Elemental sulfur as electron donor and/or acceptor

Mechanisms, applications and perspectives for biological water and wastewater treatment

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Review

Elemental sulfur as electron donor and/or acceptor: Mechanisms, applications and perspectives for biological water and wastewater treatment

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ABSTRACT

Biochemical oxidation and reduction are the principle of biological water and wastewater treatment, in which electron donor and/or acceptor shall be provided. Elemental sulfur (S^0) as a non-toxic and easily available material with low price, possesses both reductive and oxidative characteristics, suggesting that it is a suitable material for water and wastewater treatment. Recent advanced understanding of S^0-respiring microorganisms and their metabolism further stimulated the development of S^0-based technologies. As such, S^0-based biotechnologies have emerged as cost-effective and attractive alternatives to conventional biological methods for water and wastewater treatment. For instance, S^0-driven autotrophic denitrification substantially lower the operational cost for nitrogen removal from water and wastewater, compared to the conventional process with exogenous carbon source supplementation. The introduction of S^0 can also avoid secondary pollution commonly caused by overdose of organic carbon. S^0 reduction processes cost-effectively mineralize organic matter with low sludge production. Biological sulfide production using S^0 as electron acceptor is also an attractive technology for metal-laden wastewater treatment, e.g. acid mine drainage. This paper outlines an overview of the fundamentals, characteristics and advances of the S^0-based biotechnologies and highlights the functional S^0-related microorganisms. In particular, the mechanisms of microorganisms accessing insoluble S^0 and feasibility to improve S^0 bio-utilization efficiency are critically discussed. Additionally, the research knowledge gaps, current process limitations, and required further developments are identified and discussed.

1. Introduction

Elemental sulfur (often written as S^0, S_8) is one of the predominant sulfur forms (i.e. sulfide, S^0, and sulfate) in the terrestrial crust (Rabus et al., 2013), and it is a central intermediate in the geochemical sulfur cycle (Hao et al., 2014). For instance, S^0 is a crucial intermediate during biological sulfide oxidation to sulfate (Klok et al., 2012). S^0 can also be a final product of biological sulfide oxidation by phototrophic bacteria (Lin et al., 2018) or sulfide-oxidizing bacteria (SOB) with O_2, nitrate, or ferric ions as electron acceptors (Di Capua et al., 2019). In other words, S^0 can be bio-utilized as either electron acceptor (S^0 reduction) or electron donor (S^0 oxidation) in biological reactions. This suggests S^0 may have a broad application in water and wastewater treatment processes.

Applying biological S^0 reduction and/or oxidation in water and wastewater treatment has received increasing attention in the past few decades. Various S^0-based biotechnologies, such as sludge reduction, denitrification and metal-laden wastewater treatment, have been developed to solve complicated environmental problems that occur within current water and wastewater treatment processes in a more cost-

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effective approach (Sahinkaya et al., 2015; Sun et al., 2018; Zhang et al., 2018b). However, there is still a lack of comprehensive summary and discussion of the key advances in the emerging S^0-biotechnologies. On one hand, there are substantial distinctions among these S^0-based bioprocesses, such as microbial communities (e.g. S^0-reducing bacteria, S^0-oxidizing bacteria), fundamental mechanisms (e.g., S^0 reduction, oxidation), and scenarios employed (e.g., treatment of domestic wastewater, industrial wastewater, groundwater, agricultural wastewater, metallurgical wastewater). It is thus necessary to have a clear picture on the fundamental knowledge of respective S^0-based biotechnologies.

On the other hand, there are a number of reviews related to sulfur oxidation metabolism (e.g., sulfide, S^0, sulfite and thiosulfate) (Frigaard and Dahl, 2008; Ghosh and Dam, 2009; Gregersen et al., 2011). However, we notice that sulfur metabolism, especially S^0 metabolism, has not been used to explain and link with process design and optimization. It is known that the water solubility of S^0 is extremely low (5 μg/L at 25 °C) (Boulegue, 1978), which limits the bioavailability of S^0. This could be a bottleneck for its scale-up and wide applications in water and wastewater treatment. Thus, systematical assessment on accessibility of microorganisms to S^0 and their corresponding metabolism would help develop feasible strategies that could facilitate S^0 bio-utilization efficiency, thereby substantially improving process performance.

This review summarizes and critically discusses the S^0-related microbiology, and the mechanisms how sulfur-respiring bacteria access and metabolize the almost insoluble sulfur. The present applications of S^0-based biotechnologies for water and wastewater treatment are comprehensively reviewed. The chemical and biochemical mechanisms involved in specific scenarios and their process optimization are reported. This review is expected to enrich the knowledge on the emerging S^0-based water and wastewater treatment biotechnologies, and to provide technical guidelines for their potential engineering applications. The insights gained are used to define a research agenda.

2. Principles of S^0-based biotechnologies and S^0-respiring bacteria

2.1. S^0 reduction

2.1.1. S^0-reducing bacteria

S^0-reducing bacteria (S^0RB) oxidize organic matter using S^0 as electron acceptor with sulfide being the by-product (Eq. (1)). As such, S^0 reduction can be employed for organic removal from wastewater. The biogenic sulfide can precipitate metals via forming insoluble metals sulfides, indicating its feasibility for metal-laden wastewater treatment. In fact, S^0RB thrive in a variety of environments from acidic to haloalkaline as well as saline and thermophilic conditions (Table 1), suggesting their diverse inhabiting environments. This versatility allows them to participate in water and wastewater treatment in various environments.

$$Acetate^- + 4S^0 + 2H_2O \rightarrow 2CO_2 + 4HS^- + 3H^+ \Delta G^0 = -39\ kJ/mol \quad (1)$$

There are 69 sulfur-reducing genera that have been identified in the *Bacteria* domain, affiliated with nine phyla, such as *Aquificiae, Chrysiogenetes, Deferribacteres, Firmicutes, Proteobacteria, Thermodesulfobacteria, Thermotogae, Spirochaetes, and Synergistetes*. In the *Archaea* domain, 37 genera affiliated with two phyla: *Euryarchaeota* and *Crenarchaeota,* have been identified as S^0 reducers (Florentino et al., 2016b). S^0-reducing archaea are generally hyperthermophilic, and they have been seldom reported for mesophilic wastewater treatment processes. S^0RB utilize a broad spectrum of organics, such as acetate, formate, sugars, lactate, propionate, ethanol, and yeast extract (Hedderich et al., 1998). Besides, some S^0RB can utilize other electron acceptors, such as sulfite, thiosulfate, oxygen, ferric, nitrate, and nitrite (Table 1). Notably, a small fraction of sulfate-reducing bacteria (SRB) (such as *Desulfurobacterium, Desulfuromusa, Desulfovibrio, Desulfitibacter*, and *Desulfobacter*) are also capable of S^0 reduction (Table 1).

Table 1
Typical genera capable of S^0 reduction in wastewater treatment processes. ND: Not determined.

Genus	pH range	Temperature range (°C)	Electron acceptor	Reference
Geobacter	5.5-8.0	4-50	S^0/Fe (III)/ graphite/ NO_3^-/ NO_2^-/Mn (IV)	(Murillo et al., 1999; Coates *et al.*, 2001; Strycharz *et al.*, 2008; Florentino et al., 2016b)
Pelobacter	6.0-8.0	4-45	S^0/Fe(III)	(Lovley *et al.*, 1995; Narasingarao and Häggblom, 2007)
Sulfurospirillum	6.0-8.0	20-36	S^0/ $S_2O_3^{2-}$/ SO_3^{2-}/ NO_3^-/ NO_2^-/O_2	(Stolz *et al.*, 1999; Kodama and Watanabe, 2007)
Pseudomonas	ND [7.0 (optimum)]	20-36	S^0/ $S_2O_3^{2-}$/ SO_3^{2-}// NO_3^-/ NO_2^-	(Balashova, 1985; Almeida *et al.*, 1995; Kesserű *et al.*, 2002)
Clostridium	5.8-9.0	18-45	S^0/SO_4^{2-}/ $S_2O_3^{2-}$/ SO_3^{2-}/ NO_3^-/ NO_2^-	(Hasan and Hall, 1975; Sallam and Steinbüchel, 2009)
Desulfurella	3.0-7.5	20-77	S^0/$S_2O_3^{2-}$	(Florentino et al., 2016a; Sun *et al.*, 2019)
Desulfuromonas	6.5-8.5	25-35	S^0/Fe(III)	(Roden and Lovley, 1993; Finster *et al.*, 1994)
Desulfomicrobium	ND [6.6-7.5 (optimum)]	2-41	S^0/SO_4^{2-}/ $S_2O_3^{2-}$/ SO_3^{2-}	(Barton and Hamilton, 2007; Florentino et al., 2016b)
Desulfovibrio	5.5-8.0	10-40	S^0/SO_4^{2-}/ $S_2O_3^{2-}$/ SO_3^{2-}/ O_2/NO_3^-/ NO_2^-	(Surkov *et al.*, 2001)
Desulfobulbus	6.0-8.6	10-43	S^0/SO_4^{2-}/ $S_2O_3^{2-}$/ SO_3^{2-}/ NO_3^-/ NO_2^-/O_2/ Fe(III)	(Sass et al., 2002; Holmes et al., 2004)
Desulfobacter	ND [6.6-7.3 (optimum)]	5-38	S^0/SO_4^{2-}/ $S_2O_3^{2-}$/ SO_3^{2-}	(Widdel, 1987; Lien and Beeder, 1997)

2.1.2. Main enzymes associated with S^0 reduction

The biochemical mechanisms underlying S^0 reduction and the nature of the involved enzymes remain incompletely understood. Three main enzymes (polysulfide reductase (PSR), sulfur reductase (SRE), and sulfide dehydrogenase (SUDH)) involved in S^0 reduction (Fig. 1) have been purified and characterized in a limited number of S^0 reducers (Florentino et al., 2016b), such as *Wolinella succinogenes, Pyrococcus furiosus, Acidianus ambivalens*, and *Desulfurellaceae* family (Ma and Adams, 1994; Hedderich et al., 1998; Florentino et al., 2017).

The membrane-bound polysulfide reductase consists of three subunits (PsrABC). PsrA is the catalytic subunit of the Psr protein for polysulfide reduction to sulfide. PsrB on the periplasmic side of the

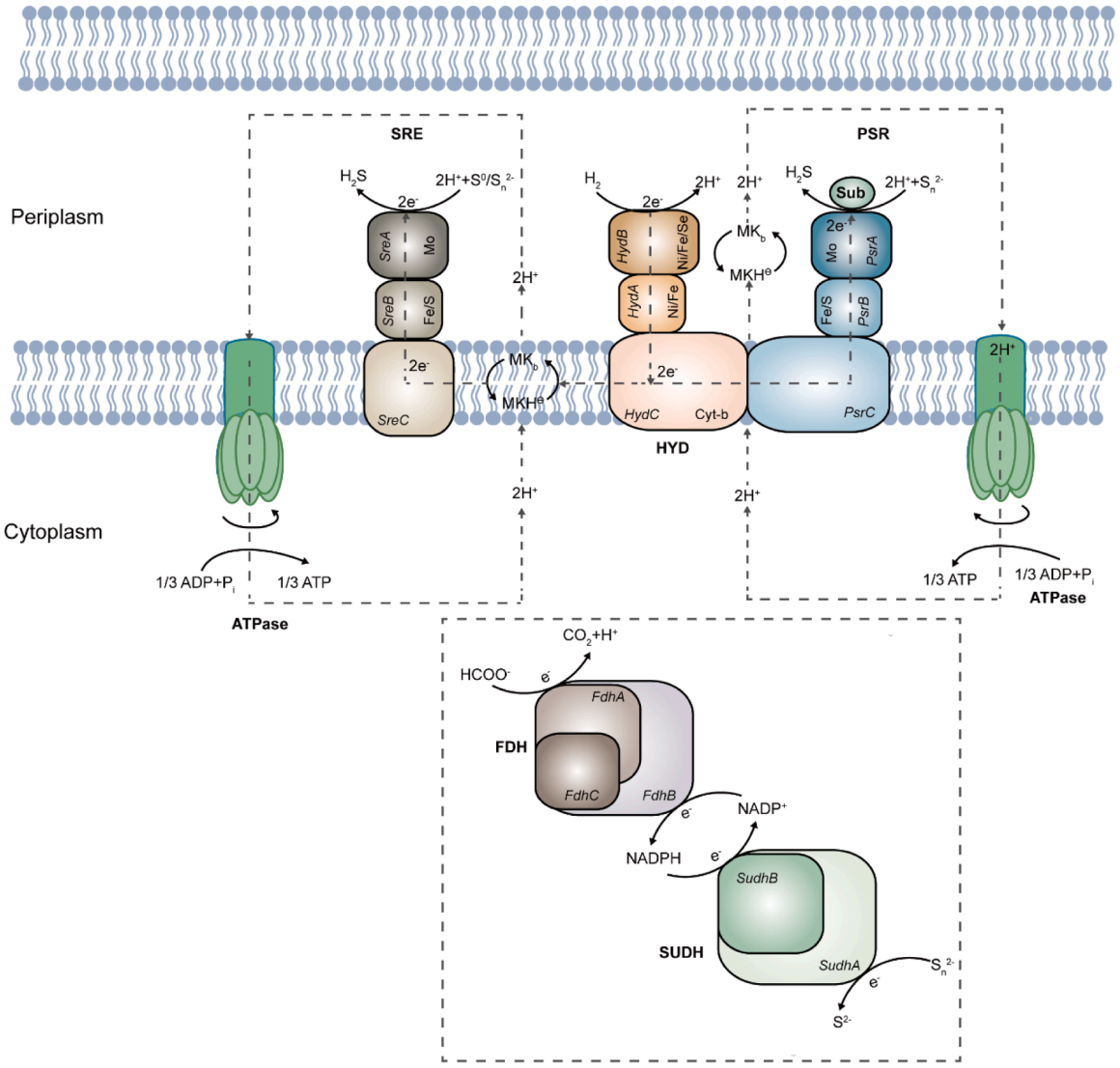

Fig. 1. Putative mechanisms of S^0 reduction. Electrons may be transferred from hydrogenases (HYD) to polysulfide reductases (PSR) or sulfur reductases (SRE) via menaquinones (MK). Sub is a polysulfide transferase. If sulfide dehydraogenase (SUDH) is involved in S^0 respiration, the electrons may be transferred from the intracellular formate dehydrarogenase (FDH) to SUDH with $NADP^+$/NADPH as intermediates (in case of formate as the electron donor) (adapted from Hedderich et al. (1998) and Florentino et al. (2019)). Note that not all the S^0RB contain all the enzymes or pathways described in this figure.

membrane contains several [Fe–S] clusters, likely mediating the electron transfer from the membrane anchor (PsrC) to the catalytic subunit (PsrA) of the Psr (Florentino et al., 2016b). The Psr contains menaquinone, which could serve as electron acceptor from hydrogenase, and could also be electron donor for polysulfide/S^0 reduction. Membrane-bound sulfur reductase is analogous to polysulfide reductase and consists of following subunits predicted by the operon SreABCDE; catalytic subunit (SreA), [Fe–S] subunit (SreB), membrane anchor subunit (SreC), and two subunits (SreDE) with unknown functions (Liu et al., 2012). The SRE reduces S^0/polysulfide to sulfide with hydrogenase, quinones, cytochromes, and NADPH as the electron donors (Florentino et al., 2017). When polysulfide is transported to cytoplasmic space, it can be reduced to sulfide by the cytoplasmic SUDH using NADPH as the electron donor (Ma and Adams, 2001). This reductase consists of two subunits (SudhAB) and contains two flavins and three different [Fe–S] clusters (2Fe-2S, 3Fe-4S, and 4Fe-4S) (Ma and Adams, 1994). However, how these [Fe–S] clusters participate in electron transfers in S^0 reduction still remains unknown. The enzymes associated with S^0 reduction include but not limited to the three discussed above. Further research can be focused on the S^0 reduction functions of these enzymes and discovering new enzymes responsible for S^0 reduction.

2.2. S^0 oxidation

2.2.1. S^0-oxidizing bacteria

S^0-oxidizing bacteria (S^0OB) can reduce oxidative substances (nitrate, nitrite, ferric ion etc.) using S^0 as electron donor with sulfate being the by-product (taking nitrate as an example, Eq. (2)). In principle, S^0 oxidation can be employed for nitrate, perchlorate, and oxidative metals removal from water and wastewater. Phototrophic sulfide-oxidizing bacteria can oxidize S^0, but they are only employed for sulfide instead of S^0 oxidation in specific environmental biotechnologies for wastewater treatment (Pokorna and Zabranska, 2015). This is not be further discussed in this review.

$$S^0 + 1.2NO_3^- + 0.4H_2O \rightarrow SO_4^{2-} + 0.6N_2 + 0.8H^+ \Delta G^0 = -547.6\ \mathrm{kJ/mol} \quad (2)$$

Most chemotrophic S^0OB are affiliated with the phylum *Proteobacteria*, particularly distributed in the class γ-*Proteobacteria* (Muyzer et al., 2013). The chemotrophic S^0OB (*Acidithiobacillus, Thiobacillus,*

Sulfurimonas, Halothiobacillus etc.) are also capable of oxidizing sulfide, thiosulfate, and sulfite (Table S1) (Inagaki et al., 2003; Ito et al., 2004; Muyzer et al., 2013; Qiu et al., 2020). Besides neutrophilic, their inhabiting environments also include psychrophilic and thermophilic conditions (Odintsova et al., 1996; Muyzer et al., 2013). As such, the applications of S^0OB-based environmental technology are not restricted to neutral conditions, but include bioleaching of heavy metals under acidic conditions (Liu et al., 2008) and others. The detailed information about the typical chemotrophic S^0OB is shown in Table S1.

2.2.2. *Main enzymes associated with S^0 oxidation*

Microbial S^0 oxidation is a complicated process that is associated with various enzymes/proteins and sulfur intermediates in different cellular compartments, and several related genes and proteins have so far been characterized (Kletzin et al., 2004; Ghosh and Dam, 2009; Yin et al., 2014; Wang et al., 2019b). Thus, the possible S^0-oxidizing pathways have been depicted for some S^0OB, particularly for *Acidithiobacillus* spp. (Fig. 2), such as *A. thiooxidans*, and *A. ferrooxidans* (Yin et al., 2014; Wang et al., 2019b). The S^0 metabolic process is generally summarized into two different pathways, i.e., sulfur-oxidizing enzyme (Sox)-dependent (i.e., *A. thiooxidans*) and Sox-independent S^0 oxidation (i.e., *A. ferrooxidans*). Generally, extracellular S^0 can first be activated by thiol-containing OMP while forming thiol-bound sulfane sulfur atoms (R-S-S_nH), and is then transported to the periplasmic space (Rohwerder and Sand, 2003), where various enzymes, such as Sox system (not present in Sox-independent S^0 oxidation pathway), tetrathionate hydrolase (TetH), thiosulfate dehydrogenase (TSD), participate the further oxidation of R-S-S_nH (Fig. 2).

In the cytoplasmic membrane, sulfide:quinone oxidoreductase (SQR) oxidizes hydrogen sulfide to S^0, which may be re-oxidized or mobilized into the cytoplasmic space (Chen et al., 2012). In the cytoplasmic space, S^0 is further oxidized to sulfite by a series of enzymes, such as sulfur oxygenase reductase (SOR), rhodanese (TST), Hdr-like complex (HDR) and sulfur dioxygenase (SDO) (Wang et al., 2019b). Sulfite is then oxidized to sulfate either via the sulfite : acceptor oxidoreductase (SAOR) (Rohwerder and Sand, 2003; Ghosh and Dam, 2009) or the adenosine-5'-phosphosulfate (APS) oxidation pathway (Kletzin et al., 2004). Under neutral or alkaline conditions, if sulfide is present in the extracellular environments, polysulfide-involved S^0 oxidation may occur. Polysulfide could cross the cell membrane to the periplasm, followed by the complete oxidation to sulfate via a series of enzymes or enzyme complexes and electron transport chain reactions, likely similar to those in *Acidithiobacillus* spp. (Ghosh and Dam, 2009). For more detailed and comprehensive information about S^0 oxidation systems and the related electron transfer pathways, readers can refer to Kletzin et al. (2004), Dahl and Friedrich (2008), Wang et al. (2019b), and Yin et al. (2014).

3. S^0-based biotechnologies for water and wastewater treatment

Over the past few decades, biotechnologies driven by S^0 reduction and/or oxidation have been fruitfully developed for water and wastewater treatment. It is vital to notice that such applications are cost-efficient and practically feasible. These S^0-based biotechnologies can

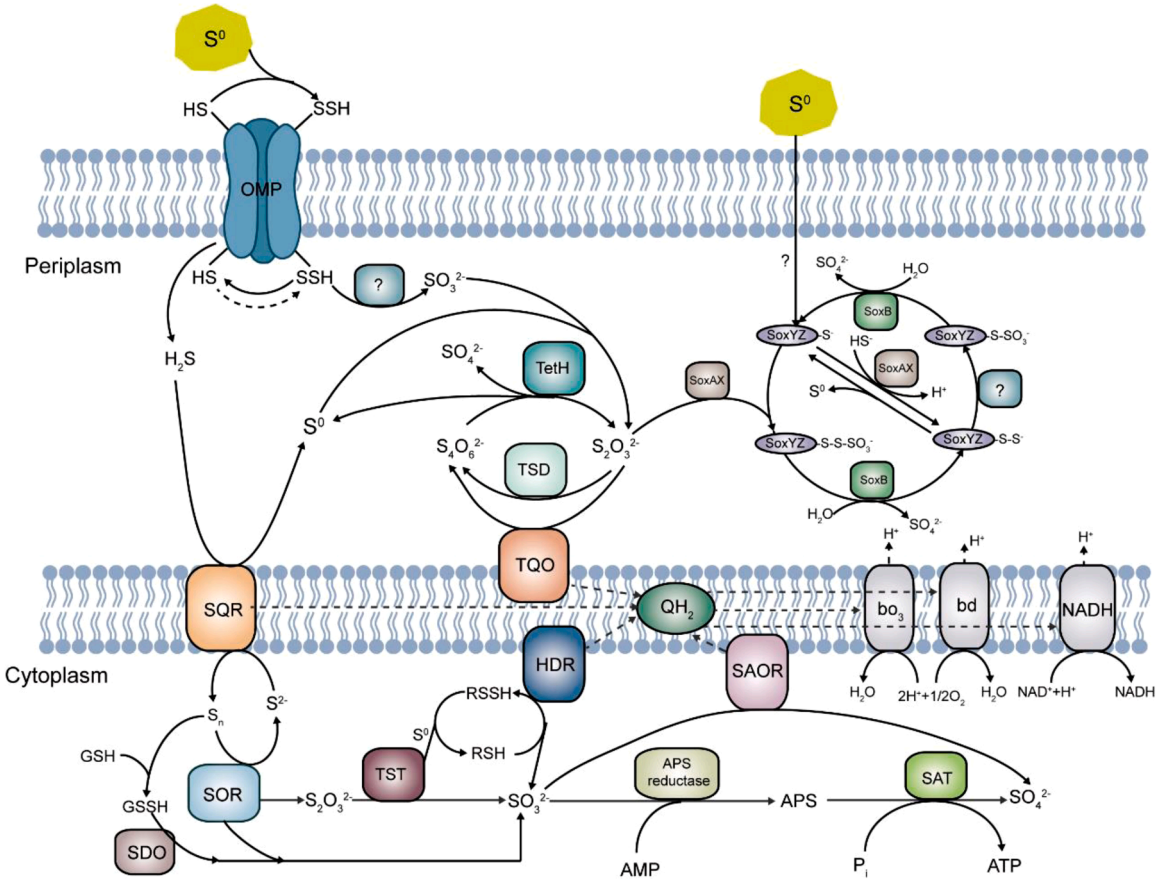

Fig. 2. Overview of the proposed S^0 oxidation metabolisms in *Acidithiobacillus* spp. (Modified from the Kletzin et al. (2004), Wang et al. (2019b) and Yin et al. (2014)). Abbreviation: OMP: outer-membrane proteins; SDO: sulfur dioxygenase; SQR: sulfide:quinone oxidoreductase; SOR: sulfur oxygenase reductase; TetH: tetrathionate hydrolase; TSD: thiosulfate dehydrogenase; TQO: thiosulfate quinone oxidoreductase; TST: rhodanese; HDR: Hdr-like complex; QH_2: quinol pool; bo_3 and bd: terminal oxidases; DADH: NADH dehydrogenase complex I. SAOR: sulfite : acceptor oxidoreductase; SAT: ATP sulfurylase; APS: adenosine-5'-phosphosulfate; AMP: adenosine monophosphate. Note that not all the S^0OB contain all the enzymes or pathways described in this figure.

be categorized into three groups: a) S^0 used as electron acceptor for organic removal; b) S^0 used as electron donor for nitrate, perchlorate, and oxidative metals removal; and c) S^0 used to generate sulfide for heavy metal precipitation.

3.1. Achieving sludge reduction during high-rate organic carbon removal

The treatment and disposal of excess activated sludge have always been a challenging issue in wastewater treatment plants (WWTPs). Minimizing sludge production by anaerobic wastewater treatment processes is a promising approach (Guo et al., 2013). S^0 reduction process has recently been demonstrated to be an efficient anaerobic wastewater treatment process with a high organic removal rate of 1.71 kg COD/m^3-d and a hydraulic retention time (HRT) of 3 h (COD: chemical oxygen demand) (Zhang et al., 2018b). The sludge yield was 0.16 kg VSS/kg COD, which is much lower than that of conventional activated sludge process (0.35–0.47 kg VSS/kg COD) (Wei et al., 2003). The obtained high-rate performance was attributed to the polysulfide-involved indirect S^0 reduction (Zhang et al., 2018c). The S^0 reduction during domestic wastewater treatment was mainly mediated by the S^0 reducers *Geobacter* and *Desulfomicrobium* (Zhang et al., 2018b).

The produced sulfide could be further used as the electron donor for autotrophic denitrification, which is particularly suitable for the treatment of low C/N ratio wastewater (Show et al., 2013; Huang et al., 2021). However, S^0 derived compounds (i.e., sulfate) still remain in the effluent, likely causing secondary sulfate pollution. A promising solution is to terminate sulfide oxidation at S^0 via controlling the molar ratio of nitrate/sulfide (N/S) at an optimal level (i.e., $\leq$0.4) (Lin et al., 2018). The biogenic sulfide can also be recovered and recycled via micro-aeration. Zhang et al. (2018d) developed an internal sulfur cycling (ISC) process consisting of a S^0-reducing reactor and a sulfide-oxidizing reactor to recover S^0 (Fig. 3). The 200 days of stable operation of the lab-scale ISC system demonstrated that 94% of the influent COD (~300 mg/L) were removed without excessive sludge withdrawal, and 76% of the produced sulfide were selectively oxidized to S^0 for recycling. *Clostridium* played an important role in S^0 reduction, while *Halothiobacillus* and *Thiomonas* contributed to sulfide oxidation to S^0 under micro-aerobic conditions.

3.2. Cost-efficient removal of nitrate from low C/N ratio wastewater

Reduced sulfur species (i.e., sulfide, S^0 and thiosulfate) have been often reported to participate in chemolithotrophic denitrification as electron donors (Cui et al., 2019). The denitrification rate with the three types of electron donors follows the order of thiosulfate > sulfide > S^0. Considering operational cost and sulfate production, elemental sulfur-based autotrophic denitrification (SADN) has competitive advantages over thiosulfate- or sulfide-driven autotrophic denitrification. For instance, thiosulfate-driven autotrophic denitrification has the highest operation cost and sulfate production ($0.72/kg nitrate and 11.07 g SO_4^{2-}/g NO_3^--N) (Di Capua et al., 2019). Although the operational cost and sulfate production during sulfide-driven denitrification are lower than that of SADN (5.58 g SO_4^{2-}/g NO_3^--N vs. 7.54 g SO_4^{2-}/g NO_3^--N, $0.19/kg nitrate vs. $0.43/kg nitrate) (Cui et al., 2019), the use of sulfide chemicals induces safety issues and a negative public image. S^0 oxidation can achieve complete denitrification and partial denitrification under favorable conditions, which are discussed further.

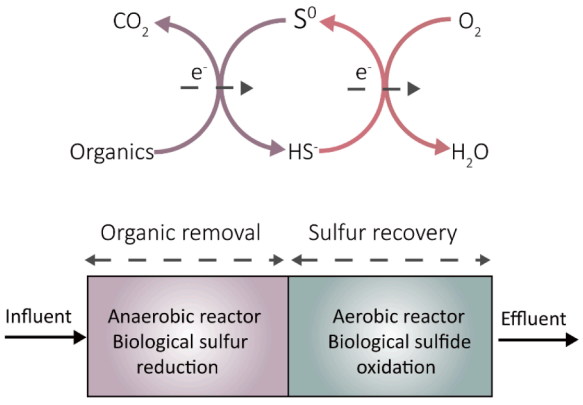

Fig. 3. Schematic diagram and flow chart of internal sulfur cycling (ISC) process.

3.2.1. Complete denitrification

SADN is an appealing low-cost process for nitrogen removal (Eq. (2)) from drinking water, stormwater runoff, groundwater, and low C/N ratio wastewater (Sierra-Alvarez et al., 2007; Shao et al., 2010; Wang et al., 2016). Equally important, SADN produces less or similar N_2O compared to heterotrophic denitrification (0.01–0.6% vs. 0.005–1.2% of the nitrogen load) (Zhang et al., 2015b; Zhu et al., 2018; Kampschreur et al., 2009). *Thiobacillus* and *Sulfurimonas* are often reported to be two dominant autotrophic denitrifiers in SADN systems (Table S2). SADN removes one gram nitrate consuming 4.57 g alkalinity (as $CaCO_3$) (Cui et al., 2019). Thus, it declines system pH and possibly influences nitrogen removal. S^0-packed bed reactors (S^0-PBRs) supplemented with limestone called S^0-limestone autotrophic denitrification (SLAD) are often employed in which limestone is served as the neutralizer for balancing pH and inorganic carbon for microbial synthesis. Solid S^0 and limestone can also serve as carriers to support biofilm development, which is beneficial for biomass retention and performance improvement of nitrate removal. Other solid-phase buffers (calcite, crushed oyster shells etc.) are also used (Cui et al., 2019). In addition, membrane bioreactors (MBRs) with bicarbonate have been applied to achieve high biomass retention and increase effluent quality (Sahinkaya et al., 2015; Zhang et al., 2015a; Ucar et al., 2020; Ucar et al., 2021). SADN has been applied at pilot-scale for drinking water production from nitrate-contaminated groundwater (Shao et al., 2010; Wang et al., 2019c). It has also been applied in pilot- and full-scale systems for treating WWTP effluent (Sahinkaya et al., 2014). More information about SADN achieved in different systems as well as process performance is presented in Table S2.

Sulfate generation is a major concern during SADN process. Although sulfate concentration is not regulated by the wastewater discharge standards, the maximum sulfate concentration in drinking water set by US EPA is 250 mg/L (Zhang et al., 2015a), which is equivalent to the amount of sulfate produced from complete removal of around 33 mg N/L nitrate (Sahinkaya et al., 2011). A high sulfate concentration can result in a noticeable taste (>400 mg/L) or even diarrhea (1000–1200 mg/L) (Huang et al., 2018). One compromised approach is to couple S^0-based autotrophic denitrification and heterotrophic denitrification to form mixotrophic denitrification, as heterotrophic denitrification produces alkalinity and reduces sulfate production (Zhang et al., 2015a). Integrating SADN with iron-based autotrophic denitrification is another solution with more competitive advantages. In addition to avoid the secondary pollution caused by residual organics in mixotrophic denitrification, Zhu et al. (2018) demonstrated that a SADN system supplemented with siderite ($FeCO_3$) not only reduced sulfate production but also significantly improved nitrate removal rate (Eq. (3)). It was due to the synergistic effects of S^0 and $FeCO_3$ on denitrification. This process was also demonstrated in a pilot-scale system (Wang et al., 2019c). Similar with siderite, iron sulfides (e.g. pyrrhotite, Eq. (4)) can also be used to reduce sulfate production and improve nitrate removal in SADN systems (Hu et al., 2020; Li et al., 2020a). Of note, the produced Fe(III) could further remove phosphate via sorption process (Wilfert et al., 2015; Kumar et al., 2019).

$$FeCO_3 + 0.2NO_3^- + 1.6H_2O \rightarrow Fe(OH)_3 + 0.1N_2 + 0.8CO_2 + 0.2HCO_3^- \ \Delta G^0 = -54.3 \text{ kJ/mol} \quad (3)$$

$$FeS + 1.8NO_3^- + 1.6H_2O \rightarrow Fe(OH)_3 + 0.9N_2 + SO_4^{2-} + 0.2H^+ \ \Delta G^0 = -3817 \text{ kJ/mol} \quad (4)$$

According to Eq. (2), a theoretical stoichiometric ratio of sulfur to nitrogen for complete nitrate reduction to N_2 gas is 1.9 (g S/g N). However, NO_2^- and N_2O would be accumulated if this ratio is applied. It could be attributed to the limited mass transfer from solid to liquid phase caused by the low aqueous solubility of S^0, and/or the different competitive capabilities of enzymes associated with nitrogen oxides reduction (e.g., NO_3^-, NO_2^-, N_2O) for limited electron donors (Oberoi et al., 2021). Biogenic S^0 (hereafter referred to as bio-S^0) has higher bioavailability than chemically synthesized S^0 (hereafter referred to as chem-S^0). Hence, bio-S^0 would result in lower accumulation of intermediates during nitrate reduction. To minimize nitrite accumulation, S^0 is usually present in a large stoichiometric excess to supply sufficient electron donors (Zhang et al., 2015a; Ucar et al., 2020).

3.2.2. Partial denitrification coupled with anammox process

Partial denitrification ($NO_3^- \rightarrow NO_2^-$) coupled with anaerobic ammonium oxidation (anammox) process (PD/A) is an attractive autotrophic nitrogen removal process for energy-efficient industrial and domestic wastewater treatment Du et al., 2019). The anammox process inevitably generates 11% of nitrate relative to the influent ammonium concentration. Heterotrophic denitrification process can be supplemented to remove nitrate from anammox system. To apply the right amount of organics for denitrification is very complicated, likely leading to secondary pollution. Alternatively, SADN process could replace full denitrification to supplement anammox process with nitrite as only after conversion to nitrite, further denitrification to nitrogen gas occurs (Eqs. (5) and ((6)) (Chen et al., 2018).

S/N ratio is a critical factor influencing nitrite accumulation during SADN. As stated above, when S^0 is supplied according to the stoichiometric S/N ratio, nitrite build-up can be achieved. However, it is highly uncertain if efficient conversion of nitrate to nitrite can be obtained. Thus, in practice, S^0 is excessive in reactors. How to optimize the S/N ratio to achieve effective nitrite accumulation still needs further investigation. Feed pH and temperature are the other two key factors. Weak alkaline conditions could encourage nitrate conversion while minimizing nitrite removal (Chen et al., 2018). Although temperature has a minor impact on nitrate and nitrite removal, appropriate temperature would be beneficial for denitrifying activity, and could result in satisfactory nitrite accumulation owing to the faster rate of nitrate reduction over nitrite reduction. For example, Chen et al. (2018) obtained more than 95% of nitrite accumulation with an influent nitrate concentration of 100 mg N/L in a SADN system at pH 8.5 and 35 °C. The feasibility of S^0-driven autotrophic PD/A process has been further demonstrated by treating fluorine-containing semiconductor wastewater rich in ammonia and nitrate (Li et al., 2019). The total nitrogen removal efficiency reached 98% with an influent total nitrogen loading rate of 4.19 kg/m^3-d.

$$3NO_3^- + S^0 + H_2O \rightarrow 3NO_2^- + SO_4^{2-} + 2H^+ \Delta G^0 = -277.8 \text{kJ/mol} \quad (5)$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \ \Delta G^0 = -357.8 \text{ kJ/mol} \quad (6)$$

3.3. Treating drinking water resources contaminated with nitrate and perchlorate

Autotrophic S^0 oxidation can also be employed to efficiently remove perchlorate from surface water, groundwater, and drinking water via reduction process with chloride ions being the end-product (Eq. (7)) (Ju et al., 2007; Sahu et al., 2009; Wan et al., 2017; Wan et al., 2019a). Perchlorate often co-contaminates with nitrate in surface water and groundwater (Wan et al., 2017). Microbial community analysis showed that *Sulfuricella*, *Sulfuritalea, Thiobacillus,* and *Sulfurimonas* were the effective autotrophic denitrifiers/perchlorate-reducing bacteria. Wan et al. (2017) observed that a lab-scale S^0-PBR operated at a low HRT (0.75 h) simultaneously removed >97% of the influent nitrate (22 mg N/L) and perchlorate (472 μg/L or 22 mg/L). However, when perchlorate concentration decreased to a low level, S^0 disproportionation to sulfide and sulfate initiated (Eq. 8), resulting in excessive sulfate production and alkaline consumption (Wan et al., 2019a; Wan et al., 2019b). Combining heterotrophic denitrification and SADN could be a solution to prohibit S^0 disproportionation, in which a heterotrophic reactor was followed by a S^0 autotrophic reactor (CHSAS) (Wan et al., 2019a). In the CHSAS, both heterotrophic and autotrophic processes remove perchlorate and nitrate, thereby reducing sulfate production. Lowering the HRT as much as possible without sacrificing the system's performance could also restrict S^0 disproportionation. However, the right amount of organic dosage is difficult to control. The feasibility of the CHSAS still needs to be further demonstrated before practical application.

$$3ClO_4^- + 4S^0 + 4H_2O \rightarrow 4SO_4^{2-} + 3Cl^- + 8H^+ \Delta G^0 = -2397.7 \text{ kJ/mol} \quad (7)$$

$$4S^0 + 4H_2O \rightarrow SO_4^{2-} + 3HS^- + 5H^+ \Delta G^0 = 10.2 \text{ kJ/mol} \quad (8)$$

3.4. Metal removal from groundwater and wastewater

Either biological S^0 reduction or oxidation can be employed for metal removal from water and wastewater. Biogenic sulfide produced from S^0 reduction can directly precipitate metals (Cu(II), Pb(II), Fe(II), Cd(II), Zn (II), Ni(II), Hg(II), etc.) via forming insoluble metal sulfides. When treating arsenic- and mercury-contaminated wastewater, S^0 reduction exhibited competitive advantages over sulfate reduction (discussed separately). The immobilization of chromium is also highlighted separately, because hexavalent chromium (Cr(VI)) can be reduced by biogenic sulfide or autotrophic S^0 oxidation.

3.4.1. S^0-driven sulfidogenic process for metal-laden wastewater treatment

Sulfate reduction has been widely applied for metal-laden wastewater treatment in practice, such as metallurgical wastewater and AMD Neculita et al., 2007). However, organic content in metal-laden wastewater is generally too low to achieve high-rate sulfate reduction process (Neculita et al., 2007). Supplementing additional carbon sources would certainly increase the operational cost and in return limit its wide application. S^0 reduction could be a promising alternative that requires only two electrons per sulfide instead of the eight electrons demanded by sulfate reduction (Eqs. (9) vs. ((10)). This theoretically reduces 75% of organic consumption compared to sulfate reduction. An experimental study on the laboratory-scale AMD system showed the S^0 reduction could reduce 25.6–78.9% of the chemical cost for sulfide production compared to sulfate reduction (Sun et al., 2020a). *Desulfomicrobium* as a facultative S^0RB was found to predominate in the reactor, which preferentially utilized S^0 rather than sulfate as the electron acceptors in the presence of sulfide. It may be due to the presence of polysulfide (S_n^{2-}) in such scenario, where it is rapidly formed by the abiotic reaction between sulfide and S^0 (Eq. (11)), and the facultative S^0 reducers tend to grow on polysulfide instead of sulfate (Eq. (12)). It is because the activation of sulfate consumes ATP, whereas polysulfide does not (Poser et al., 2013). As such, even sulfate is more bioaccessible than S^0, high-rate S^0-driven sulfidogenic processes (53–63 mg S/L-h) are still viable (Table 2). Li et al. (2021) has recently tested this concept in a pilot-scale system treating real Cu-laden electroplating wastewater. However, the S^0 reduction rate was lower than those obtained in laboratory-scale studies (Table 2). It may be due to the fact that S^0RB could only use simple organics, which is commonly used in the laboratory-scale studies, and

Table 2
Comparisons between S^0-driven and sulfate-driven sulfidogenic reactors.

Reactor	Wastewater type	Carbon source	Sulfur source	pH	HRT (h)	Sulfide production rate (mg S/L-h)	Reference
Neutral conditions							
S^0-PBR	Synthetic Cr(VI)-contaminated wastewater	Ethanol	S^0	8.1	8.6-24	12-36	(Sahinkaya et al., 2012)
S^0-PBR	Synthetic pretreated AMD	Sodium acetate and glucose	S^0	7.3 ± 0.3	9.6	63	(Sun et al., 2020b)
S^0-PBR	Cu-laden electroplating wastewater	Domestic wastewater	S^0	~7.5	10-21	19-21	(Li et al., 2021)
FBR	Synthetic wastewater	Sodium acetate, glucose, and yeast extract	S^0	~8.0	3	126	(Zhang et al., 2018c)
SBR	Synthetic pretreated AMD	Sodium acetate and glucose	S^0	~7.0	12	53	(Sun et al., 2018)
SBR	Synthetic mercury-contaminated wastewater	Sodium acetate, glucose, and yeast extract	S^0	7.0	48	7-12	(Wang et al., 2019a)
CSTR	Synthetic wastewater	Ethanol	SO_4^{2-}	7.0-7.2	240	1.7	(Sahinkaya, 2009)
An-RBC	Growth medium	Sodium lactate	SO_4^{2-}	7.0	24	~10	(Kiran et al., 2017)
UASB	Synthetic wastewater	Lactate	SO_4^{2-}	7.0	96	3.3	(Gopi Kiran et al., 2016)
EGSB	Synthetic wastewater	Sodium acetate	SO_4^{2-}	8.0-8.5	5.5	87.5	(De Smul and Verstraete, 1999)
FBR	Synthetic wastewater	Sodium lactate	SO_4^{2-}	6.8	16	23	(Kaksonen et al., 2003)
FBR	Growth medium	Ethanol and yeast extract	SO_4^{2-}	7.0	5.1	13	(Nagpal et al., 2000)
AnMBR	Synthetic wastewater	Ethanol	SO_4^{2-}	6.5-7.0	26.4	23-28	(Sahinkaya et al., 2018)
Acidic conditions							
S^0-PBR	Synthetic wastewater	Sodium acetate and glucose	S^0	4.3	6.7	18	(Sun et al., 2019)
S^0-PBR	Synthetic pretreated AMD	Sodium acetate, glucose, and yeast extract	S^0	2.6-3.5	9.6	36	(Sun et al., 2020b)
EGSB	Synthetic AMD	Acetate	SO_4^{2-}	6.0	15	40	(Liu et al., 2018)
FBR	Synthetic metal-contaminated wastewater	Lactate	SO_4^{2-}	2.0-4.0	22.9	24	(Sahinkaya and Gungor, 2010)
FBR	Synthetic wastewater	Lactate	SO_4^{2-}	4.0-6.0	2-7	24	(Villa-Gomez et al., 2015)
UAMB	AMD	Sodium lactate and yeast extract	SO_4^{2-}	2.8	23	24	(Bai et al., 2013)
UASB	Synthetic AMD	Domestic wastewater	SO_4^{2-}	4.5	24-48	14	(Sánchez-Andrea et al., 2012)
PBR	Growth medium	Lactate	SO_4^{2-}	4.0	10-35.5	18	(Jong and Parry, 2006)
PBR	Acidic lignite mine water	Methanol	SO_4^{2-}	3.0	12	45	(Glombitza, 2001)
ASBR	Synthetic AMD	Ethanol	SO_4^{2-}	4.0	48	7.5	(Costa et al., 2017)

S^0-PBR: S^0-packed bed reactor; SBR: Sequencing batch reactor; CSTR: Completely stirred tank reactor; An-RBC: Anaerobic rotating biological contactor; UASB: Up-flow anaerobic sludge blanket; EGSB: Expanded granular sludge-blank reactor; FBR: fluidized-bed reactor; AnMBR: Anaerobic membrane reactor; UAMB: Up-flow anaerobic multiple-bed reactor; PBR: Packed bed reactor; ASBR: Anaerobic sequencing batch reactor.

the hydrolysis/fermentation of macromolecular organic compounds in domestic wastewater could a rate-limiting step.

When treating AMD without pH amelioration, the S^0 reduction rate can still be up to 36 mg S/L-h under extremely acidic conditions (pH 2.6–3.5) (Sun et al., 2020b), which is comparable to sulfate reduction processes operated under neutral conditions (Table 2). The S^0 reduction was due to the suppression of sulfate reduction and the flourish of S^0RB (i.e., *Desulfurella*) (Sun et al., 2020b). Direct cell-S^0 contact and extracellular electron transfer (EET) were proposed to be responsible for S^0 utilization under acidic conditions. Additionally, an acidic S^0-reducing bioreactor can further reduce chemical needs for alkaline supply, and warrants further investigation to verify its feasibility in pilot- or full-scale applications.

$$SO_4^{2-} + 10H^+ + 8e^- \rightarrow H_2S + 4H_2O \quad (9)$$

$$S^0 + 2H^+ + 2e^- \rightarrow H_2S \quad (10)$$

$$HS^- + \frac{n-1}{8}S_8 \rightarrow S_n^{2-} + H^+ \quad (11)$$

$$HCO_2^- + S_n^{2-} + H_2O \rightarrow HCO_3^- + HS^- + S_{n-1}^{2-} + H^+ \quad (12)$$

3.4.2. Arsenic reduction and precipitation

Arsenic contamination of groundwater has been recognized as a major concern in many developing countries, especially in Bangladesh Hossain, 2006). Arsenite (As(III)) and arsenate (As(V)) are the most common arsenic species in aquatic environment, and the former has much higher mobility and toxicity than the latter (Straif et al., 2009). Biogenic sulfide produced from sulfate reduction can remove As(III) via the formation of insoluble arsenic sulfide precipitates (As_2S_3 and AsS) (Eqs. (13) and ((14)) (de Matos et al., 2018). Sulfide can also chemically reduce As(V) to As(III), finally immobilize As as As_2S_3 and AsS (Gorny et al., 2015). However, in practice, sulfate reduction could not achieve satisfactory and stable removal of arsenic from wastewater owing to the generation of soluble thioarsenite ($As(OH)S_2^{2-}$). It is a result of the elevated pH and excessive HS^- produced during sulfate reduction which promote the production of thioarsenic complexes (Eq. (15)) (Couture and Van Cappellen, 2011). To minimize the formation of thioarsenic complexes, the pH should be less than 6.9 at a high As/S molar ratio (0.67) or lower than 5.5 at a low As/S molar ratio (0.05) (Sun et al., 2019).

S^0 reduction under acidic conditions is a promising alternative as this process produces high amount of sulfide without elevating pH. Sun et al. (2019) found that the effluent from a S^0-packed reactor operated at pH of approximately 4.3 could efficiently remove arsenic (>99%) without thioarsenite formation with a wide As/S molar ratio range (0.05–0.55). However, the produced sulfide is still in surplus even after complete arsenic removal. The sulfide likely results in equipment corrosion especially under acidic conditions, which means that counteractive measures need to be employed to avoid such corrosion issues. Additional process, such as sulfide oxidation, is required to remove or convert the

formed sulfide into benign form such as S^0 or sulfate (Zhang et al., 2018d).

$$2H_3AsO_3 + 3HS^- \rightarrow As_2S_3 + 3H_2O + 3OH^- \quad (13)$$

$$H_3AsO_3 + HS^- + 2H^+ \rightarrow AsS + 3H_2O \quad (14)$$

$$As_2S_3 + HS^- + 3OH^- \rightarrow 2As(OH)S_2^{2-} + H_2O \quad (15)$$

3.4.3. Hg(II) removal and methylmercury elimination

Mercury ions (Hg(II)) present in aquatic environments are highly toxic to humans and other organisms. Although sulfate reduction could remove mercury via forming mercury sulfide precipitate, most SRB are mercury methylators, which means that they can transform mercury ions (Hg(II)) to neurotoxic methylmercury (MeHg) in the presence of organics and sulfate (Fig. 4) (King et al., 2002). S^0 reduction has recently achieved efficient mercury removal without MeHg accumulation (Wang et al., 2018). A 326-day study of S^0 reduction process treating mercury-contaminated wastewater showed that such process efficiently removed Hg(II) with concentration ranging from 0 to 50 mg/L without MeHg detection (Wang et al., 2019a). A significant decrease in the abundance of mercury-methylation functional gene (*HgcA*) was observed over time, but it did not completely disappear. *Geobacter* and *Desulfumicrobium* were the main identified S^0RB, and some members of them are mercury methylators (Podar et al., 2015). However, the mechanisms accounting for the absence of MeHg in the process are still unknown. The study proposed that the absence of MeHg may be possibly ascribed to the binding between dissolved organic matter (DOM) and Hg (II), thereby inhibiting mercury methylation (Fig. 4) (Wang et al., 2018). Therefore, the mechanisms towards the absence of MeHg should be further investigated. The excessive sulfide should also be removed via additional process, as mentioned above.

3.4.4. Chromium reduction and immobilization

Chromium is often detected in various industrial wastewaters (e.g., leather tanning, mine tailing, and electroplating), surface water, and groundwater. Cr(VI) and Cr(III) are the primary forms of chromium in nature. Cr(VI) is soluble in water, teratogenic, and carcinogenic. In contrast, Cr(III) generally exists in the form of insoluble amorphous hydroxide ($Cr(OH)_3$) under neutral conditions. The common practice for detoxifying chromium is to reduce Cr(VI) to Cr(III), followed by immobilization as $Cr(OH)_3$ (Sahinkaya et al., 2012). Sulfide generated from S^0 reduction can chemically reduce Cr(VI) to Cr(III) (Sahinkaya et al., 2012) (Eq. (16)). Autotrophic S^0 oxidation can also be employed to promote the Cr(VI) reduction (Shi et al., 2019). In the process, autotrophic bacteria (e.g., *Thiobacillus, Ferrovibrio*) first synthesize volatile fatty acids (VFAs) via bicarbonate reduction with S^0 oxidation to sulfate. Heterotrophic metal-reducing microbes (e.g., *Geobacter*) then reduce such metals with VFAs as the electron donors and carbon sources. Such process is very promising for oxidative metal removal from contaminated surface water or groundwater without adding organic carbon. The same research group also demonstrated that autotrophic S^0 oxidation can be employed to reduce vanadium (V(V)) to V(IV), further immobilized as $VO(OH)_2$ with similar mechanisms with Cr(VI) bio-reduction (Zhang et al., 2018a). We could expect that the S^0 oxidation could be extended to reduce other oxidative metals, such as selenate and antimonite.

$$2CrO_4^{2-} + 3HS^- + 7H^+ \rightarrow 2Cr(OH)_3 + 3S^0 + 2H_2O \quad (16)$$

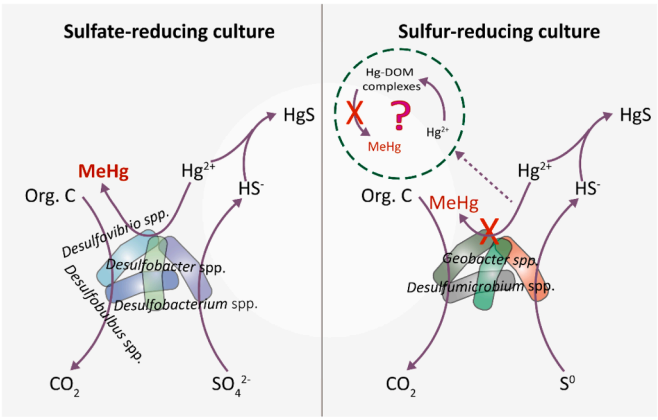

Fig. 4. Hg(II) removal and MeHg production by sulfate-reducing or S^0-reducing culture.

4. Pathways of S^0-respiring bacteria accessing S^0 and strengthened strategies

The extremely low water solubility of S^0 suggests a poor bioaccessibility, thereby impairing the applicability of S^0-based biotechnologies. Understanding how microbes access S^0 is beneficial for process optimization and scale-up. So far, there are four putative possible mechanisms for making S^0 bioavailable to microorganisms (Fig. 5): (1) direct cell-S^0 contact (pathways 1 and 2); (2) polysulfide involvement (pathway 3); and (3) extracellular electron transfer (EET) (pathways 3 and 4). It is of note that all or part of the pathways could occur concomitantly depending on the microbial community and environment.

4.1. Direct cell-S^0 contact

Physical attachment of S^0-respiring bacteria to S^0 particles is an important way for microbial S^0 uptake (Fig. 5). There are two possible pathways for microbial S^0 utilization when microorganisms attach onto the surface of S^0 particles. First, the interaction between sulfur particles and the thiol groups present on special outer-membrane proteins can generate thiol-bound sulfane sulfur atoms, which is then transported into the periplasm (Rohwerder and Sand, 2003). Second, S^0-respiring bacteria could directly uptake the polymeric sulfur. Commercial sulfur products consist of thermodynamically stable octasulfane ring (S_8) and chain-like macromolecules of polymeric sulfur. The binding energy between S–S bonds in S_8 rings is 2.4 kJ/mol stronger than that of polymeric sulfur (sulfur chain) (Franz et al., 2007). Polymeric sulfur may be more bioaccessible than cyclo-octasulfur (S_8) to S^0-respiring bacteria.

S^0 type, concentration and particle size are important factors influencing the direct S^0-cell efficacy. Mineral sulfur, chem-S^0, bio-S^0, and colloidal S^0 are the most common types of elemental sulfur (Table 3). Mineral sulfur usually contains impurities, such as arsenic, selenium, clay, and calcite. Thus, it is not suitable for wastewater treatment in practice. Chem-S^0 (also called sulfur flower) is generally obtained by Claus-process (Fischer, 1978) or by sublimation and condensation of mineral sulfur or industrial sulfur. Bio-S^0 is produced in biological treatment of sulfide-containing wastewaters (Huang et al., 2017; Huang et al., 2021), flue gases (Lin et al., 2018; Blázquez et al., 2019a), or H_2S-rich biogas (Pokorna and Zabranska, 2015). It should be noted that bio-S^0 produced by sulfide-oxidizing bacteria (*Thiobacillus*, Acidithiobacillus etc.) can be stored intracellularly or extracellularly (Kleinjan et al., 2003; Huang et al., 2021). When sulfide supply is sufficient, the intracellularly produced S^0 can be secreted by bacteria to outside the bacterial cells (Li et al., 2020b). Colloidal S^0 is produced via the grinding of S^0 particles with a colloidal mill machine or by the acidification of polysulfide or thiosulfate (Florentino et al., 2015). The high cost of colloidal S^0 production limits its application in water and wastewater treatment. Hereinafter, we only discuss chem-S^0 and bio-S^0 in the context of water and wastewater treatment.

Chem-S^0 is strongly hydrophobic and not easily dispersed in water, limiting mass transfer from the solid to aqueous phase and reducing microbial utilization rate (Sierra-Alvarez et al., 2007). In contrast, bio-S^0

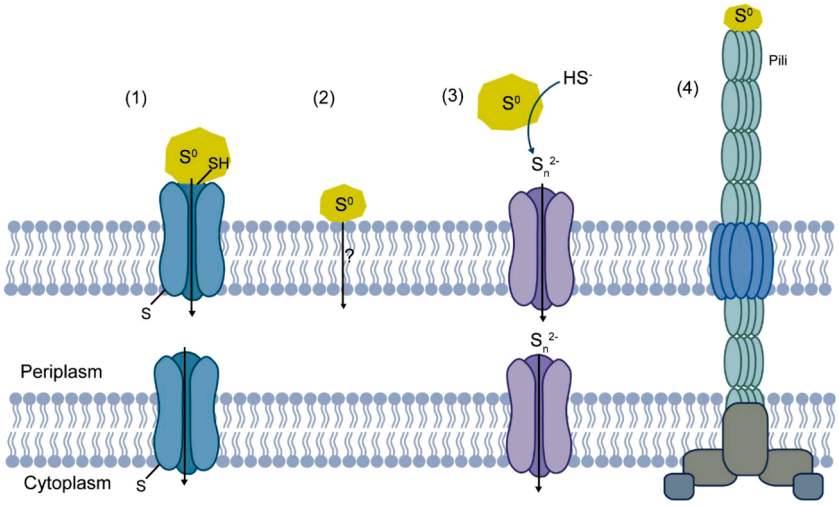

Fig. 5. Envisaged mechanisms towards S^0 uptake by S^0-respiring bacteria. (1) attachment of cell- S^0 and interaction between S^0 and thiol-containing outer-membrane proteins to generate soluble polysulfanes; (2) direct uptake of polymeric sulfur; (3) Nucleophilic attack of S^0 by sulfide to generate polysulfide; (4) pili formation resulting in extracellular electron transport (adapted from Florentino et al. (2019)).

Table 3
Comparisons among the common S^0 types.

S^0 type	Source	Bioavailability	Hydrophilicity*
Sulfur mineral	Nature	Low	4
Chem-S^0	Chemical production	Low	3
Colloidal S^0	Chemical production	High	1
Bio-S^0	Biological production	High	2

* number 1-4 represent the scale of hydrophilicity from strong to weak.

particles primarily consist of orthorhombic S^0 rings surrounded by a layer of long-chain polymers (i.e. polysulfide and polythionates) with hydrophilic properties (Di Capua et al., 2019). Due to the microcrystallinity structure, high specific surface area and solubility, bio-S^0 particles have higher bioavailability than chem-S^0 (Florentino et al., 2015; Kostrytsia et al., 2018). Although bio-S^0 is superior to chem-S^0, a limited number of batch studies demonstrated its feasibility to support denitrification. Only two studies so far reported autotrophic denitrification based on bio-S^0 in MBRs (Ucar et al., 2020; Ucar et al., 2021), in which bio-S^0 did not result in a significantly different microbial community from that with chem-S^0. However, it should be noted that bio-S^0 is not always available at every site. Considering the local availability of sulfur sources, chem-S^0 has been more frequently utilized in real applications.

Increasing S^0 concentration is beneficial for physical attachment of microbes onto S^0 particles. There is a S^0 concentration threshold by which S^0 mass transfer is no longer the rate-limiting step (Sierra-Alvarez et al., 2007). Such threshold is actually influenced by many factors, such as particle size, microbial activity, indicating that it is case-specific. For example, Sierra-Alvarez et al. (2007) found that the denitrification rate had a positive correlation with the concentration of fine S^0 particles (10–130 μm), only ranging from 0–234 mg/L. Additionally, decreasing S^0 particle size can increase the specific surface area, enhance the mass transfer efficiency, and also provide greater area for biofilm growth (Moon et al., 2006). Koenig and Liu (2001) found that the denitrification rate increased from 0.3 kg NO_3^--N/m^3-d to 0.77 kg NO_3^--N/m^3-d when the size of S^0 particles decreased from 11.2–16 mm to 2.8–5.6 mm in a S^0-PBR. The S^0 fine powder would be preferably utilized in activated sludge systems and membrane reactors (Sahinkaya et al., 2015; Zhang et al., 2018b; Qiu et al., 2020). However, in S^0-PBRs, excessively small S^0 particles would result in an extremely low bed porosity, clogging and channeling as well as limited biofilm development, which certainly deteriorate the system performance. When used for denitrification, N_2 entrapment could also occur. In practice, in order to achieve desirable process performance, S^0 particle size should be as smaller as it can on the premise of minimizing the risks of the above stated issues. According to previous studies, S^0 particle sizes at mm-level (0.5 mm–16 mm) have been frequently utilized in laboratory- and pilot-scale S^0-PBRs treating nitrate-contaminated water and wastewater (Di Capua et al., 2015; Wang et al., 2019c). As for S^0 reduction, previous studies showed that the particles sizes of 0.6–50 mm were used in PBRs (Sahinkaya et al., 2012; Zhang et al., 2018d; Sun et al., 2019; Sun et al., 2020b). Thus, in practice, S^0 particle size used for S^0 reduction can be larger than that for denitrification in PBRs, especially under neutral or alkaline conditions where the presence of abundant polysulfide ensures highly efficient system performance.

4.2. *Polysulfide as the electron shuttle*

Under neutral or alkaline conditions, the nucleophilic attack of S^0 by HS^- results in the nucleophilic cleavage of S_8 rings and the formation of small polysulfide molecules (Eq. (11)) (Florentino et al., 2016b). Polysulfide molecules can pass through the cell membrane via channels or other designated polysulfide-binding carrier proteins and react with the cytoplasmic sulfur transferases (Rabus et al., 2013; Florentino et al., 2019). Polysulfide remarkably enhances the bioavailability of S^0, and thus greatly accelerates the process rates for sulfur reduction or oxidation for the purpose of water / wastewater treatment.

Polysulfide concentration is mainly influenced by solution pH and sulfide concentration. At neutral pH, the polysulfide concentration at equilibrium does not exceed 10–50 μM when sulfide concentration increases from 1 to 10 mM. At pH 10, polysulfide is the main form of sulfide in the presence of sufficient S^0 Sorokin et al., 2010). Under neutral/alkaline conditions, polysulfide is a key electron acceptor/donor during S^0 metabolism (Berg et al., 2014, Liang et al., 2016, Zhang et al., 2018c). Zhang et al. (2018c) observed that analogous to a chain reaction (Fig. 6) (Eqs. (11) and ((12)), the rate of S^0 reduction via polysulfide accelerates until the sulfide concentration inhibits sulfidogenic activity. It was observed that with the involvement of polysulfide in sulfur reduction process, the sulfur reduction rate was approximately 40 times higher than that in the absence of polysulfide (Zhang et al., 2018c). When the pH is below 6.0 under which polysulfide formation is almost completely inhibited, the S^0 reduction/oxidation rate is significantly lower than that under neutral or alkaline conditions (Sun et al., 2019). The pH also influences the biological S^0-respiration activity. Therefore, when treating AMD, pH amelioration could not only improve S^0-reducing activity but also enhance S^0 bioaccessibility due to the

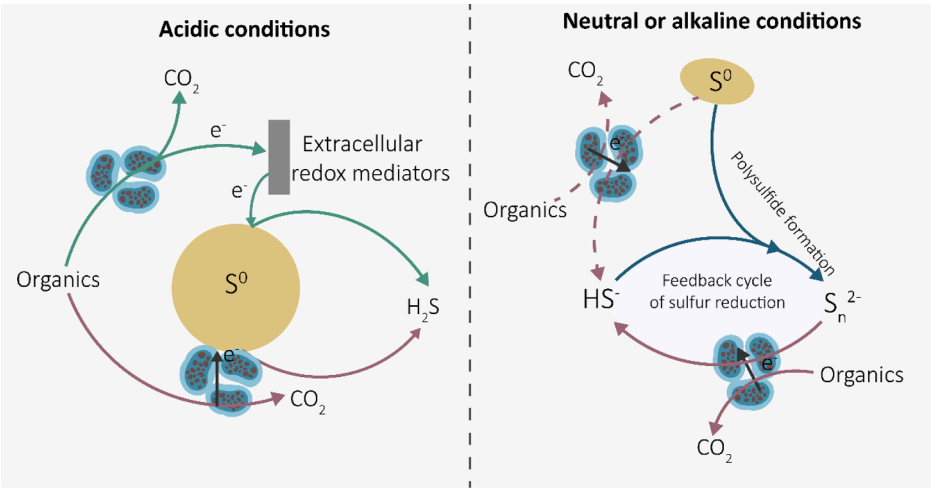

Fig. 6. Possible pathways for biological S^0 reduction under different conditions (adapted from Sun et al. (2019) and Zhang et al. (2018c)). Flavins, phenazines, humic substances, quinones, and cysteine could function as extracellular redox mediators. In the right figure, dash and solid lines represent unfavorable and favorable reactions, respectively. Dark green and red color represent chemical and biological reactions, respectively.

involvement of polysulfide. Under acidic conditions, the S^0 could be mainly utilized by EET and direct cell-S^0 contact (Fig. 6). It should be pointed out that S^0 reduction/oxidation consumes alkalinity. To counteract the decrease in pH, addition of alkaline substance is required. For instance, as stated above, during S^0-driven autotrophic denitrification, bicarbonate, limestone or other solid-phase buffers (calcite, crushed oyster shells etc.) are usually utilized (Cui et al., 2019).

Increasing sulfide concentration would also increase polysulfide concentration under neutral/alkaline conditions. Since sulfide is produced during S^0 reduction process, satisfied system performance can be achieved as long as the pH in the systems can be maintained at around neutral/alkaline conditions. As for S^0 oxidation, adding exogenous sulfide or organics is a feasible strategy for facilitating S^0-oxidizing efficiency. For instance, in addition to being directly used as electron donor, sulfide can result in polysulfide formation, thereby enhancing S^0 bioavailability, and improving nitrogen removal in SADN systems (Qiu et al., 2020). Exogenous sulfide addition can be substituted by organics, which could be due to the sulfide produced in the deep layer of biofilm growing on S^0 particles (Qiu et al., 2020). S^0 disproportionation could be another possible strategy for in-situ sulfide source (Eq. (8)), which merits further study. Although sulfur disproportionation is endergonic under standard conditions, it has been reported to occur in engineered systems and natural environments (Poser et al., 2013; Qiu et al., 2020; Wan et al., 2019a; Wan et al., 2019b; Müller et al., 2020). In addition, although temperature also influences polysulfide concentration under a certain condition, its effect may not be significant during mesophilic wastewater treatment processes. As for the mesophilic bacteria, low temperature would slow down microbial S^0 utilization rate.

4.3. *Extracellular electron transfer (EET)*

EET is the process by which microorganisms utilize insoluble electron donors or acceptors. EET pathway for biological S^0 oxidation has not been reported yet. According to the previously acquired knowledge on electroactive bacteria, such as metal-utilizing bacteria (e.g., *Shewanella, Geobacter*), and SRB (Choi and Sang, 2016), among which some members of SRB, *Shewanella* and *Geobacter* can perform dissimilatory S^0 reduction, EET could likely be applicable for S^0 reduction. Three EET pathways likely involved in S^0 reduction could be proposed (Fig. 5) (Florentino et al., 2016b). First, *c*-type cytochromes extend the respiratory chains to the cell surface to contact with S^0, on which they transport respiratory electrons in the cytoplasmic membrane through the periplasm and the outer membrane to the surface of S^0 particles. The outer membrane cytochromes then catalyze extracellular S^0 reduction at the surface of outer membrane of bacteria. Second, extracellular redox mediators, such as flavins, phenazines, humic substances, and quinones, can function as electron shuttles to transport electrons between the cells and the insoluble S^0. Polysulfide could also be considered an extracellular redox mediator to shuttle electrons between microorganisms and S^0 particles. Third, in the absence of *c*-type cytochromes (Lovley et al., 1995), microbes could form extracellular pili (also called nanowires) that are composed of protein filaments and anchored on the cell envelope (Shi et al., 2016). These nanowires build an electrical bridge connecting cells and solid materials. Therefore, adding extracellular redox mediators or conductive materials (activated carbon, carbon nanotube, iron oxides etc.) to enhance electron transfer could be practically feasible strategies for improving S^0 bio-utilization efficiency, which merits further investigation. Additionally, S^0 type, concentration and particle size also need to be considered when improving EET efficiency.

5. Future perspectives

Although the applications of S^0 during water and wastewater have drawn increasing attention and substantial progresses have been achieved in recent years, many issues related to the basic mechanisms and the applications of S^0-based biotechnologies remain unanswered and should be a goal of future research.

- Increasing S^0 bioavailability is the key step to broaden the applications of S^0-based biotechnologies. The low bioavailability of S^0 due to its extremely low aqueous solubility limits the scale-up and wide applications of S^0-based biotechnologies. Despite some viable approaches have been demonstrated to improve S^0 bio-utilization efficiency, more versatile strategies are desired to make them more competitive and robust. We propose to focus on understanding and regulation of bio(chemical) processes during the interface between S^0 particle and biofilm, exploring S^0 surface modification, introducing extracellular redox mediators or conductive materials into the S^0-based systems, and optimizing the polysulfide formation in bioprocesses.
- S^0 disproportionation is an important process in the globally biochemical sulfur cycle, and S^0-disproportionating bacteria have been detected in freshwater and marine sediments as well as bioaugmented systems. S^0 disproportionation could be potentially employed to simultaneously provide readily bioavailable electron donor and acceptor for water and wastewater treatment. However,

the engineering application of S^0 disproportionation in water or wastewater treatment processes is yet to be reported. Future research could direct towards developing novel S^0 disproportionation-based water and wastewater treatment processes.

- Bio-S^0 is a biological waste product, and has higher bioaccessibility than chem-S^0. More efforts should be dedicated to apply bio-S^0 in these biotechnologies, and to determine whether and how bio-S^0 affects sulfur metabolism. However, bio-S^0 is not universally available, the cost caused by transportation should be taken into account within cost-benefit analysis. To have a more sustainable approach, sulfur source could be recovered and recycled via additional processes. For example, the biogenic sulfide can be re-oxidized to S^0 via micro-aeration or sulfide-driven autotrophic denitrification (Show et al., 2013; Zhang et al., 2018). The by-product sulfate could also be turned into S^0 via bioelectrochemical systems (Blázquez et al., 2016; Blázquez et al., 2019b). The recovery and/or recycle of S^0 could help in closing the loop, thereby boosting the circular economy. Thus, the integrated S^0-based biotechnologies with bioelectrochemical systems can be another future research direction.
- Interspecific interactions are expected to play a more crucial role in maintaining system functions than individual populations, which could contribute to enhance S^0 bioavailability and promote system performance. For example, fermentative bacteria can decompose macromolecular organic substrates to simple ones to support the activity and respiration of S^0RB. In SADN systems, the sulfide produced by sulfidogenic bacteria can improve S^0 bioaccessibility, therefore improving denitrification mediated by nitrate-reducing bacteria. Accordingly, microbial community structure and microbial interactions at the whole community-level and molecular-level should be further investigated via emerging techniques (e.g., metagenomic, transcriptomic and proteomic analysis as well as stable isotope labeling). This information will be beneficial for optimizing process performance via calibrating operation parameters and facilitating the preferable microbial pathways.
- A suite of S^0-based technologies has recently been proposed in laboratory studies, but only S^0-driven autotrophic denitrification and S^0 reduction for metal-laden wastewater treatment have so far been demonstrated in pilot- or full-scale applications. These studies have shown a proof of concept. It is now time to establish more demonstrations to bring these technologies to full-scale applications. Development and validation of mathematical modeling to determine the key process parameters can assist their scale-up, and allow the technologies to be more practically feasible. In addition, treatment of emerging contaminants (pharmaceuticals and personal care products (PPCPs), biocides, nanoparticles and micro/nanoplastics, etc.) has been becoming a major challenge during water and wastewater treatment. In order to meet increasingly strict effluent quality criteria, the fate of emerging contaminants in the S^0-based systems and how these emerging contaminants influence the performance of S^0-based biotechnologies and S^0-related microbial community should not be overlooked.

6. Conclusions

Elemental sulfur, a non-toxic, cheap, insoluble, and easily available chemical, has been used as electron donor and/or acceptor in S^0-based biotechnologies for water and wastewater treatment. This study systematically reviews the principles, microorganisms, mechanisms and applications of S^0-based biotechnologies. S^0 can undergo oxidative and reductive conversion by a wide array of organisms. This has been recently explored in water and wastewater treatment targeting various contaminants. Compared to conventional water and wastewater treatment approaches, S^0-based biotechnologies could substantially reduce the operational cost caused by the addition of exogenous carbon sources (methanol, ethanol, acetate, glucose etc.) as the operational cost with S^0 is much lower in terms of the cost per mol electron donor provided. S^0-based biotechnologies would not result in secondary pollution caused by residual organics. The extremely low aqueous solubility of S^0 is a primary bottleneck of achieving high-level process performance, which could be overcome by shedding more light on the fundamental mechanisms underlying microbes accessibility to S^0 and microbial S^0 metabolism. Self-generated polysulfide has been proved to be an effective electron shuttle to break the bottleneck and greatly accelerate S^0-based biotechnologies. Future studies on microbial and molecular ecology as well as mathematical modeling are deemed necessary for S^0-based biotechnology scale-up. The fate of emerging pollutants and their interactions with microbial communities in S^0-based biosystems should also be investigated. Certainly, the versatility of S^0 conversion will lead to new biotechnological processes for water and wastewater treatment in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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