



Review

Incorporation of the sulfur cycle in sustainable nitrogen removal systems - A review



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ABSTRACT

In wastewater treatment systems, sulfur (S) removal processes are generally based on heterotrophic sulfate (SO_4^{2-}) reduction by sulfate reducing bacteria and S-dependent autotrophic denitrification by sulfur oxidizing bacteria. A combination of either two cycles (N and S) or three cycles (N, S and C) appears to be a viable approach to sustainable wastewater treatment, resulting in energy savings and reduction of sludge production. This review shows how the S cycle can be coupled with the other cycles in single systems for efficient N and S removal. Operating conditions, advantages, limitations and challenges of such systems are described. S removal processes are generally based on heterotrophic sulfate (SO_4^{2-}) reduction by sulfate reducing bacteria and S-dependent autotrophic denitrification by sulfur oxidizing bacteria. In terms of pH and temperature, the optimum conditions are determined by the narrowest ranges for heterotrophic SO_4^{2-} reduction (pH of 7–7.6, $T = 28\text{--}30\text{ }^\circ\text{C}$). The combined processes allow for almost complete N removal, while the efficiency of SO_4^{2-} removal can reach up to 75%. Among all the processes linking the N, S and C cycles, SANI (sulfate reduction, autotrophic denitrification and nitrification integrated) has been best recognized. Recently, the growing attention has been paid to the novel sulfammonox process, which involves SO_4^{2-} dependent, anaerobic ammonia oxidizing bacteria. Numerous systems have been developed to combine SO_4^{2-} reduction, S-dependent autotrophic denitrification and partial nitrification/anammox processes. The coexistence of several bacterial groups and their competition for the substrates is thus a key issue to be considered. Specific inhibitors for each bacterial group also need to be recognized before full-scale implementations. Moreover, modeling the transformations of S compounds has been incorporated with respect to all the processes responsible for those transformations.

1. Introduction

High concentrations of ammonium ($\text{NH}_4\text{-N}$) lead to eutrophication of surface waters and pose a threat to the aquatic life and human health (Qin et al., 2021a,b). $\text{NH}_4\text{-N}$ can effectively be converted to nitrogen gas by combined nitrification-denitrification, but this method has a few important disadvantages, including a high demand of energy and carbon, and high sludge production. On the other hand, sulfate (SO_4^{2-}) is a type of the secondary pollutant because reduction of sulfide (S^{2-}) under anaerobic conditions is harmful for the aquatic environment (Hao et al., 2014). S compounds have not been widely used as substrates in wastewater treatment processes. Simultaneous removal of these two compounds (N and S) from wastewater, with or without involving the carbon

(C) cycle, can be a viable approach to the sustainable wastewater management. In particular, this approach may be an effective alternative in the case of many types of industrial wastewater, which are characterized by high concentrations of pollutants, such as $\text{NH}_4\text{-N}$, SO_4^{2-} (>1000 mg/L of both N and S) and chemical oxygen demand (COD) (>60,000 mg COD/L) (Rikmann et al., 2016; Jarvis and Younger, 2000; Chapman, 1992).

A viable sustainable approach to biological wastewater treatment comprises a combination of nitrogen (N), sulfur (S) and carbon (C) removal. Lower operating costs result from the use of some products in one process as the substrates in other processes and the use of shared reactors. Moreover, no carbon is needed for S-dependent autotrophic denitrification, less sludge is generated, and the environmentally neutral

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compounds, such as nitrogen gas (N_2) and elemental sulfur (S^0), are the final products of biochemical reactions (Lin et al., 2018).

Conventional nitrification/denitrification for N removal is now being replaced by more sustainable N-shortcut processes, such as “nitrite shunt” or deammonification. In the case of S compounds, biological removal is based on heterotrophic SO_4^{2-} reduction by sulfate reducing bacteria (SRB) and S-dependent autotrophic denitrification by sulfur oxidizing bacteria (SOB). Recently, the growing attention has been paid to the novel sulfate reducing ammonia oxidizing (sulfammox) process, which involves anaerobic ammonium oxidizing bacteria (AAOB). These bacteria use SO_4^{2-} , instead of nitrite nitrogen (NO_2^- -N), as an electron acceptor to oxidize NH_4^+ -N under anaerobic conditions.

The growing importance of using the combined N, S and C cycles in biological wastewater treatment processes has been confirmed by the increasing number of review papers on various aspects of S transformations. According to Web of Science database, 15, 12 and 3 review papers have been published specifically on S-dependent autotrophic denitrification, heterotrophic reduction of SO_4^{2-} and sulfammox (–see Fig. S1 in the Supporting Information (SI)). Several papers focused on particular issues, including a detailed description of mechanisms of the individual processes, responsible microorganisms, reactors used, optimal operational conditions or inhibiting factors in S-dependent autotrophic denitrification (Wu et al., 2021; Cui et al., 2019; Lin et al., 2018), heterotrophic sulfate reduction (Sinharoy et al., 2020b) and sulfammox (Liu et al., 2021; Grubba et al., 2021).

However, only a combination of either two cycles – (N and S) or three cycles (N, S and C) would be the rational approach to wastewater treatment in order to save energy and the amount of sludge generated, especially for NH_4^+ -N and SO_4^{2-} rich industrial wastewater. Due to the variety of N, S and C removal processes, the research interests have been shifting to the use of single- and multi-stage systems based on the combination of several processes, such as heterotrophic sulfate reduction, S-dependent autotrophic denitrification, nitrification, denitrification, anaerobic ammonia oxidation (anammox) and sulfammox (Wu et al., 2020, Yuan et al., 2020, Sun et al., 2018, Liu et al., 2017, Qian et al., 2015a, b, c, Jiang et al., 2013, Wang et al., 2009b).

Only two review papers (Hao et al., 2014; Show et al., 2013) described simultaneously S-dependent autotrophic denitrification and heterotrophic sulfate reduction. Hao et al. (2014) described a relationship between the N, S, C and P cycles in biological wastewater treatment systems. These authors focused on the acceptors and electrons used in the transformations of S compounds, key microorganisms, developed technologies, factors influencing the process performance, and achieved SO_4^{2-} reduction efficiencies. In the review of Show et al. (2013), existing models of the transformations of S compounds were additionally described (see – Table S1 in SI).

The present review provides updated results of research on S transformations, which have been revised and extended with new understanding and discoveries. A novel aspect is the inclusion of sulfammox in these transformations as no paper has synthesized autotrophic S-dependent denitrification, heterotrophic sulfate reduction and the sulfammox process in one review. In addition, the present study describes how sulfammox can increase the efficiency of N and S removal. Various process configurations and technologies, which are based on the three (N–S–C) cycles, are described and compared in terms of their efficiency. Moreover, modeling the transformations of N, S and C compounds has been incorporated with respect to all processes responsible for those transformations. Such a review provides a deeper insight into the conversions of S in biochemical processes, including sulfammox.

2. Single S-dependent biochemical processes integrating N, S and C conversions

There are three known processes combining sulfur and nitrogen conversions: S-dependent autotrophic denitrification, heterotrophic sulfate reduction and autotrophic sulfammox. The detailed description

of those processes, including the metabolic mechanisms, biochemical reactions, influencing environmental factors can be found in the SI (S1–S3).

S-dependent autotrophic denitrification consists of oxidation of S compounds, including S^{2-} , S^0 , thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}), coupled with reduction of NO_3^- -N and/or NO_2^- -N. *T. denitrificans*, *Thiomicrospira denitrificans*, *Thiobacillus versutus*, *Thiosphaera pantotropha* and *P. denitrificans* are the known microorganisms responsible for that process. *P. denitrificans* is the chemotrophic α -proteobacteria which can grow on organic monocarbon compounds (methanol, methylamine) while using reduced forms of S and hydrogen as electron donors in denitrification (Baker et al., 1998). *T. denitrificans* belongs to β -proteobacteria that can use $S_2O_3^{2-}$ and thiocyanates under aerobic conditions, and additionally S^{2-} and S^0 under anaerobic conditions. *Sulfurimonas denitrificans* belongs to the ϵ -proteobacteria and is capable of oxidizing SO_3^{2-} , $S_2O_3^{2-}$ and S^0 , while both NO_3^- -N and oxygen are used as electron acceptors. *T. thioparus* is one of the representatives of autotrophic denitrifiers that reduce NO_3^- -N to NO_2^- -N by oxidation of S^{2-} (Tang et al., 2009). Although autotrophic denitrifying bacteria are chemolithotrophic, there are many denitrifying bacteria capable of adapting to autotrophic, heterotrophic and even mixotrophic growth (*P. versutus*, *P. denitrificans*, *Beggiatoa* sp.) (Pokorna and Zabranska, 2015).

Heterotrophic sulfate reduction is SO_4^{2-} reduction which takes place in two independent different paths. The first is the use of organic electron donors, which are also the carbon source for the SRB. The second is the use of inorganic electron donors, which must be supplemented with a carbon source, such as CO_2 (Sinharoy et al., 2020a). The SRB can be divided into 7 phylogenetic lines, including five for bacteria and two for archaea. Most of the SRB found in sulfate reduction reactors belong to 23 genera within *Deltaproteobacteria* (*Desulfovibrio*, *Desulfobacteraceae*, *Desulfobulbaceae*, *Syntrophobacteraceae*, *Desulfomicrobium*, *Desulfolobium*). Another SRB belong to the gram-positive genera *Clostridia* (*Desulfotomaculum*, *Desulfosporosinus* and *Desulfosporomusa*). Three lineages, *Nitrospirae* (*Thermodesulfovibrio*), *Thermodesulfobacteria* (*Thermodesulfobacterium*) and *Thermodesulfobiaceae* (*Thermodesulfobium*), contain only thermophilic SO_4^{2-} reducing agents. Archaeal SRB are *Euryarchaeota* and *Crenarchaeota* (Muyzer and Stams, 2008).

In a novel sulfammox process, NH_4^+ -N is oxidized to N_2 , whereas SO_4^{2-} plays the role of an electron acceptor which is reduced to S^0 under anaerobic conditions. *Brocadia Anammoxoglobus Sulfate* (Liu et al., 2008) is a functional microorganism responsible for simultaneous removal of NH_4^+ -N and SO_4^{2-} and ended the conversion of NH_4^+ -N and SO_4^{2-} by producing NO_2^- -N as an intermediate. The second isolated species, *Bacillus Benzoevorans*, is responsible for carrying out the entire sulfammox reaction (Cai et al., 2010). *Verrucomicrobia* has also been reported to be involved in the sulfammox process (Rikmann et al., 2016). Some *Proteobacteria*, which may potentially perform sulfammox, include the following species: *Sulfurimonas*, *Desulfuromonadales*, *Desulfovibrio*, *Desulfuromonas*, *Desulfobulbus*, *norank Rhodobacteraceae* and *Thiobacillus* (Rios-Del Toro et al., 2018; Wang et al., 2017).

The key issues and challenges of S-dependent autotrophic denitrification, heterotrophic sulfate reduction and sulfammox are presented in Table 1. Fig. 1 below shows the interactions between S-dependent autotrophic denitrification, heterotrophic sulfate reduction and sulfammox process.

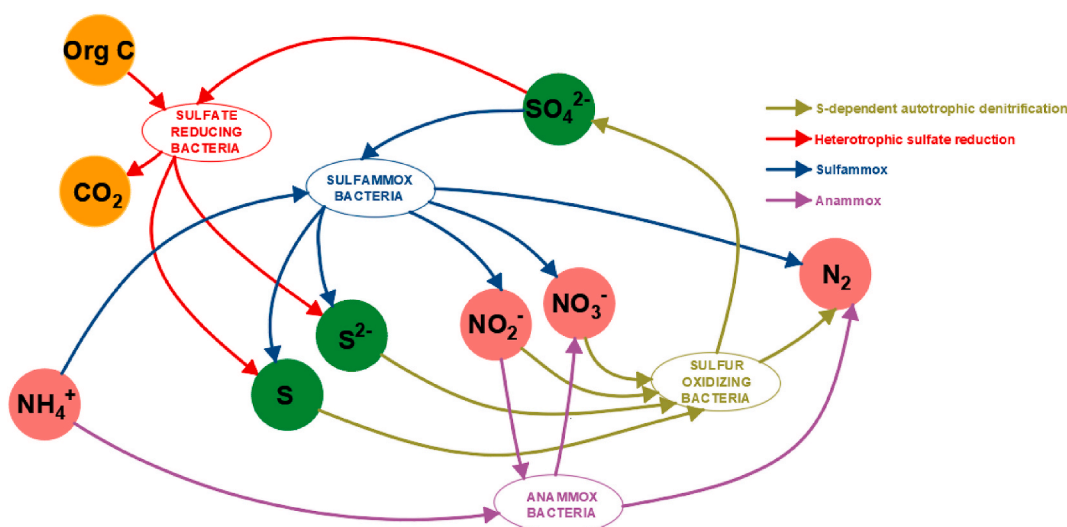
3. Operational conditions and performances of single S-dependent processes

Each of the discussed processes (S-dependent autotrophic denitrification, heterotrophic sulfate reduction and sulfammox) can be carried out independently, as evidenced by numerous studies (Tables 2 and 3). However, the challenge is to combine these processes, in either single- or multi-stage systems, in order to make biological wastewater treatment systems more efficient.

Table 1

Key issues and challenges of S-dependent autotrophic denitrification, heterotrophic sulfate reduction and sulfamox.

Topic	Process		
	S-dependent autotrophic denitrification	Heterotrophic sulfate reduction	Sulfamox
Key issues	consists of oxidation of S compounds, including S^{2-} , S^0 , thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}), coupled with reduction of NO_3^- -N and/or NO_2^- -N	SO_4^{2-} reduction, which involves the use of organic electron donors or inorganic electron donors, which must be supplemented with a carbon source	NH_4^+ -N is oxidized to N_2 , whereas SO_4^{2-} plays the role of an electron acceptor and is reduced to S^0 under anaerobic conditions
Challenges and opportunities	a) a good alternative to heterotrophic denitrification due to the lack of carbon dosing; b) reduction of toxic S_2 ; c) the possibility of treating wastewater poor in organic content; d) residual SO_4^{2-} in wastewater; e) a long incubation time is needed before a fully adapted culture is obtained; f) precise control strategy (from S_2 - to S^0) and novel S^0 recovery technology at the source; g) acclimation and adjustment of microorganisms: the concentration of S_2 - should be controlled; maintaining the denitrification efficiency of autotrophic denitrification systems at low temperatures; alkalinity and pH control is necessary to prevent the formation of NO_2^- -N; influence of the N/S ratio on the reactions and bioproducts, the optimal N/S ratio = 0.5–0.9 for S oxidation and NO_3^- -N reduction (see Eqs. 1–8 in the SI); h) when the dissolved oxygen (DO) concentration is > 1.6 mg O_2 /L, denitrification is completely inhibited	a) SO_4^{2-} reduction, especially in SO_4^{2-} rich industrial wastewater; b) use of wastewater rich in organic compounds; c) high concentrations of SO_4^{2-} inhibit SRB activity; d) elevated levels of heavy metals may reduce or terminate SRB activity	a) anaerobic oxidation of NH_4^+ -N without carbon addition; b) SO_4^{2-} reduction in wastewater; c) knowledge of microorganisms, mechanisms and their metabolic pathway is still limited; d) temperature, DO and pH would influence its practical applications; e) inhibition of sulfamox activity due to S^{2-} accumulation; f) with a high concentration of NO_3^- -N, SO_4^{2-} concentration may increase due to autotrophic denitrification

**Fig. 1.** Interactions between S-dependent autotrophic denitrification, heterotrophic sulfate reduction, anammox and sulfamox process.

3.1. S-dependent autotrophic denitrification

In S-dependent autotrophic denitrification, the most frequently used electron donors are S^0 and S^{2-} (Table 2). The experiments were mainly carried out in packed bed reactors, but several other types of reactors were also used. The reported rates of denitrification varied in a wide range - from 0.03 to 8.13 kg N/m³/d, depending mainly on the temperature and influent NO_3^- -N concentrations. The effects of pH in the investigated range (6.0–9.0) and S concentrations were less significant. For a detailed description of previous research related to S-dependent autotrophic denitrification, see the SI (S1). This process allowed for the efficient (>90%) removal of N and S^{2-} (Yang et al., 2016; Jing et al., 2010) with the NO_3^- -N concentration in the range of 20–1230 mg N/L (Zhu et al., 2019; Zou et al., 2016; Kim et al., 2004).

During S-dependent autotrophic denitrification, SO_4^{2-} can be

produced from different electron donors. Frequently, the S balance in the process is not 1/1 for the removed electron donor to SO_4^{2-} produced (Zou et al., 2016). In Table 2, the initial donor concentrations and the amount of SO_4^{2-} produced are similar. The observed imbalances result from the production of other S intermediates. The most common electron acceptor is NO_3^- -N, but several studies comparing NO_3^- -N and NO_2^- -N have been reported (Sun and Nemat, 2012; Moraes et al., 2012; Jing et al., 2010).

Different aspects of S-dependent autotrophic denitrification have been addressed in several reviews (Wu et al., 2021; Cui et al., 2019; Lin et al., 2018; Sabba et al., 2016). Wu et al. (2021) summarized all types of biofilm denitrification in terms of the reactor configuration, microbial transformations, factors influencing the process, and especially focused on N_2O emissions. The coexistence of S-dependent denitrification with anammox was also reported and S-driven denitrifiers were identified,

Table 2

Process conditions and observed NO_3^- utilization rates during S-dependent autotrophic denitrification in different types of reactors.

Reactor type	Electron donor	Temperature	pH	S-compound	Initial NO_3^- concentration	SO_4^{2-} production	Denitrification rate	References
		(°C)	(-)	(mg S/L)	(mg N/L)	(mg S/L)	(kg N/m ³ /d)	
Fluidized-bed reactor	$\text{S}_2\text{O}_3^{2-}$	20–30	7	184–2260	100–1230	150–320	1.24–3.25	Zou et al. (2016)
Fluidized-bed reactor	S^0	28–30	7.2–9	na	25–75	100–600	0.07–0.2	Sahinkaya and Dursun (2015)
Fluidized-bed reactor	$\text{S}^0/\text{S}_2\text{O}_3^{2-}$	20	6.8–8.2	na	20–700	na	2.53–3.37	Kim et al. (2004)
Packed-bed reactor	S^0	28–30	6–8	na	50–75	200–600	0.07–0.1	Sahinkaya and Kilic (2014a)
Packed-bed reactor	S^0	10–26	6–8	na	30–60	191–483	0.03–0.24	Sahinkaya et al. (2014b)
Packed-bed reactor	S^0	15.2–29	6.7–8.4	592.42–5924.17	20–25	640	0.2	Kimura et al., 2002
Packed-bed reactor	S^0	20–25	8.3–8.7	na	60–251	na	0.27–0.87	Koenig and Liu (2002)
Packed-bed reactor	S^0	20–25	na	na	60–400	na	0.48–0.77	Koenig and Liu (2001)
Up-flow continuous reactor	S^{2-}	29–31	7	160–1000	30.4–169.6	na	0.15–0.61	Jing et al. (2010)
Up-flow anaerobic sludge blanket	S^{2-}	30	7.5	0.62 ^a	0.33 ^a	na	0.09–0.31	Yang et al. (2016)
Up-flow column reactor	S^0	30	7.3	na	20	6.15–7.92 ^b	0.22	Zhu et al. (2019)
Vertical fixed-bed reactors	S^{2-}	30	7–7.5	49.3	20	20	na	Moraes et al. (2012)

^a kg/m³/d.^b g/g $\text{N}_{\text{removal}}$.

including *Thiobacillus denitrificans* and *Thiobacillus thioparus*.

Cui et al. (2019) described S-dependent autotrophic denitrification in terms of the functional enzymes, electron donors, types of reactors, and operational factors. They also emphasized a significant advantage regarding S-dependent autotrophic denitrification compared to heterotrophic denitrification with respect to N_2O emissions. It was shown that autotrophic denitrification mediated by S compounds (S^0 , S^{2-}) emitted significantly less N_2O than heterotrophic denitrification with methanol, ethanol or acetate.

Sabba et al. (2016) focused mainly on SO_3^{2-} and its occurrence in the environment, chemistry, microbiology, and the role in denitrification. It was emphasized that SO_3^{2-} is an intermediate in the S oxidation pathway and should be chosen as the most economical electron donor. Lin et al. (2018) focused primarily on S oxidation, including biological gas desulfurization, phototrophic S^{2-} oxidation, S-dependent autotrophic denitrification, biological sulfur oxidation associated phosphorous removal, dye treatment. They also indicated viable applications of the products, such as Li batteries, production of S concrete by mixing S^0 with aggregates, biologically produced S fertilizer, oxidation of S^{2-} in microbiological fuel cells, and reclamation of metals from sewage sludge.

3.2. Heterotrophic sulfate reduction

Table 3 presents the diversity of research carried out so far on heterotrophic SO_4^{2-} reduction in terms of the electron donor, type of reactor and operating conditions. Most studies have been carried out in the gas lift reactor and fluidized-bed reactor. Both organic and inorganic donors were used, including carbon monoxide, methane, methanol, ethanol, hydrogen, crab shell, compost and many others. The use of different donors resulted in a different SO_4^{2-} reduction efficiency. A detailed description of the research can be found in SI (S2). The use of different electron donors and SO_4^{2-} content resulted in a wide range of SO_4^{2-} removal efficiencies (51–98%) and rates (0–3400 mg SO_4^{2-} /L/d). Nielsen et al. (2019) used methanol and ethylene glycol which resulted in reduction of SO_4^{2-} by 71.2% and 36.9%, respectively. The decrease of SO_4^{2-} concentration was limited to 13.8 and 5.3%, respectively, with the use of peat and straw. Low temperatures (below 10 °C) significantly affected the SO_4^{2-} removal rates. For example, Virpiranta et al. (2019)

carried out studies at various temperatures (22 °C, 16 °C, 6 °C) and found gradually decreasing SO_4^{2-} removal rates, i.e. 169, 98 and 13–42 mg SO_4^{2-} /L/d, respectively.

Sulfate reduction is less popular compared to S-dependent autotrophic denitrification, but that process has also been addressed in several reviews (Kumar et al., 2021; Costa et al., 2020; Sinharoy et al., 2020b; Serrano et al., 2019; Van den Brand et al., 2015). Kumar et al. (2021) and Costa et al. (2020) focused on the use of SO_4^{2-} reduction for treatment of metal-rich wastewater and recovery of these metals, showing a high degree of SO_4^{2-} reduction (>90%) along with the efficient (>99%) recovery of metals (Fe, Zn, Cd, Cu).

Similarly, Sinharoy et al. (2020b) described treatment of acid mine drainage (AMD) with biological reduction of SO_4^{2-} . Heavy metals present in AMD can be removed by S^{2-} precipitation. The review discussed various gaseous substrates, such as H_2 , CO, CH_4 , as electron donors that could be used in this process. It was emphasized that only the microorganisms capable of using gaseous substrates are appropriate for the AMD treatment systems.

Serrano et al. (2019) focused on the optimum conditions for SRB. They presented the recommended conditions for biomass, electron donor and acceptor and an experimental setup of three SRB tests: (1) to assess the activity of SRB culture, (2) to determine the reduction potential of an electron donor, and (3) to determine the possibility of using various sources of SO_4^{2-} as an electron acceptor. They collected methodologies and results from many publications and recommended setup and monitoring conditions to increase the comparability and reproducibility of the SRB tests. Sodium sulfate and lactate were used as an electron acceptor and electron donor, respectively.

Van den Brand et al. (2015) analyzed important parameters, such as pH, organic substrates, COD/ SO_4^{2-} ratio, substrate composition, SO_4^{2-} , salt, temperature and DO. They found that the presence of SRB reduced pathogens, heavy metals and sludge produced. Sulfate reduction, autotrophic denitrification and nitrification integrated (SANI) was identified as a process combining the advantages of SRB and S-dependent autotrophic denitrification. However, they indicated that in order to ensure the benefits of using SRB, a sufficient SO_4^{2-} concentration in the influent wastewater would be required to maintain the COD/ SO_4^{2-} ratio below 0.67.

Table 3

Reactor types, operational (environmental) conditions, influent S concentrations and efficiency of heterotrophic SO_4^{2-} reduction and sulfamox.

Reactor type	Electron donor	Temperature	pH	SO ₄ ²⁻ concentration	SO ₄ ²⁻ removal efficiency or rate	References	
		(°C)		(mg/L)			
HETEROTROPHIC SULFATE REDUCTION							
Gas lift reactor	Carbon monoxide	30	7	250–1000	62.5–97.5%	Sinharoy et al.. (2020a)	
Moving bed biofilm reactor	Carbon monoxide	30	7	250–1000	67.1–95.2%	Sinharoy et al.. (2019)	
Batch	Succinic acid, yeast extract	22	–	1700	169 mg SO ₄ ²⁻ /L/d	Virpiranta et al.. (2019)	
		16			98 mg SO ₄ ²⁻ /L/d		
		6			13–42 mg SO ₄ ²⁻ /L/d		
Batch	Methanol	5	7	–	26.7 mg SO ₄ ²⁻ /L/d	Nielsen et al. (2019)	
Packed bed reactor	Ethylene glycol	30	7	250–1000	4.1 mg SO ₄ ²⁻ /L/d	Kumar et al.. (2018)	
Inverse fluidized bed reactor	Scourer	30	7	700	34 mg SO ₄ ²⁻ /gVSS/d	Reyes-Alvarado et al. (2018)	
		Cork			6.1 mg SO ₄ ²⁻ /gVSS/d		
Packed bed reactor	Molasses	4–8	6.5–7.1	287–548.2	0–22 mg SO ₄ ²⁻ /L/d	Nielsen et al. (2018)	
Batch	Crab shell	30	7	721–738	6–9 mg SO ₄ ²⁻ /gVSS/d	Reyes-Alvarado et al. (2017)	
		Potato			764–766	22–34 mg SO ₄ ²⁻ /gVSS/d	
		Filter paper			752–823	50–65 mg SO ₄ ²⁻ /gVSS/d	
Fluidized-bed reactor	Glycerol	23	5.5–8.5	2000–3000	167 mg SO ₄ ²⁻ /gVSS/d	Bertolino et al.. (2014)	
Stirred tank reactor	Hydrogen + carbon dioxide	30	6.95–7.05	–	3400 mg SO ₄ ²⁻ /L/d	Sáez-Navarrete et al., 2012	
		Fluidized-bed reactor	Ethanol	35	7.5	–	211 mg SO ₄ ²⁻ /gVSS/d
	Ethanol + lactate				2016 mg SO ₄ ²⁻ /gVSS/d		
Gas lift reactor	Hydrogen	30–35	7–7.5	5000–30000	7080 kg SO ₄ ²⁻ /d	Van Houten et al.. (2009)	
Anaerobic filter	Ethanol, spent manure	6	2.5–4.3	900	961–1345 mg SO ₄ ²⁻ /L/d	Tsakamoto et al. (2004)	
					1057–1441 mg SO ₄ ²⁻ /L/d		
SULFAMMOX							
Upflow anaerobic sludge bed reactor	Ammonium nitrogen	35	7.9–8.3	80	8.18 mg S/L/d	Qin et al. (2021a)	
Circulating flow completely anaerobic reactor	Ammonium nitrogen	30	8.1–8.6	88	2–27%	Zhang et al. (2020)	
				223	2–27%		
				154	18–64%		
Self-designed circulating flow reactor	Ammonium nitrogen	35	8.1–8.3	183	approx. 40%	Zhang et al. (2019a)	
				216	approx. 0%		
				116	approx. 30%		
				100	approx. 45%		
Self-designed circulating flow reactor	Ammonium nitrogen	30	8.1–8.6	90	approx. 30%	Zhang et al. (2019b)	
				170	approx. 30%		
				360	approx. 5%		
Sequencing batch reactor	Ammonium nitrogen	–	–	261	19%	Prachakittikul et al. (2016)	
Batch	Ammonium nitrogen	30	8.5	163	40%	Cai et al. (2010)	
Upflow anaerobic sludge blanket reactor	Ammonium nitrogen	35	7.5–8.5	240	30%	Yang et al. (2009)	
Non-woven rotating biological contactor	Ammonium nitrogen	35	8–8.2	–	–	Liu et al. (2008)	

3.3. Sulfamox

Sulfamox is a new process that has been addressed in the literature, especially review papers, only very recently. Sulfamox has mainly been carried out in an upflow anaerobic sludge bed reactor and circulating flow reactor (Table 3). The obtained SO_4^{2-} removal efficiencies are normally much lower compared to heterotrophic sulfate reduction. However, sulfamox is an important process linking the N and S cycles, therefore the effect of sulfamox on the overall reduction of SO_4^{2-} and NH_4^+ -N should not be neglected. In the studied systems, the typical influent concentrations of SO_4^{2-} ranged from 80 to 360 mg/L (Qin et al., 2021a,b; Zhang et al., 2019b) and the highest obtained SO_4^{2-} removal efficiency was 45% (Zhang et al., 2019a). A detailed description of the research can be found in the SI (S3).

Liu et al. (2021) summarized the current understanding of sulfamox, including the mechanisms, responsible microorganisms and factors influencing the process. It was emphasized that the understanding of sulfamox has improved significantly in recent years, but more attention should be paid to recognizing the microbial community and its metabolic pathways. In addition, a variety of sulfamox end products were described that could be substrates for various N and S (anammox, S-dependent autotrophic denitrification) processes and coexist together in wastewater treatment systems. However, a challenge for the process is to ensure optimal environmental factors, such as temperature, pH, DO,

for its practical applications. It was also emphasized that residual organic carbon could have a significant positive effect on sulfamox, but this requires further research. A significant limitation of sulfamox is that the process was mostly investigated under laboratory scale. Practical applications should focus on implementations at low temperatures in full-size reactors.

In order to increase the efficiency of S removal in the sulfamox process, it is important to maintain the optimal pH of 8.5 and temperature of 30 °C (Cai et al., 2010). The N/S ratio is also an important factor affecting that efficiency. When increasing the influent NH_4^+ -N concentration from 166 to 666 mg N/L to 1000–2000 mg N/L, then the SO_4^{2-} removal efficiency increased from 64% to 71%. However, after increasing the influent NH_4^+ -N concentration further to >3000 mg/L, the SO_4^{2-} reduction efficiency decreased to 28% (Wang et al., 2017). Also, reducing the concentration of SO_4^{2-} from 223 to 154 mg/L had a positive effect on the removal of SO_4^{2-} in the sulfamox process (Zhang et al., 2020). The N/S ratio also influenced the SO_4^{2-} removal efficiency, as the SO_4^{2-} removal efficiency at N/S = 2:1 and 4:1 was 38.8% and 30.5%, respectively (Zhang et al., 2019a).

3.4. Optimal conditions for S-dependent autotrophic denitrification, heterotrophic sulfate reduction and the sulfamox process

Fig. 2 shows a summary of the reported pH and temperature ranges

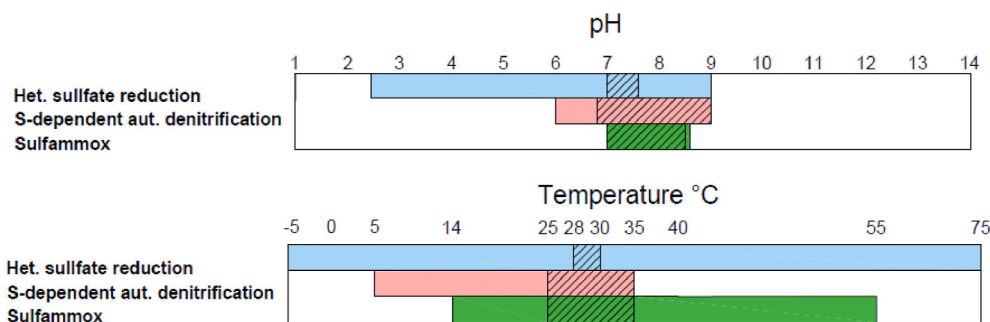


Fig. 2. Ranges of pH and temperatures and their optimal values (“[]” – optimum conditions) reported in literature for the S-dependent processes.

and their optimal values for the three S-dependent processes. The overall optimum conditions are explicitly determined by the narrowest ranges for heterotrophic sulfate reduction, which are 7–7.6 and 28–30 °C for pH and temperature, respectively. The processes of S-dependent autotrophic denitrification, heterotrophic sulfate reduction and sulfamox can occur simultaneously with deammonification or its component processes, i.e. partial nitrification and anammox.

For comparison, for partial nitrification, the optimal ranges were 25–35 °C for temperature (Zhu et al., 2008; Kanders et al., 2014) and 7–8.6 for pH, with the optimal value of 8 (Jaroszynski et al., 2011). On the contrary, too low temperatures (10–15 °C) cause the excessive activity of NOB (Kouba et al., 2017), which can grow faster than AOB under such conditions (Hellenga et al., 1998). The optimal pH range for NOB is 6–7.5, with the maximum at 7 (Yin et al., 2016). For the anammox process, the optimal temperature and pH is respectively 35–40 °C (Dosta et al., 2008) and 6.7–8.3 (Jetten et al., 2001). The recommended ranges for efficient deammonification are as follows: T = 20–35 °C (Kanders et al., 2014) and pH of 7.5–8 (Oshiki et al., 2011).

When coupling sulfamox with S-dependent autotrophic denitrification and heterotrophic SO_4^{2-} reduction to increase the efficiency of S removal, it is important to keep the optimal temperature of 28–30 °C and pH of 7–7.6. The N/S ratio should be adjusted based on the stoichiometry of all the processes involved, so that products of one process can be the substrates for another process. Deviations from the optimal ratio can cause either production of unwanted residues or bacterial competition for the substrates. SRB can compete with sulfamox bacteria for SO_4^{2-} . Moreover, heterotrophic SO_4^{2-} reduction and sulfamox contribute to formation of S^{2-} and/or S^0 , which is the substrate for S-dependent autotrophic denitrification. Too intensive production of S^{2-} may lead to the persistence of this toxic compound in the effluent. The presence of carbon in heterotrophic SO_4^{2-} reduction may also contribute to the development of heterotrophic bacteria responsible for heterotrophic denitrification. Then NO_3^- -N and/or NO_2^- -N may become limited due to their use in both autotrophic and heterotrophic denitrification. In such a case, it is recommended to use full or partial nitrification to produce NO_3^- -N and/or NO_2^- -N. The competition and interactions of microorganisms participating in the aforementioned processes are shown in Fig. 1.

4. Wastewater treatment systems integrating the N–S–C cycles

4.1. Systems integrating the sulfur cycle with nitrification-denitrification - sulfate reduction, autotrophic denitrification and nitrification integrated (SANI) and its modifications

Biological SO_4^{2-} reduction along with biological oxidation of S in the form of SO_3^{2-} , S^0 or $\text{S}_2\text{O}_3^{2-}$ are two main pathways responsible for S conversions in wastewater treatment systems (Cardoso et al., 2006). An integrated process for SO_4^{2-} reduction, autotrophic denitrification and nitrification (SANI) was aimed to primarily remove organic compounds and N (Wang et al., 2009b). This process was originally developed for

saline wastewater in Hong Kong and demonstrated there in full-scale (Wu et al., 2016; Wang et al., 2009b).

With that innovative approach, the conventional wastewater treatment, incorporating C and N cycles, can be extended with the S cycle, as shown in Fig. 3. In the first anaerobic zone, COD is removed by SRB, which results in SO_4^{2-} reduction to S^{2-} . In the second anoxic zone, autotrophic reduction of NO_3^- -N occurs with dissolved S^{2-} formed in the first zone. In the third aerobic zone, NH_4^+ -N is oxidized to NO_3^- -N, which is then recirculated to the second anoxic zone (Wang et al., 2009b). The SANI process and its modifications combine the advantages of energy saving, reduced sludge production and smaller footprint. Wang et al. (2009b) noted that the total cost reduction for SANI would be >50% for a WWTP with an influent flow rate of 10,000 m³/d.

The SANI process can be used for treatment of SO_4^{2-} -poor wastewater provided that low-cost and S-rich sources are available. For example, wet flue gas desulfurisation (FGD) systems used in boilers, coal-fired furnaces and power plants, can be reduced to alkaline flue gas sorption for production of liquid waste containing SO_4^{2-} and SO_3^{2-} (Srivastava and Jozewicz, 2001). Such a waste stream can be co-treated in the main wastewater stream in wet FGD-SANI after removing suspended solids and heavy metals (Qian et al., 2013).

The Mixed Denitrification (MD) - SANI process has also been proposed (Qian et al., 2015a,b,c). MD-SANI generates $\text{S}_2\text{O}_3^{2-}$, S^{2-} , and some volatile fatty acids (VFA), which are subsequently converted in both heterotrophic denitrification (VFA) and autotrophic denitrification (S^{2-} and $\text{S}_2\text{O}_3^{2-}$) (Qian et al., 2015a). It should be noted that the latter process is induced faster by $\text{S}_2\text{O}_3^{2-}$ than S^{2-} (Cardoso et al., 2006). Fig. 3b-d shows the SANI, FGD-SANI and MD-SANI processes depending on the available substrates.

4.2. Systems integrating the S cycle with anammox-based nitrogen removal processes

In recent years, the growing attention has been paid to N removal using the anammox process. The anammox process completely eliminates the need for organic C source, reduces the amount of sludge produced by 80% and related energy costs for aeration by 60% compared to conventional nitrification/denitrification. The anammox process also has economic advantages in the context of co-treatment of wastewater containing S compounds, especially S^{2-} (Kosugi et al., 2019).

The anammox-based systems for combined N and S removal comprise (1) Sulfate Reduction, Denitrification/Anammox and Partial Nitrification (SRDAPN), (2) Partial Nitrification/Anammox and S-dependent autotrophic Denitrification (PNASD), (3) Anammox and S-dependent autotrophic Denitrification (ASD), and (4) S-dependent autotrophic Partial Denitrification/Anammox (SPDA).

The SRDAPN process is similar to the SANI process, but enhanced with anammox (Fig. 4a). As a consequence, instead of full nitrification, only PN is needed to produce NO_2^- -N (Kosugi et al., 2019).

The PNASD process uses PN/A to remove NH_4^+ -N under aerobic (PN) – anoxic (anammox) conditions. With S-dependent autotrophic

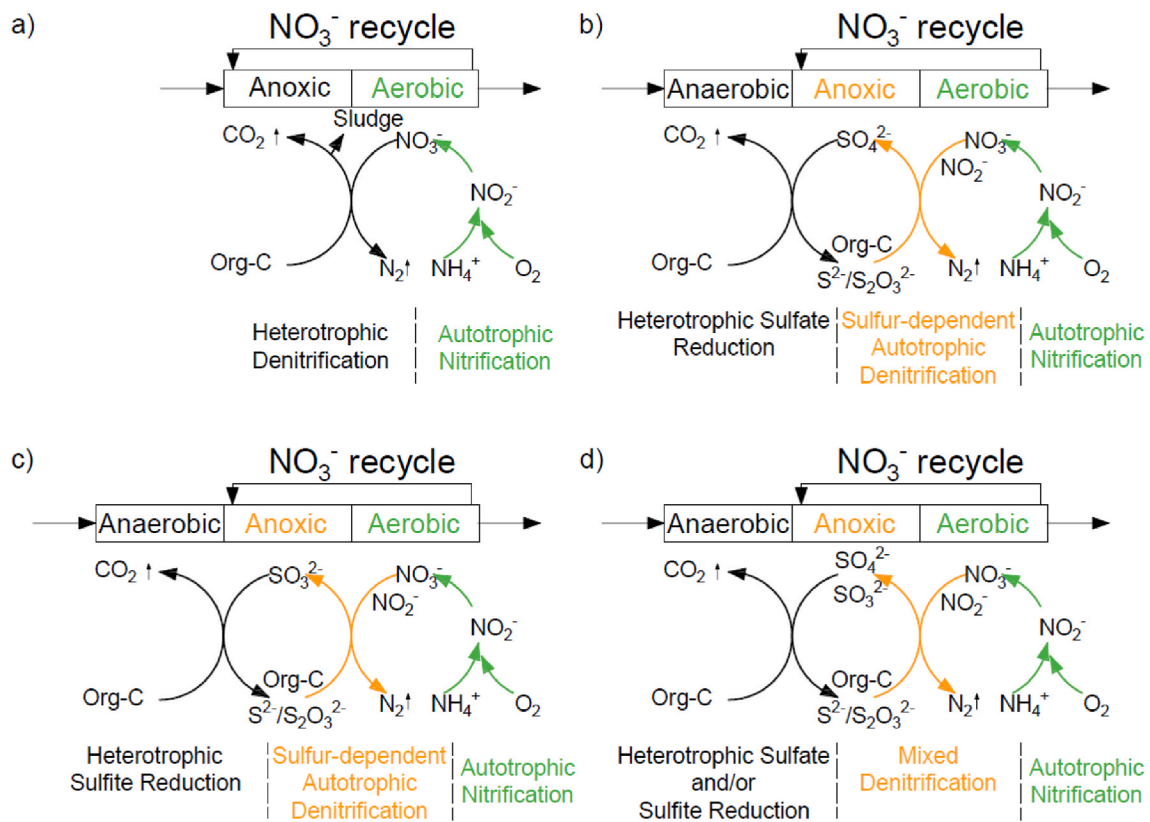


Fig. 3. Biological wastewater treatment systems using a) conventional heterotrophic denitrification with autotrophic nitrification b) SANI c) FGD-SANI d) MD-SANI.

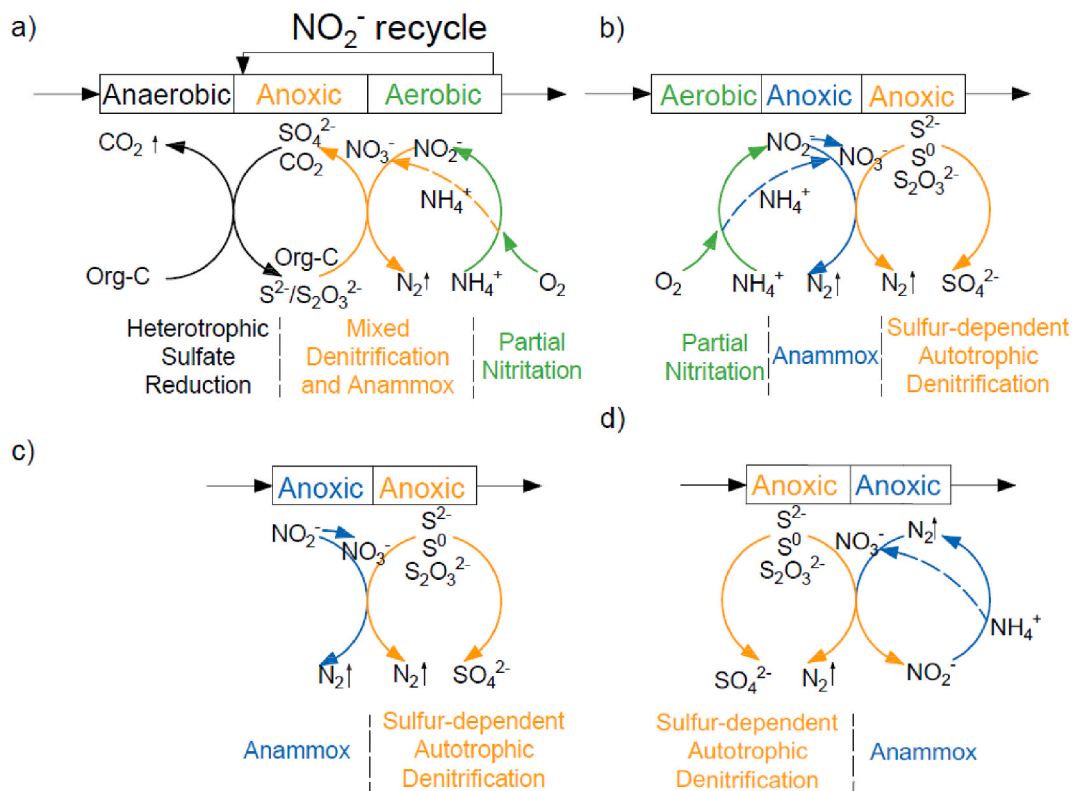


Fig. 4. Wastewater treatment systems using the anammox process a) SRDAPN b) PNASD c) ASD d) SPDA.

denitrification, the produced NO_3^- -N can further be reduced to N_2 , as shown in Fig. 4b. The PNASD process has been implemented as both two-stage (Dasgupta et al., 2017) and one-stage system (Yuan et al., 2020).

The PNASD system can also be limited to an ASD system that ignores the share of PN, as shown in Fig. 4c. Then the NO_2^- -N acceptor for anammox is not obtained from the conversion of NH_4^+ -N, but supplied from external sources. Accordingly, the costs of energy used to produce NO_2^- -N by AOB in PN are neglected, but the costs of process substrates increase. The residual NO_3^- -N from anammox can be removed along with S compounds (S^{2-} , S^0 , $\text{S}_2\text{O}_3^{2-}$) by S-dependent autotrophic denitrification. The ASD process has been implemented in both one-stage (Guo et al., 2016) and two-stage (Sun et al., 2018) systems.

If NO_2^- -N can be obtained by partial autotrophic denitrification of NO_3^- -N with oxidation of S compounds (S^{2-} , S^0 , $\text{S}_2\text{O}_3^{2-}$), then it can be used as a substrate in the anammox process. Liu et al. (2017) and Wu et al. (2019) used a UASB reactor to perform S-dependent denitrification with S^{2-} (Liu et al., 2017) and $\text{S}_2\text{O}_3^{2-}$ (Wu et al., 2019) for NH_4^+ -N removal from wastewater, as shown in Fig. 4d.

4.3. Systems including the sulfamnox process

Both sulfamnox and anammox incorporate “anaerobic” oxidation of NH_4^+ -N. The coexistence of both processes was found in marine sediments (Rios-Del Toro et al., 2018) and anaerobic sludge (Rikmann et al., 2016). In conventional sulfamnox, SO_4^{2-} is an electron acceptor, which is reduced to S^0 or S^{2-} , while NH_4^+ -N is oxidized to N_2 , NO_2^- -N and/or NO_3^- -N. Sulfamnox may occur on its own, as shown in Fig. 5a. Alternatively, the formed NO_2^- -N may be used as an electron acceptor for anammox in the combined Sulfamnox/Anammox (SA) system (Fig. 5b).

As NO_2^- -N and NO_3^- -N are generated in sulfamnox, the process can be combined with autotrophic S-dependent denitrification in an Sulfamnox - S-dependent autotrophic Denitrification (SSD) system, as

shown in Fig. 5c (Liu et al., 2021; Grubba et al., 2021). The formed S^0 and S^{2-} in sulfamnox can be oxidized again to SO_4^{2-} , while NO_x -N are reduced to N_2 . The SSD system can be expanded with anammox in SASD (Sulfamnox - Anammox - S-dependent autotrophic denitrification), as shown in Fig. 5d. In this case, NO_2^- -N can be reduced by both AAOB and autotrophic denitrifiers (Liu et al., 2021; Grubba et al., 2021).

5. Operational conditions and performances of the systems integrating the N-S-C cycles

The biochemical processes associated with the C, N and S conversions and the microorganisms responsible for those conversions can be found in the SI (Fig. S2).

5.1. SANI, FGD-SANI, MD-SANI

The S cycle, which is part of the SANI process, ensures a more efficient use of electrons (Wu et al., 2020) and eliminates the production of toxic S^{2-} (Qian et al., 2015c). In addition, it reduces sludge production by 90% compared to the conventional biological N removal processes. This is possible due to very low yield coefficients of the microorganisms responsible for SO_4^{2-} reduction, autotrophic denitrification and nitrification, i.e., 0.02 kg VSS/kg COD, 0.01 kg VSS/kg NO_3^- -N and 0.07 kg VSS/kg NH_4^+ -N, respectively (Lu et al., 2011; Wang et al., 2009b). In addition, there are other significant reductions, including energy consumption by 35% (Lu et al., 2011), greenhouse gas emission (GHG) by 36% (Lu et al., 2011), and the space required for the process of wastewater treatment and sludge handling by 30%–40% (Liu et al., 2016).

As shown in Table 4, SANI shows a relatively high level of performance compared to the conventional systems. The efficiencies of SO_4^{2-} , total nitrogen (TN) and COD removal vary in the ranges of 72–98%, 55–74% and 82–97%, respectively (Hao et al., 2015; Lu et al., 2009). The SANI modifications (FGD-SANI and MD-SANI), which use

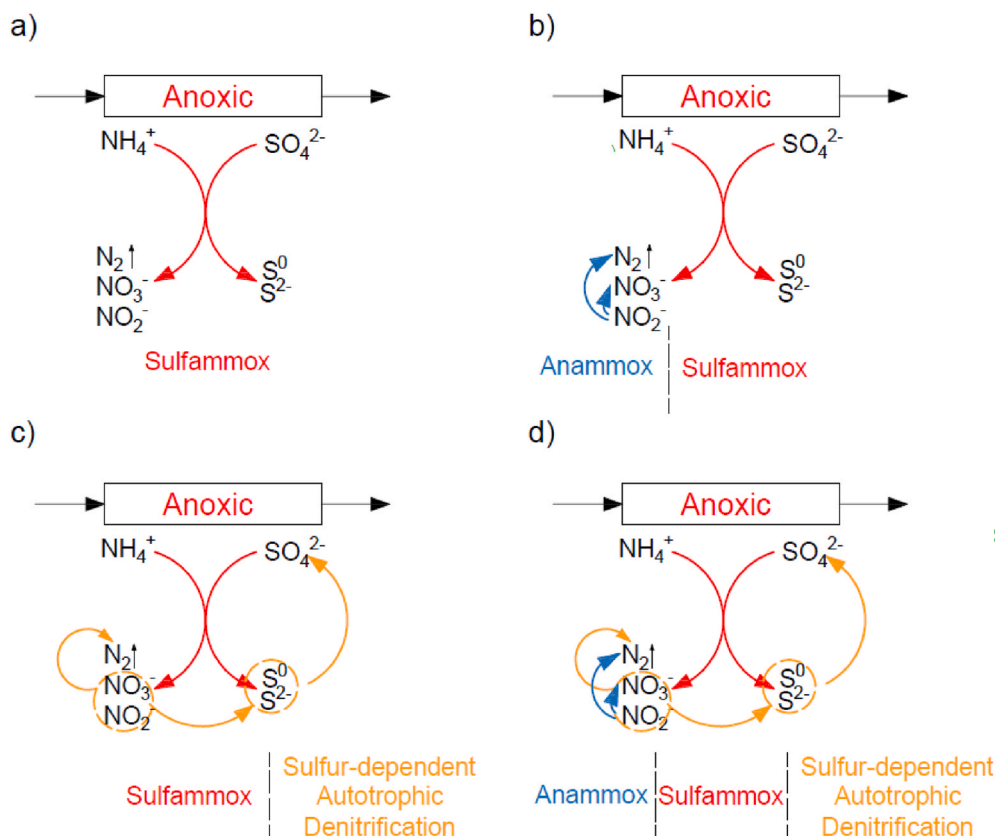


Fig. 5. Wastewater treatment systems incorporating the sulfamnox process a) Sulfamnox b) SA c) SSD d) SASD.

Table 4
Technologies for integrated S, N, COD removal and the observed removal efficiencies for S, N and COD.

Process	Reactor type	S removal efficiency	N removal efficiency (N form)	COD removal efficiency	References
Sulfate reduction, Autotrophic denitrification and Nitrification Integrated (SANI)	Up-flow anaerobic sludge bed, an anoxic filter, an aerobic filter	16–68 mg S ²⁻ /L	74% (TN)	95%	Wang et al. (2009b)
	Up-flow sludge bed reactor, an anoxic reactor and an aerobic reactor	98% S ²⁻	55% (TN)	87%	Lu et al. (2012)
SANI	Up-flow anaerobic sludge bed, an anoxic filter and an aerobic filter	97% S ²⁻	74% (TN)	97%	Lu et al. (2009)
SANI	Sulfate-reducing up-flow sludge bed	75% SO ₄ ²⁻	–	90%	Hao et al. (2013)
SANI	Sulfate-reducing up-flow sludge bed	72% SO ₄ ²⁻	–	82%	Hao et al. (2015)
Flue gas desulphurization - Sulfate reduction, Autotrophic denitrification and Nitrification Integrated (FGD-SANI)	Sulfite-reducing upflow anaerobic sludge bed	~54% S ²⁻	~98% (TN)	94%	Jiang et al. (2013)
Mixed Denitrification - Sulfate reduction, Autotrophic denitrification and Nitrification Integrated (MD-SANI)	Sulfate/sulfite reducing upflow sludge bed and anoxic up-flow sludge bed	–	100% (NO ₃ ⁻ -N)	80%	Qian et al. (2015a)
MD-SANI	Sulfur-reducing upflow sludge bed and the anoxic upflow sludge bed	~100% SO ₃ ²⁻	100% (TN)	81%	Qian et al. (2015b)
Sulfate reduction, denitrification/anammox and partial nitrification (SRDAPN)	Laboratory scale up-flow anaerobic-anoxic biological filter reactor	400–500 mg S ²⁻ /d	79% (TN)	500–2300 mg/d	Kosugi et al. (2019)
Partial Nitrification/Anammox and S-dependent autotrophic Denitrification (PNASD)	PN/A reactor and an elemental sulfur-supported packed bed autotrophic denitrification	–	97% (TN)	–	Dasgupta et al. (2017)
PNASD	Single reactor under mainstream conditions	~100% S ²⁻	84% (TN)	–	Yuan et al. (2020)
Anammox and S-dependent autotrophic Denitrification (ASD)	Expanded granular sludge bed	90–100% S ₂ O ₃ ²⁻	98% (TN)	–	Sun et al. (2018)
ASD	Up-flow anaerobic sludge blanket reactor	99.6% S ²⁻ , 330 mg S ²⁻ /L	88% (TN), 252 mg NH ₄ ⁺ -N/L	–	Guo et al. (2016)
S-dependent autotrophic Partial Denitrification and Anammox (SPDA)	Up-flow anaerobic sludge blanket reactor	~100% S ₂ O ₃ ²⁻	>90% (TN)	–	Wu et al. (2019)
SPDA	Up-flow anaerobic sludge blanket reactor	70% S ⁰	90% (NO ₂ ⁻ -N)	–	Liu et al. (2017)
Sulfamox/Anammox (SA) with COD	Moving Bed Biofilm Reactor	10% SO ₄ ²⁻	30% (NH ₄ ⁺ -N)	–	Rikmann et al. (2016)

wastewater streams from wet flue gas desulphurization, reveal even a greater performance potential (Qian et al., 2015a, b, Jiang et al., 2013). The biological reduction of SO₃²⁻ in FGD-SANI and MD-SANI provides more energy for bacterial growth, which is associated with a higher sludge efficiency compared to the biological reduction of SO₄²⁻ (Jiang et al., 2013). Moreover, SO₃²⁻ is an intermediate in SO₄²⁻ reduction, which may result in faster reduction by SRB.

Jiang et al. (2013) found that the removal rates of specific organics in the SO₃²⁻ and SO₄²⁻ reducing reactors were similar. At the extremely low temperatures (<10 °C), incomplete reduction of SO₃²⁻ in an anaerobic reactor (Fig. 3c) resulted in accumulation of S₂O₃²⁻ and reduction in the removal rate of organics. However, the anoxic and aerobic reactors (Fig. 3c) still provided a high removal efficiency of organics (>94%), while NH₄⁺-N and NO₃⁻-N were almost completely removed.

The MD reaction can lead to a much higher reduction of NO₃⁻-N and NO₂⁻-N compared to the S²⁻ based SANI process (Qian et al., 2015a). Qian et al. (2015b) reported that the denitrification rate increased sevenfold in MD-SANI compared to SANI. Furthermore, in comparison with SANI, FGD-SANI shows higher TN and COD removal efficiencies (98% and 94%). The complete removal of SO₃²⁻ and TN was achieved in MD-SANI, while the COD removal efficiency in that process was 81% (Table 4).

5.2. SRDAPN and PNASD - challenges resulting from the combination of aerobic and anaerobic conditions

The presence of S²⁻ in the influent wastewater imposes a significant risk of inhibition of the AAOB responsible for anammox. Threshold levels of S²⁻ inhibiting AAOB were found in the range of <1–64 mg S/L (Jin et al., 2013; Carvajal-Arroyo et al., 2013; Dapena-Mora et al.,

2007). The study by Wisniewski et al. (2019) determined the half maximal inhibitory concentration (IC₅₀) under two different S²⁻ conditions. The IC₅₀ was 4.25 mg H₂S–S/L at a constant S²⁻ concentration of 11 mg TS–S/L and pH in the range 7–7.9 vs. 4.67 mg H₂S–S/L at a varying concentration of S²⁻ ranging from 1 to 15 mg TS–S/L and a constant pH of 7. The decrease in AAOB activity was due to the pH-dependent non-ionized form of H₂S. In addition, heterotrophic bacteria may coexistence with AAOB but also outcompete AAOB at high influent C/N ratios (Chamchoi et al., 2008).

The PNASD process has been implemented in both one- and two-stage systems. The two stage-systems are easier to maintain and allow to avoid the negative impact of S²⁻ on AAOB and the competition between AOB and SOB for DO (Sahinkaya and Kilic, 2014).

Zhang et al. (2020) used S⁰ for denitrification and observed only a small effect, when DO was kept at the level of 0.4–0.8 mg/L. When the DO concentration increased to 1.2 mg O₂/L, the concentrations of NO₃⁻-N and SO₄²⁻ also increased. This indicates excessive oxidation of S²⁻ or its reduced compounds in aerobic systems. Under non-limited DO conditions, autotrophic SOB can readily utilize oxygen, which leads to accumulation of SO₄²⁻. On the other hand, too low DO concentrations in the PNASD process can reduce the NO₂⁻-N production rate in PN.

5.3. ASD, SPDA and sulfamox systems - coexistence of AAOB and denitrifiers

Under anaerobic conditions, the combination of anammox process and S-dependent autotrophic denitrification can work with high removal efficiencies of TN (88–96%) and S (90–100%) (Table 4). AAOB and T. denitrificans can assist in the combined N and S removal without inhibition by S²⁻ (Guo et al., 2016). In that study, most of S²⁻ was

oxidized to S^0 at the influent ratios of NH_4^+-N/S^{2-} and NO_2^-N/S^{2-} at 1.74 and 2.2–2.27, respectively. Two S forms can accumulate depending on the S/N ratio in the reactor, i.e., SO_4^{2-} (at S/N ratio <1) or S^0 (at S/N ratio >1) (Cardoso et al., 2006).

When NO_2^-N is fed to the anammox process, S-dependent autotrophic denitrification may occur. When both NO_2^-N (anammox substrate) and NO_3^-N (anammox product) are simultaneously present in the influent, the latter form is the preferred electron acceptor for denitrification (Guo et al., 2016). However, a small portion of NO_2^-N can also be used by *T. denitrificans* and increase the overall efficiency of N and S removal.

Instead of complete denitrification, partial reduction to NO_2^-N can be achieved. This approach is advantageous for the Partial Denitrification/Anammox (PD/A) systems by continuously producing NO_2^-N for anammox (Wu et al., 2019). In addition, the consumption of electron donors can be reduced in comparison with the conventional biological nitrogen removal processes. The reported TN removal efficiencies exceeded 90% in SPDA (Table 4).

The novel sulfamox process has been applied in SO_4^{2-} and NH_4^+-N -rich wastewater treatment systems. One of the intermediates in the sulfamox reaction is NO_2^-N , which can be used by either AAOB or S-dependent autotrophic denitrification along with the residual NO_3^-N

from anammox. Wu et al. (2020) combined sulfamox and anammox and obtained high removal efficiencies of NH_4^+-N (98.5%) and SO_4^{2-} (53%). Furthermore, the sulfamox and anammox processes can also be combined with S-dependent autotrophic denitrification (Rios-Del Toro et al., 2018).

6. Modeling N, S and C conversions in wastewater treatment systems

Modeling has been proven to be an effective tool to understand complex, interrelated N, S and C transformations (Show et al., 2013). In principle, two modeling approaches are possible, including empirical models, such as artificial neural networks (ANNs), and mechanistic models based on the Activated Sludge Model (ASM) family.

6.1. Artificial neural networks (ANNs)

The ANN model does not require a detailed process description, and it can be established by simple input and output parameters. Therefore, the ANN has been known for a long time as a tool in setting control mechanisms and performance models of biological wastewater treatment processes (Choi and Park, 2001). Wang et al. (2009a) developed an

Table 5
Overview of the reported mechanistic models linking C, S and N transformations.

No.	Reactor type	Substrate	Influent concentrations			Model structure				References
			Organic (mg COD/L)	S^{2-} (mg S ²⁻ /L)	NO_3^-N (mg -N/L)	No. of processes	No. of components	No. of parameters	S and N involved processes	
1	Bench-scale EGSB reactor	Synthetic wastewater	200–800	200–800	75–275	7	10	18	Hydrolysis: Particulate N → Organic N Ammonification: Organic N → NH_4^+-N Heterotrophic: NO_3^-N → N_2 Autotrophic: NO_3^-N → N_2	Wang et al. (2010)
2	Bench-scale EGSB reactor	Synthetic wastewater	275–2300 mg C/L	156–1490	100–800	6	8	31	Autotrophic: S^{2-} → SO_4^{2-} Autotrophic: NO_3^-N → NO_2^-N → N_2 Heterotrophic: NO_3^-N → NO_2^-N → N_2	Xu et al. (2014)
3	Bench-scale SBR	Synthetic wastewater	–	194 145	321 202	4	5	9	Autotrophic: S^{2-} → S_0 → SO_4^{2-} Autotrophic: NO_3^-N → NO_2^-N → N_2	Xu et al. (2016)
4	Bench-scale EGSB reactor	Synthetic wastewater	2700	1000 mg SO_4^{2-} -S/L	200–700	14	15	38	Autotrophic: S^{2-} → S_0 Autotrophic: NO_3^-N → NO_2^-N Heterotrophic: NO_3^-N → NO_2^-N → N_2 Heterotrophic: SO_4^{2-} → S^{2-}	Xu et al. (2017)
5	MBfR	Anaerobic digestion liquor	50–100	30	50–1000	18	17	60	Autotrophic: NH_4^+-N → NO_2^-N → NO_3^-N Autotrophic: NH_4^+-N , NO_2^-N → N_2 , NO_3^-N Heterotrophic: NO_3^-N → N_2 Autotrophic: S^{2-} → S^0 → SO_4^{2-} Autotrophic: CH_4 → CO_2	Chen et al. (2016)
6	Coastal upwelling system	Sea water	–	0.1 mmol S/m ³	0.1 mmol N/m ³	9	14	46	Autotrophic: NH_4^+-N → NO_2^-N → NO_3^-N Heterotrophic: NO_3^-N → NO_2^-N → N_2 Heterotrophic: SO_4^{2-} → S^{2-} Autotrophic: S^{2-} → SO_4^{2-}	Azhar et al. (2014)

SBR: sequencing batch reactor, EGSB: expanded granular sludge bed, MBfR: membrane biofilm reactor.

ANN model to monitor a denitrifying S^{2-} removal (DSR) process. The proposed model revealed that the comparative influences of four input factors on DSR performance were as follows: hydraulic retention time (HRT) > S^{2-} concentration > C/S ratio > N/S ratio. Even though the ANN model is capable of predicting an intricate function between input and output parameters, it cannot help in understanding mechanisms of the complex biochemical processes.

6.2. Mechanistic models

The International Water Association Activated Sludge Models (ASMs) No. 1, 2, 2d and 3 (Henze et al., 2000) describe conversions of organic C and N compounds (ASM1 and ASM3), and additionally P compounds (ASM2 and ASM2d). However, to simplify the model structure, all the ASMs only considered NO_3^- -N reduction as a one-step heterotrophic process using readily biodegradable organic compounds as electron donors. Moreover, one-step NH_4^+ -N oxidation to NO_3^- -N was the only autotrophic N transformation.

S-dependent autotrophic denitrification and the synergistic and competitive relationships among microorganisms were subsequently integrated with the ASMs. On one hand, developing realistic models is essential for practical applications in simultaneous N, C and S removal systems. On the other hand, due to the complex interactions between autotrophic and heterotrophic denitrifiers, developing an exhaustive model and appropriate control strategy becomes challenging. The existing models (Table 5) have been used in bench-scale reactors to predict the process involving intricate metabolic pathways with synthetic substrates. However, further work is still necessary to confirm the models in practical applications with real wastewater.

A detailed description of the mechanistic models can be found in SI (S4).

7. Implications of combining the N, S and C cycles in wastewater treatment systems

7.1. Processes application opportunities

S^0 and S^{2-} are considered good alternatives to organic matter in the denitrification process due to the absence of organic residues in the treated wastewater. It is thus strongly recommended to use S-dependent autotrophic denitrification instead of heterotrophic denitrification, especially for wastewater with a low organic content. Attention should also be paid to the water-insoluble S^0 , which can physically be removed from wastewater and reused for production of sulfuric acid, pesticides, fertilizers, in construction (Lin et al., 2018). It is economic, effective and readily available source of electrons. On the other hand, $S_2O_3^{2-}$ is readily bioavailable and may mediate a higher rate of denitrification compared to S^0 and H_2S . S^{2-} is often used in municipal and industrial areas requiring desulphurization. Depending on the local conditions, S-dependent autotrophic denitrification can occur with a wide spectrum of S compounds. Moreover, it can get them from the initial SO_4^{2-} reduction stage in the integrated systems combining N-S-C cycles.

Biological SRB-based methods are a sustainable way of treating AMD compared to physico-chemical methods (Sinharoy et al., 2020b). SRB are capable of using toxic metals in their metabolism, thus reducing environmental and human health problems. SRB can grow in a wide range of environmental conditions, which provides many opportunities for the development of technologies based on their metabolism, with SO_4^{2-} reduction being recognized as a key step in all S-dependent processes (Hao et al., 2014).

Among the various gaseous substrates for SO_4^{2-} reduction, H_2 is most energetic for SRB. The resources that can be recovered from this process are metal sulfides and S^0 , which has also been identified by Kumar and Pakshirajan (2020) as a potential substrate for S-dependent autotrophic denitrification.

The combination of the N, S and C cycles could lead to the

development of economically feasible and sustainable wastewater treatment systems that produce less sludge and reduce carbon footprint compared to the existing systems. The SANI process has already been used in several full-scale wastewater treatment installations in Hong Kong due to the practice of flushing toilets with seawater (Jiang et al., 2013). The process can also be applied to freshwater wastewater, even in cold inland areas that do not contain enough SO_4^{2-} or SO_3^{2-} rich wet flue gas desulphurization (Qian et al., 2015a, b, Jiang et al., 2013). It can also be adapted to treat industrial wastewater by adding SO_4^{2-} , seawater or some SO_4^{2-} -rich wastewater. Lu et al. (2009, 2012) suggested that the SANI process could be a good solution in densely populated cities to treat saline wastewater as an economic source in terms of water scarcity and wastewater treatment in water-poor coastal areas.

Other technologies that include anammox and SANI processes have discovered the advantages of AAOB coexisting with SRB, SOB, and AOB. In addition, compared to the SANI process, the combination of SO_4^{2-} reduction, denitrification/anammox and partial nitrification will further reduce aeration energy consumption due to the lack of full nitrification required for NO_3^- -N production. The presence of anammox in the SRDAPN process resulted in an increased NO_2^- -N removal efficiency by over 30% (Kosugi et al., 2019).

For wastewater with a low organic content, PNASD can be considered a viable option. The two-step PNASD system was more efficient for N and S removal, and easier to maintain than the one-step system (where bacteria competed for DO) (Dasgupta et al., 2017). Moreover, it has also been proven that the process can be applied in a single reactor under mainstream conditions (Yuan et al., 2020).

Instead of combining the heterotrophic SO_4^{2-} reduction with anammox, sulfamox can replace or accompany both processes by using a SO_4^{2-} dependent AAOB. Recent studies have proposed the use of sulfamox based on the combined reduction of NH_4^+ -N and SO_4^{2-} . If SO_4^{2-} was reduced to S^{2-} or S^0 with organic compounds, this process would be replaced with sulfamox, while eliminating the addition of external carbon. Another suggested solution is to combine the sulfamox process with heterotrophic SO_4^{2-} reduction in order to increase the reduction rate of SO_4^{2-} . Moreover, if sulfamox is used upstream of an S-dependent autotrophic denitrification reactor, it contributes to oxidation of NH_4^+ -N to N_2 (which increases the overall efficiency of NH_4^+ -N removal) or NO_2^- -N and NO_3^- -N (which can be used in S-dependent autotrophic denitrification). By combining sulfamox and anammox, the efficiency of NH_4^+ -N removal and SO_4^{2-} reduction to S^0 can be simultaneously increased (Liu et al., 2021; Grubba et al., 2021).

7.2. Advantages and disadvantages of two cycles or three cycles in wastewater treatment

The advantages and disadvantages of the systems based on the N-S-C cycles and their coupling are summarized below.

Advantages:

1. Approximately 35% reduction in energy consumption and up to 90% reduction in sludge production compared to full nitrification-denitrification.
2. Reduction or even no external carbon dosing for S-dependent autotrophic denitrification.
3. For the combined processes, almost complete N and S^{2-} removal and up to 75% efficiency of SO_4^{2-} removal.
4. Products of one process used as the substrates for another process.
5. When replacing heterotrophic denitrification with S-dependent autotrophic denitrification, carbon consumption is reduced by 100%. If heterotrophic SO_4^{2-} reduction is replaced by sulfamox, carbon consumption is also reduced by 100%.
6. Removal of a few harmful compounds (NH_4^+ , NO_2^- , SO_4^{2-} , S^{2-}) in one system.
7. Approximately 30–40% reduction of volumes required for wastewater and sludge treatment processes.

- Reduction of GHG emissions by 36% compared to conventional nitrification-denitrification.

Disadvantages:

- Limited use in cold regions due to the high optimal temperature range (28–30 °C).
- Complex interactions and competition for substrates between the functional microorganisms.
- Greater complexity of the systems potentially resulting in higher investment costs.
- The operating conditions must be compatible with all the N–S–C processes.
- Some substrates/products involved in one process may be inhibitors for other processes, e.g. S^{2-} .

7.3. Processes application limitations

One of the most important limitations of technologies combining N, S and C cycle processes is the narrow optimal range of temperature (28–30 °C) and pH (7–7.6). Thus, cold weather in inland areas also restricts the use of coupled systems.

An important factor that should be considered when implementing technologies containing the S-dependent autotrophic denitrification process is the inhibition of this process caused by S^{2-} (Cardoso et al., 2006) as well as NO_2^- -N, NO_3^- -N and free nitric acid (FNA) (Cui et al., 2019). Even though S^0 is an inexpensive and non-toxic electron donor, but it provides a low denitrification rate due to its low solubility. The use of smaller S granules with a larger surface area improves the reaction efficiency, however it can cause low porosity and clogging and fouling of the reactors due to small S grain size or cracking (Wu et al., 2021). Moreover, as S^0 and H_2S reveal a much lower rate of NO_3^- -N reduction, mainly the use of $S_2O_3^{2-}$ is recommended in the process. However, its natural content of wastewater is rather limited due to its instability (Cui et al., 2019).

In the case of heterotrophic SO_4^{2-} reduction, the presence of DO, NO_3^- -N and NO_2^- -N inhibits reduction of SO_4^{2-} and enhances oxidation of S^{2-} to S^0 or SO_4^{2-} (Mohanakrishnan et al., 2009). Moreover, the activity of SRB is inhibited by heavy metals, including Pb and Cd (Sinharoy and Pakshirajan, 2019). The toxicity of heavy metals depends mainly on the type of metal, responsible microorganisms, presence of other pollutants, and process conditions (Mal et al., 2016). Therefore, the systems based on heterotrophic SO_4^{2-} reduction cannot be used for wastewater rich in heavy metals. Moreover, a significant limitation is the limited number of microorganisms that are able to carry out SO_4^{2-} reduction with the use of gaseous substrates. Moreover, the low gas-liquid mass transfer also makes it difficult to scale-up the process.

A significant limitation in the implementation of integrated systems connecting N–S–C cycles is also the insufficient knowledge about the mechanism of sulfamox and responsible microorganisms. Until now, there has been no genomic evidence to support the ability of AAOB to use SO_4^{2-} as an electron acceptor. The growth rate of potential functional bacteria is also low, which limits their unambiguous identification (Liu et al., 2021). Moreover, the organic matter present in the wastewater stimulates the survival of heterotrophic bacteria, including denitrifiers. This leads to a competition between these bacteria and the sulfamox bacteria, thus destroying the sulfamox process.

7.4. Processes application challenges

Using specific N, S and C removal processes independently of each other is much easier to maintain than the processes combining these cycles. To link those processes in the combined technologies as presented in this review, it is important to recognize the effects of S^{2-} on N removal processes, such as autotrophic/heterotrophic denitrification and anammox, as well as the competition between AOB and SOB for DO.

S^{2-} and organic matter, which are fed to an anaerobic compartment, can inhibit AAOB in anammox-coupled systems (Kosugi et al., 2019). Chen et al. (2018) showed that DO can react with S^{2-} while reducing the NO_3^- -N removal rate. In addition, S^{2-} was reduced to S^0 and then converted to SO_4^{2-} due to the presence of DO. These findings highlight the challenges faced by single-stage integrated systems.

In order to avoid the inhibition of SO_4^{2-} reduction by heavy metals, it is recommended to use an upstream reactor in order to remove metals from AMD using S^{2-} . In order to use SO_4^{2-} reduction coupling systems, it is also necessary to consider selection of the appropriate type of reactor, use of resistant microorganisms, and presence of other pollutants. Designing novel reactor configurations with high gas-liquid mass transfer can also help in applying the process in full scale. Moreover, instead of obtaining pure gases, a cost-effective solution would be production of gaseous substrates by thermochemical or biochemical methods from various compounds (e.g. waste) (Sinharoy et al., 2020b).

In the case of sulfamox, more research is needed to identify potential applications and integration with other systems. The key enzymes involved in the metabolism of NH_4^+ -N and SO_4^{2-} should also be investigated. For this purpose, it is important to develop appropriate reactor configurations and create operational conditions that can enrich functional bacteria and allow for simultaneous removal of NH_4^+ -N and SO_4^{2-} . Under non-limited NO_3^- -N conditions, the SO_4^{2-} concentration may increase due to S-dependent autotrophic denitrification. The role of organic matter also requires further investigation with regard to the existence of the sulfamox process.

The combination of anammox, S-dependent autotrophic denitrification and sulfamox processes is challenging due to the different requirements of the microorganisms responsible for each process. The S-dependent autotrophic denitrification process may result in the production of SO_4^{2-} from S^{2-} or S^0 , which negatively affects sulfamox, where SO_4^{2-} must be reduced to S^0 (Liu et al., 2021). More focused research on the coexistence of sulfamox with other bacteria and the development of a mechanistic model are needed to better understand and predict N and S dynamics. Moreover, the S/N ratio also plays an important role in determining the S-dependent autotrophic denitrification end products, requiring a closer look at the N and S dynamics. On the other hand, in order to avoid fouling and clogging of the reactors due to the presence of S^0 , it is important to search for the appropriate sulfur grain size.

Wang et al. (2009b) identified three main challenges for the SANI process. First of all, it is the low efficiency of both SO_4^{2-} reduction during heterotrophic and S-dependent autotrophic denitrification reduction. Secondly, high concentrations of SO_4^{2-} are required, which may increase residual S^{2-} in the treated wastewater. Thirdly, transfer of NO_3^- -N from the nitrification reactor to the S-dependent autotrophic denitrification reactor can also be difficult.

8. Conclusions

In terms of sustainability, the combination of N–S–C cycles processes has a few important benefits, including energy savings and lower sludge production. The combined processes allow for almost complete N and S^{2-} removal, while the efficiency of SO_4^{2-} removal can reach up to 75%.

Among all the processes linking the N–S–C cycles, SANI has been best recognized, but is rather not applicable in the case of wastewater with low organic content. Instead, it is worth of considering the sulfamox process that can reduce SO_4^{2-} and increase NH_4^+ -N removal rate under anoxic conditions without the addition of external carbon.

Practical applications of the reviewed systems still face many challenges, especially in the single-stage configurations. In particular, the coexistence of several bacterial groups (AOB, AAOB, sulfamox bacteria, SOB, SRB) and their competition for the substrates is a key issue to be considered. Moreover, practical applications of the coupled S and N/C cycles require realistic models. However, due to the complex interactions between autotrophic and heterotrophic denitrifiers,

development of a mechanistic model and appropriate control strategy becomes challenging.

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CRediT authorship contribution statement

Dominika Grubba: Conceptualization, Investigation, Formal analysis, Writing – original draft, Preparation, Visualization, Funding acquisition, Project administration. **Zhixuan Yin:** Conceptualization, Investigation, Writing – original draft, Preparation, Visualization. **Joanna Majtacz:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Preparation, Visualization. **Hussein Ezzi Al-Hazmi:** Conceptualization, Methodology, Investigation, Writing – original draft, Preparation, Visualization. **Jacek Mąkinia:** Validation, Writing – review & editing, Supervision.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jclepro.2022.133495>.

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