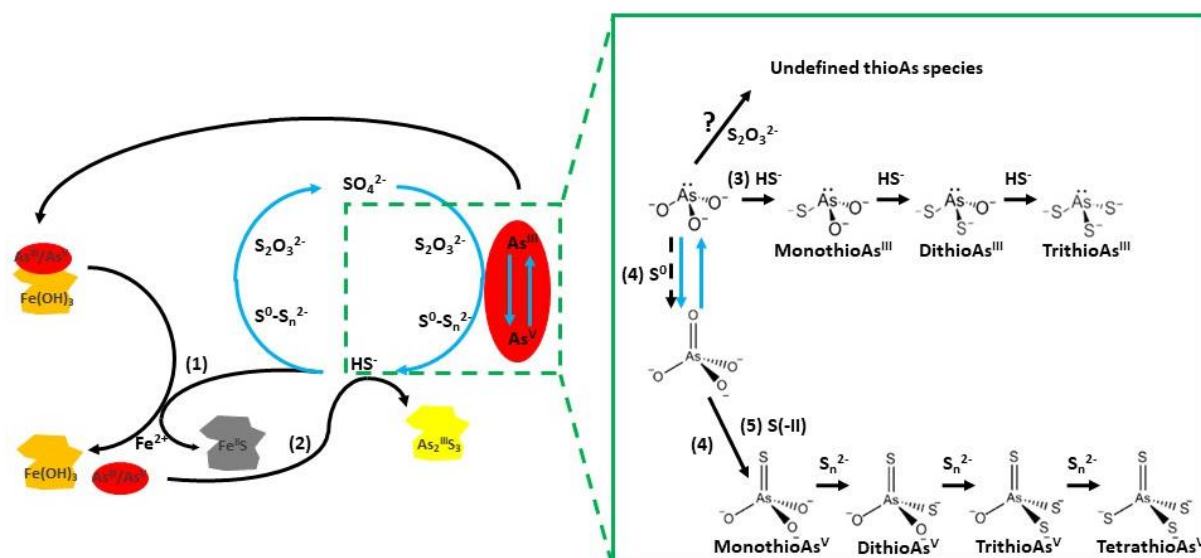
The interplay between S and As cycles can be defined at two main levels that are developed below: 1) the impact of S compounds on the bioavailability and/or conversion of As compounds, and 2) the use of shared enzymes for their metabolization. It is to note, however, that the interplay between these two compounds has also been reported at the level of transcriptional regulation with an operon coregulated by  $\text{HS}^-$  or As described in the cyanobacterium *Synechocystis* sp. PCC6803 (Nagy et al. 2014). This operon contains genes coding for an ArsR-like protein (usually associated with the *ars* genes for resistance to As), an Sqr-like enzyme for  $\text{HS}^-$  oxidation (*suoS*) and a chromate-like transporter working likely as an As(III) importer in some conditions (*suoT*). The coregulation of the whole gene cluster by the two compounds, although its role is still unknown, establishes the direct link of both compounds in the resistance and/or metabolization of S and As compounds in this bacterium.

##### 4.1. Pure chemical interaction between sulfur and arsenic compounds

Among the As-containing minerals, 20% are represented by sulfidic forms (*e.g.*, orpiment ( $\text{As}_2\text{S}_3$ ), realgar ( $\text{As}_4\text{S}_4$ ) and arsenopyrite ( $\text{FeAsS}$ )). While orpiment needs high temperatures to be formed and is therefore present mainly in volcanic areas or hot springs, arsenopyrite and realgar are found in a more diverse geological context. Co-occurring in a common environment,  $\text{HS}^-$  can easily combine with As(III) to form As-S (or Fe-As-S in the presence of iron) minerals, therefore rendering As less bioavailable. As, in the form of As(III) or As(V), can also be absorbed on a number of minerals such as Fe-oxides (Smedley 2002). On the contrary, in certain physico-chemical circumstances, As, absorbed at the surface of minerals or being part of As-minerals, can be redistributed in groundwater by the chemical reaction with reduced S (-II). In As-Ferrihydrite, for example, sulfidation can, under specific conditions, release both reduced Fe and As, which can form either secondary minerals or soluble As (**Figure 5**, paths (1) and (2)) (Cao et al. 2020; Zhang et al. 2023).

In sulfidic alkaline waters, the majority of As is rather found in thiolated soluble pentavalent forms (thioAs(V)) (**Figure 5**), but these species are widespread in a large number of different environments (see Herath et al 2018) for a comprehensive review). While mono-, di-, tri- and tetra- thioAs(V) species exist, the most stable form is the monothioAs(V). In all these thioAs(V) compounds, the S atom is in a reduced form, whereas the As atom is in the oxidized form, making them suitable as both electron donors and acceptors. Thiolated arsenites (thioAs(III)) might also be present *in situ* but they are likely quickly oxidized in contact with oxygen and they are therefore less stable and hardly detectable in laboratory experiments (Planer-Friedrich

et al. 2010). S/As ratios, S oxidation state, as well as pH and oxygen concentration represent some of the chemical parameters that influence the formation and transformation of these thioAs compounds (Wilkin et al. 2003; Stauder et al. 2005; Planer-Friedrich et al. 2015; Sun et al. 2020). Although some details are still under debate, it is proposed that thioAs(V) are formed in anoxic sulfidic aquifers in three ways (see **Figure 5**, ways (3), (4) and (5)). Firstly, under circum-neutral to alkaline conditions, exchange of the hydroxyl group of As(III) for S<sup>-</sup> group from S(-II) leads to the formation of thioAs(III). This thioAs(III) has never been measured and is considered to be rapidly oxidised to thioAs(V). In a second way, S<sup>0</sup> is reduced to S(-II) by oxidation of As(III) to As(V) leading to the formation of thioAs(V). The third method consists in producing thioAs(V) from As(V), but only under acidic conditions (Burton et al. 2013). These thioAs species have also been proposed to be formed from S<sub>2</sub>O<sub>3</sub><sup>2-</sup> (Fisher et al 2008). Thioarsenicals can also be produced from the dissolution of As-S minerals, such as orpiment and arsenopyrite, in HS<sup>-</sup> rich and neutral to alkaline solutions (Suess and Planer-Friedrich 2012).



**Figure 5.** The interplay of sulfur and arsenic biogeochemical cycles. Adapted from Planer-Friedrich (2023).

Microorganisms also play a fundamental role in these processes as the chemical reactions depend on the oxidation state of both S and As and this oxidation state is typically influenced by the microbial activity. There is therefore a strong interconnection between biotic and abiotic transformations, as shown below.

#### 4.2. Intricate chemical and biological interplay between sulfur and arsenic compounds

Bioleaching and bio/chemical generation of (Fe)As-S minerals and complexes are well known, but the exact microbial metabolism involved has often not yet been identified. The fine mechanisms are particularly difficult to comprehend because 1) the impact of S compounds on the As cycle can be the result of a complex interlacing of the biological conversion of S compounds and of the chemical transformation of As compounds, 2) the enzymatic systems involved in the biology of both elements are highly diverse and allow redox changes of the compounds in multiple ways as exposed in sections 2, and 3) often, the enzymes are not known in the specific cases reported.

Joint biological and chemical processes have been reported in the bioleaching of As-Fe-oxides by sulfate-reducing microorganisms in anoxic environments. The release of  $\text{HS}^-$  from the metabolism of sulfate reduction accelerates the release of As and Fe(II) by a purely chemical reaction on As-Ferrihydrite (as explained above), therefore contributing to the bioavailability of As (Nghiem et al. 2023). Release of As from orpiment has also been reported under the action of S-oxidising *Acidithiobacillus* species (Shen et al. 2022). In this case too, the first step is the chemical dissolution of the orpiment while the second step is biological, with bacteria oxidising the  $\text{HS}^-$ , which shifts the chemical equilibrium towards further chemical dissolution. This leads to a release of As in the mobile phase, therefore contributing to the bioavailability of this element. As *Acidithiobacillus* species are also iron oxidisers (e.g., *A. ferrooxidans*), they also contribute to the bioleaching of As from the iron containing arsenopyrite. The formed Fe(III) destroys the bonds at the surface of the mineral's crystal lattice and therefore promotes the mobilisation of  $\text{Fe}^{2+}$  and As (Tang et al. 2023).

$\text{SO}_4^{2-}$  reduction has also been proposed to be involved in the chemical formation of S-As compounds. The bacterium *Desulfotomaculum* TC-1 has been indeed described as growing anaerobically by As(III) oxidation/ $\text{SO}_4^{2-}$  reduction, a biological redox energy process that leads to formation of thioAs(V) (Wu et al. 2017). This accumulation of thioAs(V) was rationalized by the authors as being a result of a chemical reaction between the released products of the bacterial energy metabolism: As(V) (from the oxidation of As(III) by an identified Arx) and  $\text{HS}^-$  (from a  $\text{SO}_4^{2-}$  reduction pathway; **Figure 5**). However, this is not straightforward since, as mentioned above, the generation of thioAs(V) in such a way at pH 6.8 (pH of the growth) is not considered to take place. Some additional experiments are therefore needed to fully understand the intricate chemical and biological processes involved.

Cycling of S-As compounds is not restricted to anoxic sulfidic environments. *Thermocrinis* (*T. ruber* OC14/7/2) has been demonstrated to convert S-As compounds in the presence of O<sub>2</sub> (Hartig et al. 2014). In this environment, monothioAs(V) could be formed by an abiotic reaction between As(III) and S<sup>0</sup>. At the high temperature of *T. ruber* growth (80°C), this monothioAs(V) can be reversely converted to As and different S compounds but it was observed that the bacterium accelerates this conversion, with SO<sub>4</sub><sup>2-</sup> and As(V) being the end products. Although the strain has been demonstrated to be able to grow on As(III) as energy source, the authors proposed a mixed chemical/biological dominant process where monothioAs(V) would be chemically converted to As(V) and S<sup>0</sup>, S<sup>0</sup> being subsequently microbially oxidized to the observed end product SO<sub>4</sub><sup>2-</sup> (Hartig et al. 2014). In addition, they also suggested, a direct biological oxidation of a fraction of monothioAs(V) through the oxidation of the As-bound S. The presence of *hdr*, *sox* (see below) and *aio* genes in all *T. ruber* strains' genome perfectly rationalize this model (unpublished data).

Cycling of thioAs(V) can also result from the reduction of As(V) coupled with the oxidation of S compounds. What was initially considered as a simple biological oxidation of HS<sup>-</sup> coupled with a respiration of As(V) in the bacterium MLMS-1 from the alkaline hypersaline Mono Lake (Hoeft et al. 2004) is now proposed to be a mixed chemical and biological process. An initial biological transformation of the substrates As(V) (by Arr) and sulfide (by an oxidation pathway) provides As(III) and S<sup>0</sup>, respectively. These products would then chemically react to form the transiently detected monothioAs(V) (**Figure 5**). It is proposed that this monothioAs(V) is microbially consumed and transformed by disproportionation into As(III) and S<sup>0</sup>, by an unknown bacterial enzyme (Planer-Friedrich et al. 2015). The growth of the bacterium SLAS-1 (now *Halarsenatibacter silvermanii* SLAS-1) on HS<sup>-</sup>/As(V) could be explained by an identical model including abiotic and biotic processes (the strain also carries *arr* genes) although the thioAs(V) intermediates were not sought (Oremland et al. 2005). In the cases of *Sulfurihydrogenibium* (*S.*) *subterraneum*, *S. azorense* and *S. rodmanii*, isolated from hot springs, the interplay between S and As conversions is really unclear due to the lack of data. They are all thermophilic bacteria belonging to the *Aquificales* order and growing by the anaerobic oxidation of S<sup>0</sup>/S<sub>2</sub>O<sub>3</sub><sup>2-</sup> coupled to the reduction of As(V) as energy metabolism (Takai et al. 2003; Aguiar et al. 2004; O'Neill et al. 2008). ThioAs species were not investigated but, as stated above, their production at the pH of growth (pH 6-7.5), directly from As(V), is unlikely. On the other hand, an enzymatic model for a biological coupled S/As oxido-reduction is not straightforward to explain this metabolism (the authors did not propose any), as genes

homologous to *sox* (for thiosulfate oxidation) can be identified in the genomes of these strains, but not the ones for *aio* (Hamamura et al. 2010) or *arr* (unpublished results).

As highlighted above, thiolated arsenicals are attracting increasing attention as a mean for deciphering the interaction between the cycle of S and As species. A number of works have therefore been devoted specifically to decrypt the way of chemical/biological conversion of these compounds. To this end, several purple sulfur bacteria have been studied for their ability to oxidise monothioAs(V) as sole electron donor for their anoxygenic photosynthesis, releasing As(V) and  $\text{SO}_4^{2-}$  as final metabolic product. The S-oxidising Sox system has been suggested as enzymatic candidate for thioAs(V) transformation in these bacteria, due to the chemical similarities between its natural substrate for growth ( $\text{S}_2\text{O}_3^{2-}$ ) and the monothioAs(V) (Edwardson et al 2014). This hypothesis has been also proposed based on metagenomic analyses correlating the abundance of *sox* genes along hot springs' channels and thioAs(V) transformation (Qing et al. 2023). It is to note that *T. ruber*, which is able to convert monothioAs(V) as well (Hartig et al 2014), also carries the *sox* genes in its genome (unpublished results). Controversial transcriptomic results have, however, been obtained on two *Thioalkalivibrio* species grown under  $\text{S}_2\text{O}_3^{2-}$  supplemented with As(III): *sox* genes are upregulated by As(III)+ $\text{S}_2\text{O}_3^{2-}$  in only one of the two species, although formation of thioAs species has been hypothesized (and known to be possible under these experimental conditions) (Ahn et al. 2019). Hug *et al.* proposed Arr to be able to take over these thioAs(V) species (Hug et al. 2014). But, unlike the hypothesis that Sox is responsible for the conversion of thioAs(V), the hypothesis of Arr for such a role is not supported by any data to date.

#### 4.3. Involvement of sulfur enzymes in arsenic metabolism: the case of the Mo-*bis*PGD enzymes

If the possible function of the Sox system in the metabolization of As compounds could be seen as a broadening of the catalytic spectrum, there are examples of genuine functional reconversion within the Mo-*bis*PGD family. It has long been known that a large substrate variability and versatility of these enzymes contrasts with the great structural conservation of the Mo-*bis*PGD superfamily (see for examples (Grimaldi et al. 2013; Magalon et al. 2016)). But it has been demonstrated that some members of this family can even metabolize substrates different from those predicted by sequence analyses (*i.e.*, by simple sequence homology with biochemically characterized representatives of the same phylogenetic clade).



#### 4.3.1. The case of Soe-type enzymes working in the oxidation of arsenic compounds

A number of *Thioalkalivibrio* species contain one or even two *soeABC* gene clusters, belonging to two different clades. One cluster (“*soe-2*”) is upregulated in these bacteria upon growth on As(III) added to  $S_2O_3^{2-}$  (Ahn et al. 2019). It was therefore proposed that this Soe-2 could convert both  $SO_3^{2-}$  (the “*bona fide*” substrate) and As(III) or thioAs. The latter hypothesis arises from the absence of growth on sole As(III) and from the fact that thioAs could be formed from As(III) and  $S_2O_3^{2-}$ . It is to note that *T. jannaschii* ALM2<sup>T</sup> codes in its genome for an *arx* operon which, instead, is not up-regulated in the presence of As(III) under the aerobic conditions of the experiments.

#### 4.3.2. The case of Ttr-type and Psr-type enzymes working in the reduction of arsenic compounds

The archaeon *Pyrobaculum aerophilum* and the bacterium *Anaeromyxobacter* sp. PSR-1 code in their respective genomes for a Mo-*bis*PGD enzyme closely related to the Ttr (on a sequence point of view) but overexpressed upon As(V)-respiring conditions and exhibiting an As(V) reductase activity. Since these enzymes do not display any  $S_4O_6^{2-}$  reductase activity, they are considered as cases of real enzymatic “reconversion”. It is worth mentioning that the Ttr from *Sa. enterica* does not have, on its side, any As(V) reductase activity (Cozen et al. 2009; Haja et al. 2020; Muramatsu et al. 2020).

In several additional bacteria, other Mo-*bis*PGD enzymes, that are also closer to the Ttr than the canonical Arr, have been proposed as auxiliary or exclusive As reductases on the basis of biochemical studies or transcriptomic/proteomic analyses (Gavrilov et al. 2017; Tsuchiya et al. 2019). In one of them, *Melioribacter roseus*, which does not have the genes coding for an Arr enzyme, the As(V) reduction appears to be achieved by a Ttr-type enzyme, completed by the activity of a Psr/Phs-like enzyme (Gavrilov et al. 2017).

### Conclusions

As developed in this chapter, the geochemical cycles of S and As cannot be understood without taking into account the contribution of microorganisms using these two elements as energy substrates. These two biogeochemical cycles are in fact the result not only of the highly intricate contribution of purely chemical processes and biological conversions, but also of the interplay between the two. In order to adapt to any type of environment, these microorganisms have evolved a myriad of bioenergetic enzymes, whose inventory, in both the S and As cycles, is still being established despite decades of research. The precise biochemical mechanisms

involved are therefore particularly difficult to identify. In some organisms, two different enzyme systems may have a more or less redundant function. In other cases, one enzyme may have acquired specific properties. This field of research, important because of the toxic nature of both elements, is therefore far from being fully explored.

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