

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, $T = 28\text{-}30^\circ\text{C}$). 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 sulfammox 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 nitritation/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.

Keywords: sulfur-dependent autotrophic denitrification; heterotrophic sulfate reduction; sulfamox; sulfur cycle; microbial community; mechanistic model

1 **1. Introduction**

2 High concentrations of ammonium ($\text{NH}_4\text{-N}$) lead to eutrophication of surface waters
3 and pose a threat to the aquatic life and human health (Qin et al., 2021). $\text{NH}_4\text{-N}$ can effectively
4 be converted to nitrogen gas by combined nitrification-denitrification, but this method has a
5 few important disadvantages, including a high demand of energy and carbon, and high sludge
6 production. On the other hand, sulfate (SO_4^{2-}) is a type of the secondary pollutant because
7 reduction of sulfide (S^{2-}) under anaerobic conditions is harmful for the aquatic environment
8 (Hao et al. 2014). S compounds have not been widely used as substrates in wastewater treatment
9 processes. Simultaneous removal of these two compounds (N and S) from wastewater, with or
10 without involving the carbon (C) cycle, can be a viable approach to the sustainable wastewater
11 management. In particular, this approach may be an effective alternative in the case of many
12 types of industrial wastewater, which are characterized by high concentrations of pollutants,
13 such as $\text{NH}_4\text{-N}$, SO_4^{2-} (>1000 mg/L of both N and S) and chemical oxygen demand (COD) (>
14 60,000 mg COD/L) (Rikmann et al., 2016, Jarvis and Younger 2000, Chapman, 1992).

15 A viable sustainable approach to biological wastewater treatment comprises a
16 combination of nitrogen (N), sulfur (S) and carbon (C) removal. Lower operating costs result
17 from the use of some products in one process as the substrates in other processes and the use
18 of shared reactors. Moreover, no carbon is needed for S-dependent autotrophic denitrification,
19 less sludge is generated, and the environmentally neutral compounds, such as nitrogen gas
20 (N_2) and elemental sulfur (S^0), are the final products of biochemical reactions (Lin et al.,
21 2018).

22 Conventional nitrification/denitrification for N removal is now being replaced by more
23 sustainable N-shortcut processes, such as “nitrite shunt” or deammonification. In the case of S
24 compounds, biological removal is based on heterotrophic SO_4^{2-} reduction by sulfate reducing



25 bacteria (SRB) and S-dependent autotrophic denitrification by sulfur oxidizing bacteria
26 (SOB). Recently, the growing attention has been paid to the novel sulfate reducing ammonia
27 oxidizing (sulfammox) process, which involves anaerobic ammonium oxidizing bacteria
28 (AAOB). These bacteria use SO_4^{2-} , instead of nitrite nitrogen (NO_2^- -N), as an electron
29 acceptor to oxidize NH_4^+ -N under anaerobic conditions.

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

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



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

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

67 **2. Single S-dependent biochemical processes integrating N, S and C** 68 **conversions**

69 There are three known processes combining sulfur and nitrogen conversions: S-dependent
70 autotrophic denitrification, heterotrophic sulfate reduction and autotrophic sulfamox. The
71 detailed description of those processes, including the metabolic mechanisms, biochemical
72 reactions, influencing environmental factors can be found in the SI (S1-S3).

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

88 Heterotrophic sulfate reduction is SO_4^{2-} reduction which takes place in two
89 independent different paths. The first is the use of organic electron donors, which are also the
90 carbon source for the SRB. The second is the use of inorganic electron donors, which must be
91 supplemented with a carbon source, such as CO_2 (Sinharoy et al., 2020a). The SRB can be
92 divided into 7 phylogenetic lines, including five for bacteria and two for archaea. Most of the
93 SRB found in sulfate reduction reactors belong to 23 genera within *Deltaproteobacteria*
94 (*Desulfovibrio*, *Desulfobacteraceae*, *Desulfobulbaceae*, *Syntrophobacteraceae*,
95 *Desulfomicrobium*, *Desulfohalobium*). Another SRB belong to the gram-positive genera
96 *Clostridia* (*Desulfotomaculum*, *Desulfosporosinus* and *Desulfosporomusa*). Three lineages,
97 *Nitrospirae* (*Thermodesulfovibrio*), *Thermodesulfobacteria* (*Thermodesulfobacterium*) and

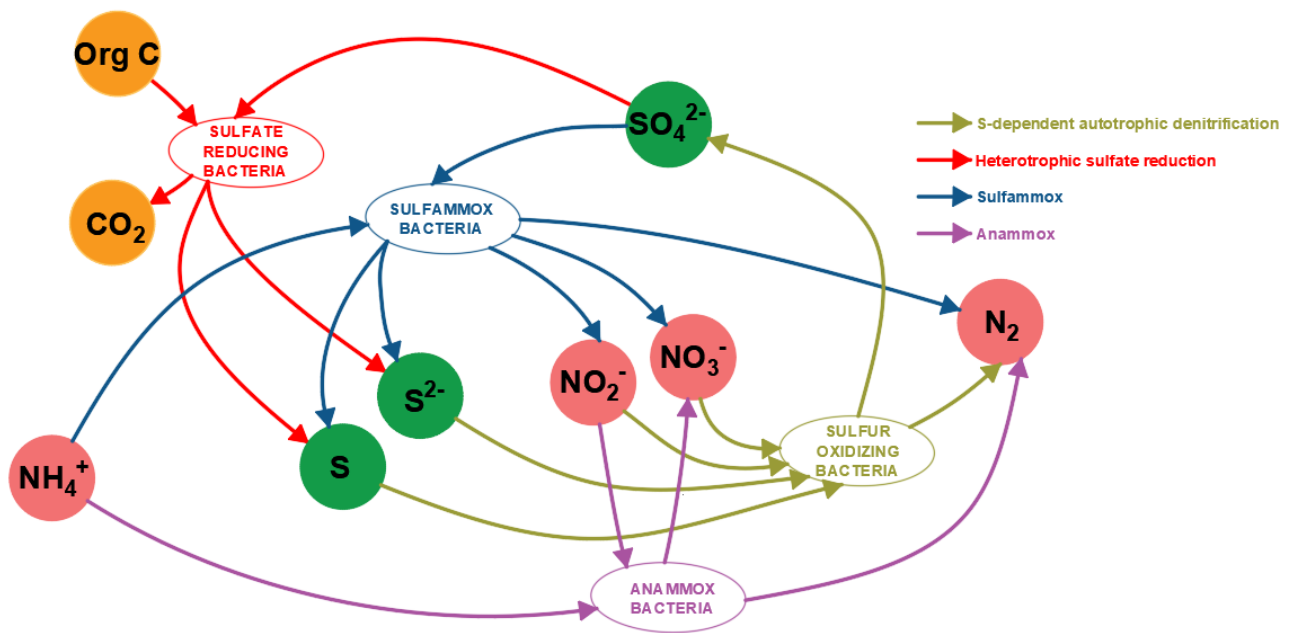


98 *Thermodesulfobiaceae* (*Thermodesulfobium*), contain only thermophilic SO_4^{2-} reducing
99 agents. Archaeal SRB are *Euryarchaeota* and *Crenarchaeota* (Muyzer and Stams, 2008).

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

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





115

116 Figure 1. Interactions between S-dependent autotrophic denitrification, heterotrophic sulfate
 117 reduction, anammox and sulfamnox process

118

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

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

125 3.1. S-dependent autotrophic denitrification

126 In S-dependent autotrophic denitrification, the most frequently used electron donors are S⁰
 127 and S²⁻ (Table 2). The experiments were mainly carried out in packed bed reactors, but several
 128 other types of reactors were also used. The reported rates of denitrification varied in a wide
 129 range - from 0.03 to 8.13 kg N/m³/d, depending mainly on the temperature and influent NO₃⁻-

130 N concentrations. The effects of pH in the investigated range (6.0-9.0) and S concentrations
131 were less significant. For a detailed description of previous research related to S-dependent
132 autotrophic denitrification, see the SI (S1). This process allowed for the efficient (>90%)
133 removal of N and S²⁻ (Yang et al., 2016, Jing et al., 2010) with the NO₃⁻-N concentration in
134 the range of 20-1230 mg N/L (Zhu et al., 2019, Zou et al., 2016, Kim et al., 2004).

135 During S-dependent autotrophic denitrification, SO₄²⁻ can be produced from different
136 electron donors. Frequently, the S balance in the process is not 1/1 for the removed electron
137 donor to SO₄²⁻ produced (Zou et al., 2016). In Table 2, the initial donor concentrations and the
138 amount of SO₄²⁻ produced are similar. The observed imbalances result from the production of
139 other S intermediates. The most common electron acceptor is NO₃⁻-N, but several studies
140 comparing NO₃⁻-N and NO₂⁻-N have been reported (Sun and Nemati, 2012, Moraes et al.,
141 2012, Jing et al 2010).

142 Different aspects of S-dependent autotrophic denitrification have been addressed in
143 several reviews (Wu et al. 2021, Cui et al. 2019, Lin et al. 2018, Sabba et al. 2016). Wu et al.
144 (2021) summarized all types of biofilm denitrification in terms of the reactor configuration,
145 microbial transformations, factors influencing the process, and especially focused on N₂O
146 emissions. The coexistence of S-dependent denitrification with anammox was also reported
147 and S-driven denitrifiers were identified, including *Thiobacillus denitrificans* and
148 *Thiobacillus thioparus*.

149 Cui et al. (2019) described S-dependent autotrophic denitrification in terms of the
150 functional enzymes, electron donors, types of reactors, and operational factors. They also
151 emphasized a significant advantage regarding S-dependent autotrophic denitrification
152 compared to heterotrophic denitrification with respect to N₂O emissions. It was shown that



153 autotrophic denitrification mediated by S compounds (S^0 , S^{2-}) emitted significantly less N_2O
154 than heterotrophic denitrification with methanol, ethanol or acetate.

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

164 **3.2. Heterotrophic sulfate reduction**

165 Table 3 presents the diversity of research carried out so far on heterotrophic SO_4^{2-} reduction
166 in terms of the electron donor, type of reactor and operating conditions. Most studies have
167 been carried out in the gas lift reactor and fluidized-bed reactor. Both organic and inorganic
168 donors were used, including carbon monoxide, methane, methanol, ethanol, hydrogen, crab
169 shell, compost and many others. The use of different donors resulted in a different SO_4^{2-}
170 reduction efficiency. A detailed description of the research can be found in SI (S2). The use of
171 different electron donors and SO_4^{2-} content resulted in a wide range of SO_4^{2-} removal
172 efficiencies (51-98%) and rates (0-3400 mg SO_4^{2-} /L/d). Nielsen et al. (2019) used methanol
173 and ethylene glycol which resulted in reduction of SO_4^{2-} by 71.2% and 36.9%, respectively.
174 The decrease of SO_4^{2-} concentration was limited to 13.8 and 5.3%, respectively, with the use
175 of peat and straw. Low temperatures (below 10°C) significantly affected the SO_4^{2-} removal
176 rates. For example, Virpiranta et al. (2019) carried out studies at various temperatures (22°C,

177 16°C, 6°C) and found gradually decreasing SO_4^{2-} removal rates, i.e. 169, 98 and 13-42 mg
178 $\text{SO}_4^{2-}/\text{L}/\text{d}$, respectively.

179 Sulfate reduction is less popular compared to S-dependent autotrophic denitrification,
180 but that process has also been addressed in several reviews (Kumar et al. 2021, Costa et al.,
181 2020, Sinharoy et al., 2020b, Serrano et al., 2019, Van den Brand et al., 2015). Kumar et al.
182 (2021) and Costa et al. (2020) focused on the use of SO_4^{2-} reduction for treatment of metal-
183 rich wastewater and recovery of these metals, showing a high degree of SO_4^{2-} reduction (>
184 90%) along with the efficient (> 99%) recovery of metals (Fe, Zn, Cd, Cu).

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

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

199 Van den Brand et al. (2015) analyzed important parameters, such as pH, organic
200 substrates, $\text{COD}/\text{SO}_4^{2-}$ ratio, substrate composition, SO_4^{2-} , salt, temperature and DO. They



201 found that the presence of SRB reduced pathogens, heavy metals and sludge produced.
202 Sulfate reduction, autotrophic denitrification and nitrification integrated (SANI) was
203 identified as a process combining the advantages of SRB and S-dependent autotrophic
204 denitrification. However, they indicated that in order to ensure the benefits of using SRB, a
205 sufficient SO_4^{2-} concentration in the influent wastewater would be required to maintain the
206 COD/ SO_4^{2-} ratio below 0.67.

207 **3.3. Sulfammox**

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

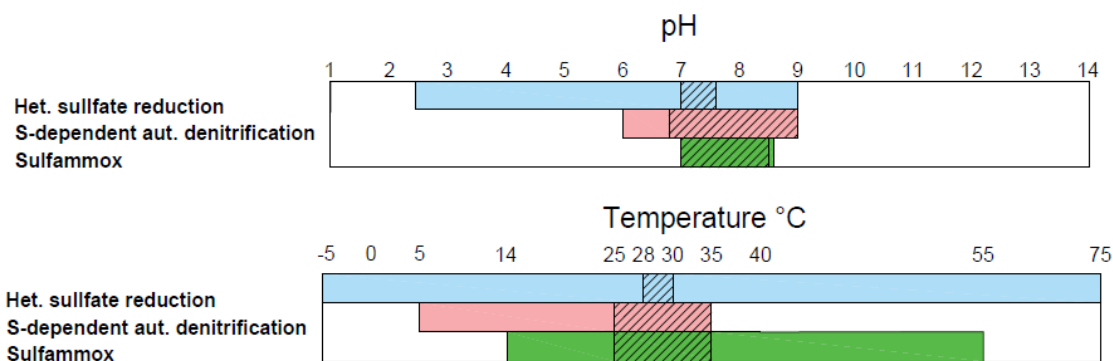
217 Liu et al. (2021) summarized the current understanding of sulfammox, including the
218 mechanisms, responsible microorganisms and factors influencing the process. It was
219 emphasized that the understanding of sulfammox has improved significantly in recent years,
220 but more attention should be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

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

229 In order to increase the efficiency of S removal in the sulfamox process, it is important to
230 maintain the optimal pH of 8.5 and temperature of 30°C (Cai et al., 2010). The N/S ratio is
231 also an important factor affecting that efficiency. When increasing the influent $\text{NH}_4^+\text{-N}$
232 concentration from 166-666 mg N/L to 1000-2000 mg N/L, then the SO_4^{2-} removal efficiency
233 increased from 64% to 71%. However, after increasing the influent $\text{NH}_4^+\text{-N}$ concentration
234 further to >3000 mg/L, the SO_4^{2-} reduction efficiency decreased to 28% (Wang et al., 2017).
235 Also, reducing the concentration of SO_4^{2-} from 223 to 154 mg/L had a positive effect on the
236 removal of SO_4^{2-} in the sulfamox process (Zhang et al., 2020). The N/S ratio also influenced
237 the SO_4^{2-} removal efficiency, as the SO_4^{2-} removal efficiency at N/S = 2:1 and 4:1 was 38.8%
238 and 30.5%, respectively (Zhang et al., 2019a).


239 ***3.4. Optimal conditions for S-dependent autotrophic denitrification, heterotrophic sulfate*** 240 ***reduction and the sulfamox process***

241 Figure 2 shows a summary of the reported pH and temperature ranges and their optimal
242 values for the three S-dependent processes. The overall optimum conditions are explicitly
243 determined by the narrowest ranges for heterotrophic sulfate reduction, which are 7-7.6 and
244 28-30°C for pH and temperature, respectively. The processes of S-dependent autotrophic
245 denitrification, heterotrophic sulfate reduction and sulfamox can occur simultaneously with
246 deammonification or its component processes, i.e. partial nitrification and anammox.



247

248

249 Figure 2. Ranges of pH and temperatures and their optimal values (“[

251

252 For comparison, for partial nitrification, the optimal ranges were 25-35°C for
 253 temperature (Zhu et al. 2008, Kanders et al., 2014) and 7-8.6 for pH, with the optimal value of
 254 8 (Jaroszyński et al., 2011). On the contrary, too low temperatures (10 - 15°C) cause the
 255 excessive activity of NOB (Kouba et al. 2017), which can grow faster than AOB under such
 256 conditions (Hellinga et al. 1998). The optimal pH range for NOB is 6 - 7.5, with the
 257 maximum at 7 (Yin et al., 2016). For the anammox process, the optimal temperature and pH
 258 is respectively 35-40°C (Dosta et al., 2008) and 6.7-8.3 (Jetten et al., 2001). The
 259 recommended ranges for efficient deammonification are as follows: T = 20 - 35°C (Kanders
 260 et al. 2014) and pH of 7.5-8 (Oshiki et al., 2011).

261 When coupling sulfamox with S-dependent autotrophic denitrification and heterotrophic
 262 SO₄²⁻ reduction to increase the efficiency of S removal, it is important to keep the optimal
 263 temperature of 28-30°C and pH of 7-7.6. The N/S ratio should be adjusted based on the
 264 stoichiometry of all the processes involved, so that products of one process can be the
 265 substrates for another process. Deviations from the optimal ratio can cause either production

266 of unwanted residues or bacterial competition for the substrates. SRB can compete with
267 sulfamox bacteria for SO_4^{2-} . Moreover, heterotrophic SO_4^{2-} reduction and sulfamox
268 contribute to formation of S^{2-} and/or S^0 , which is the substrate for S-dependent autotrophic
269 denitrification. Too intensive production of S^{2-} may lead to the persistence of this toxic
270 compound in the effluent. The presence of carbon in heterotrophic SO_4^{2-} reduction may also
271 contribute to the development of heterotrophic bacteria responsible for heterotrophic
272 denitrification. Then NO_3^- -N and/or NO_2^- -N may become limited due to their use in both
273 autotrophic and heterotrophic denitrification. In such a case, it is recommended to use full or
274 partial nitrification to produce NO_3^- -N and/or NO_2^- -N. The competition and interactions of
275 microorganisms participating in the aforementioned processes are shown in Figure 1.

276 **4. Wastewater treatment systems integrating the N-S-C cycles**

277 ***4.1. Systems integrating the sulfur cycle with nitrification-denitrification - Sulfate reduction,*** 278 ***Autotrophic denitrification and Nitrification Integrated (SANI) and its modifications***

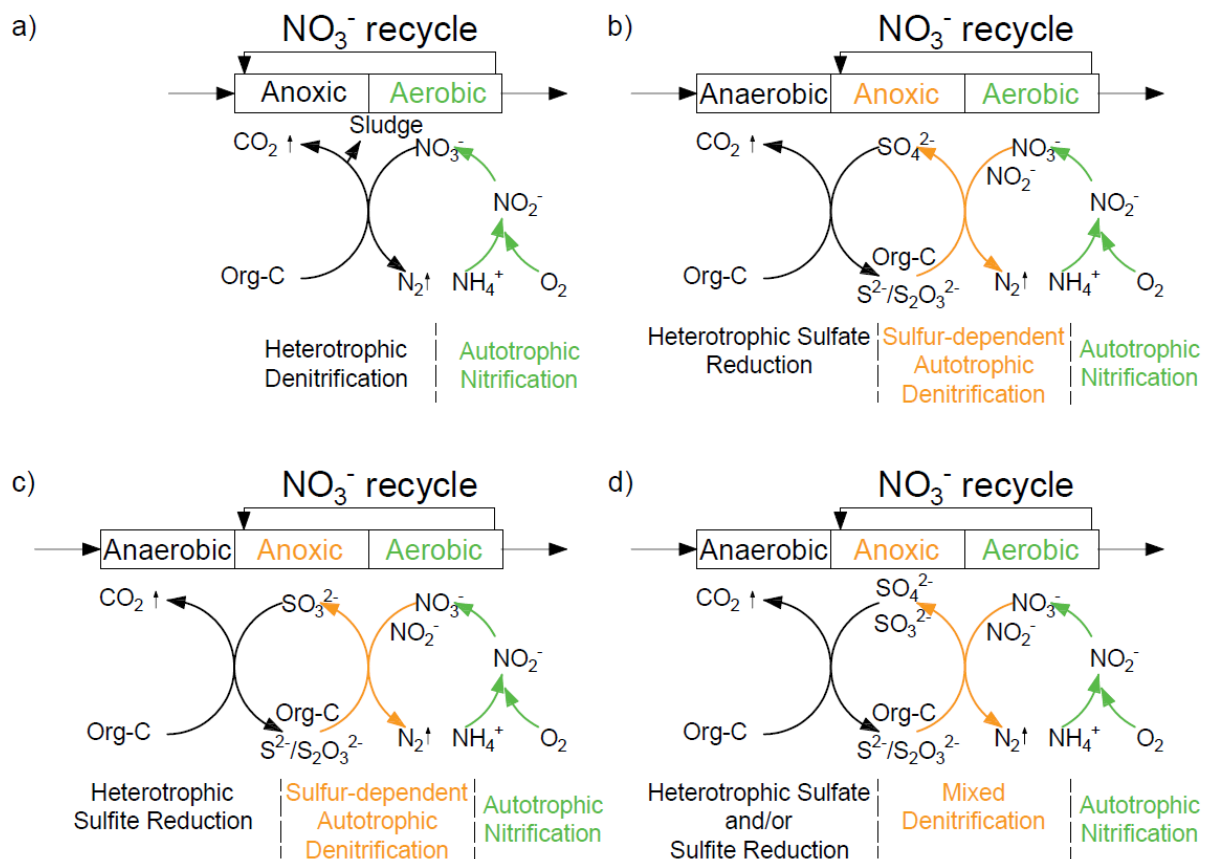
279 Biological SO_4^{2-} reduction along with biological oxidation of S in the form of SO_3^{2-} , S^0 or
280 $\text{S}_2\text{O}_3^{2-}$ are two main pathways responsible for S conversions in wastewater treatment systems
281 (Cardoso et al., 2006). An integrated process for SO_4^{2-} reduction, autotrophic denitrification
282 and nitrification (SANI) was aimed to primarily remove organic compounds and N (Wang et
283 al., 2009b). This process was originally developed for saline wastewater in Hong Kong and
284 demonstrated there in full-scale (Wu et al., 2016, Wang et al., 2009b).

285 With that innovative approach, the conventional wastewater treatment, incorporating
286 C and N cycles, can be extended with the S cycle, as shown in Figure 3. In the first anaerobic
287 zone, COD is removed by SRB, which results in SO_4^{2-} reduction to S^{2-} . In the second anoxic
288 zone, autotrophic reduction of NO_3^- -N occurs with dissolved S^{2-} formed in the first zone. In
289 the third aerobic zone, NH_4^+ -N is oxidized to NO_3^- -N, which is then recirculated to the second
290 anoxic zone (Wang et al., 2009b). The SANI process and its modifications combine the

291 advantages of energy saving, reduced sludge production and smaller footprint. Wang et al.
292 (2009b) noted that the total cost reduction for SANI would be >50% for a WWTP with an
293 influent flow rate of 10,000 m³/d.

294 The SANI process can be used for treatment of SO₄²⁻-poor wastewater provided that
295 low-cost and S-rich sources are available. For example, wet flue gas desulfurisation (FGD)
296 systems used in boilers, coal-fired furnaces and power plants, can be reduced to alkaline flue
297 gas sorption for production of liquid waste containing SO₄²⁻ and SO₃²⁻ (Srivastava and
298 Jozewicz, 2001). Such a waste stream can be co-treated in the main wastewater stream in wet
299 FGD-SANI after removing suspended solids and heavy metals (Qian et al., 2013).

300 The Mixed Denitrification (MD) - SANI process has also been proposed (Qian
301 2015a,b,c). MD-SANI generates S₂O₃²⁻, S²⁻, and some volatile fatty acids (VFA), which are
302 subsequently converted in both heterotrophic denitrification (VFA) and autotrophic
303 denitrification (S²⁻ and S₂O₃²⁻) (Qian et al., 2015a). It should be noted that the latter process is
304 induced faster by S₂O₃²⁻ than S²⁻ (Cardoso et al., 2006). Figure 3b-d shows the SANI, FGD-
305 SANI and MD-SANI processes depending on the available substrates.



306

307 Figure 3. Biological wastewater treatment systems using a) conventional heterotrophic

308 denitrification with autotrophic nitrification b) SANI c) FGD-SANI d) MD-SANI

309

310 4.2. Systems integrating the S cycle with anammox-based nitrogen removal processes

311 In recent years, the growing attention has been paid to N removal using the anammox process.

312 The anammox process completely eliminates the need for organic C source, reduces the

313 amount of sludge produced by 80% and related energy costs for aeration by 60% compared to

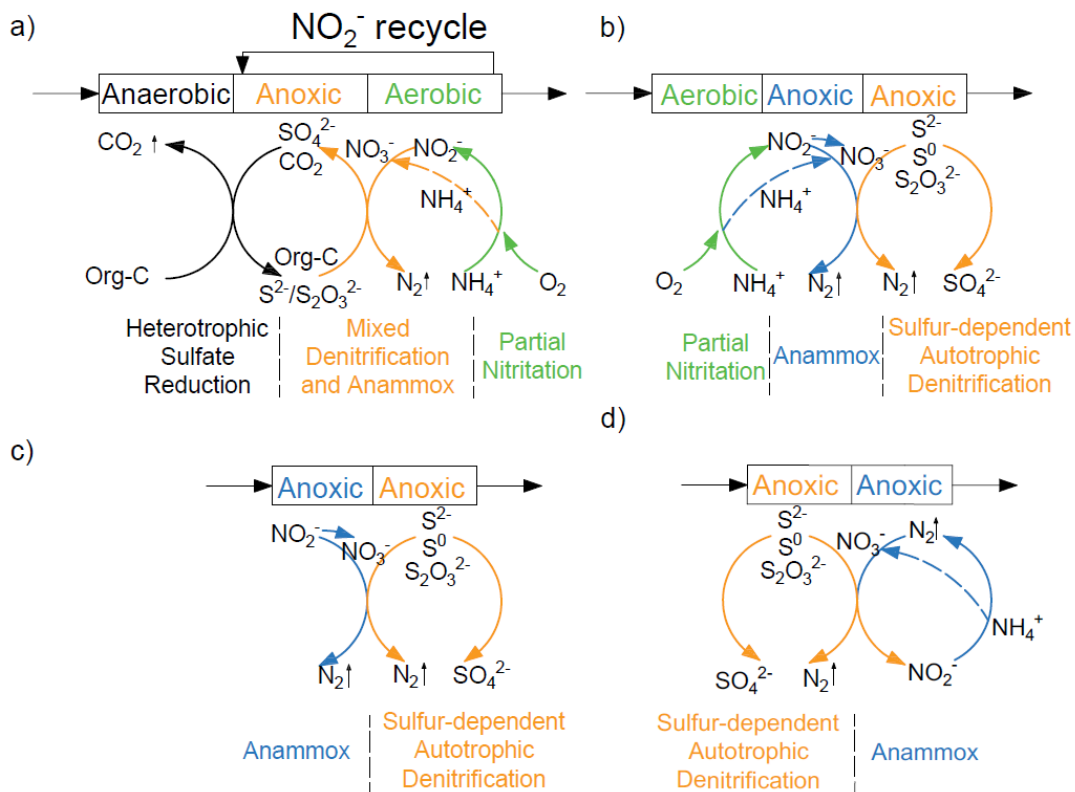
314 conventional nitrification/denitrification. The anammox process also has economic

315 advantages in the context of co-treatment of wastewater containing S compounds, especially

316 S^{2-} (Kosugi et al., 2019).

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

322 The SRDAPN process is similar to the SANI process, but enhanced with anammox
 323 (Figure 4a). As a consequence, instead of full nitrification, only PN is needed to produce NO_2^-
 324 $-\text{N}$ (Kosugi et al., 2019).



325
 326 Figure 4. Wastewater treatment systems using the anammox process a) SRDAPN b) PNASD
 327 c) ASD d) SPDA
 328

329 The PNASD process uses PN/A to remove NH_4^+ -N under aerobic (PN) – anoxic
330 (anammox) conditions. With S-dependent autotrophic denitrification, the produced NO_3^- -N
331 can further be reduced to N_2 , as shown in Figure 4b. The PNASD process has been
332 implemented as both two-stage (Dasgupta et al., 2017) and one-stage system (Yuan et al.,
333 2020).

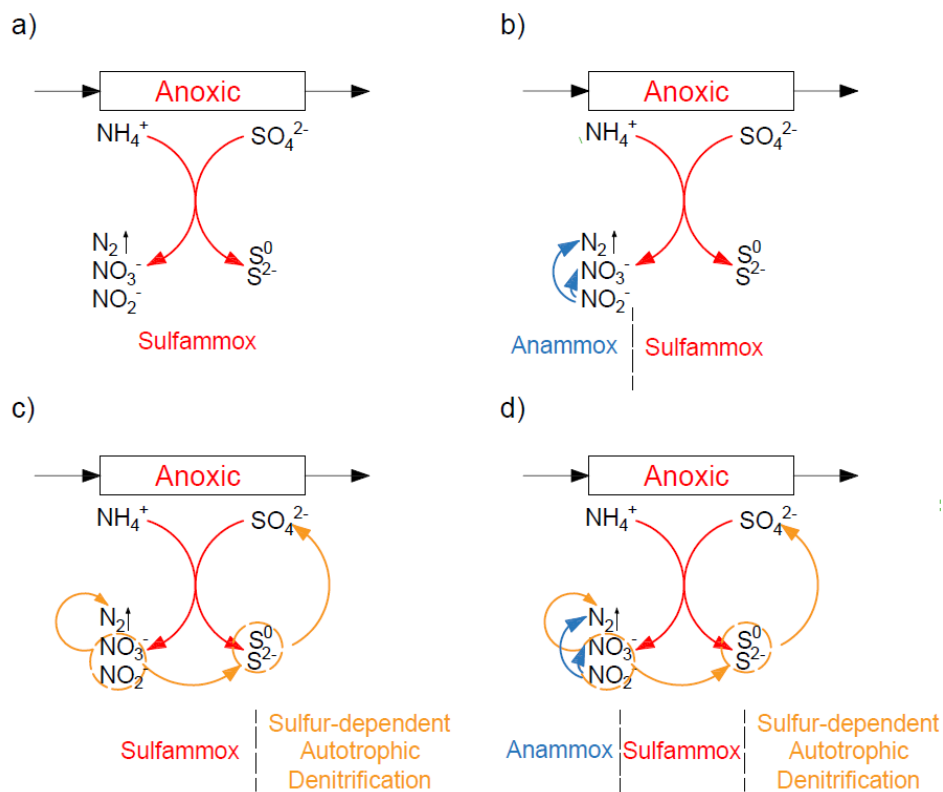
334 The PNASD system can also be limited to an ASD system that ignores the share of
335 PN, as shown in Figure 4c. Then the NO_2^- -N acceptor for anammox is not obtained from the
336 conversion of NH_4^+ -N, but supplied from external sources. Accordingly, the costs of energy
337 used to produce NO_2^-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

341 If NO_2^- -N can be obtained by partial autotrophic denitrification of NO_3^- -N with
342 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
343 process. Liu et al. (2017) and Wu et al. (2019) used a UASB reactor to perform S-dependent
344 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
345 from wastewater, as shown in Figure 4d.

346 ***4.3. Systems including the sulfammox process***

347 Both sulfammox and anammox incorporate “anaerobic” oxidation of NH_4^+ -N. The
348 coexistence of both processes was found in marine sediments (Rios-Del Toro et al., 2018) and
349 anaerobic sludge (Rikmann et al., 2016). In conventional sulfammox, SO_4^{2-} is an electron
350 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^- -
351 N. Sulfammox may occur on its own, as shown in Figure 5a. Alternatively, the formed NO_2^- -

352 N may be used as an electron acceptor for anammox in the combined Sulfamnox/Anammox
 353 (SA) system (Figure 5b).



354
 355 Figure 5. Wastewater treatment systems incorporating the sulfamnox process a) Sulfamnox
 356 b) SA c) SSD d) SASD
 357

358 As NO_2^- -N and NO_3^- -N are generated in sulfamnox, the process can be combined with
 359 autotrophic S-dependent denitrification in an Sulfamnox - S-dependent autotrophic
 360 Denitrification (SSD) system, as shown in Figure 5c (Liu et al., 2021, Grubba et al., 2021).
 361 The formed S^0 and S^{2-} in sulfamnox can be oxidized again to SO_4^{2-} , while NO_x -N are
 362 reduced to N_2 . The SSD system can be expanded with anammox in SASD (Sulfamnox –
 363 Anammox - S-dependent autotrophic denitrification), as shown in Figure 5d. In this case,

364 NO₂⁻-N can be reduced by both AAOB and autotrophic denitrifiers (Liu et al., 2021, Grubba
365 et al., 2021).

366 **5. Operational conditions and performances of the systems integrating the N-S-C** 367 **cycles**

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

370 **5.1. SANI, FGD-SANI, MD-SANI**

371 The S cycle, which is part of the SANI process, ensures a more efficient use of electrons (Wu
372 et al., 2020) and eliminates the production of toxic S²⁻ (Qian et al., 2015c). In addition, it
373 reduces sludge production by 90% compared to the conventional biological N removal
374 processes. This is possible due to very low yield coefficients of the microorganisms
375 responsible for SO₄²⁻ reduction, autotrophic denitrification and nitrification, i.e., 0.02 kg
376 VSS/kg COD, 0.01 kg VSS/kg NO₃⁻-N and 0.07 kg VSS/kg NH₄⁺-N, respectively (Lu et al.,
377 2011, Wang et al., 2009b). In addition, there are other significant reductions, including energy
378 consumption by 35% (Lu et al., 2011), greenhouse gas emission (GHG) by 36% (Lu et al.,
379 2011), and the space required for the process of wastewater treatment and sludge handling by
380 30%–40% (Liu et al., 2016).

381 As shown in Table 4, SANI shows a relatively high level of performance compared to
382 the conventional systems. The efficiencies of SO₄²⁻, total nitrogen (TN) and COD removal
383 vary in the ranges of 72-98%, 55-74% and 82-97%, respectively (Hao et al., 2015, Lu et al.,
384 2009). The SANI modifications (FGD-SANI and MD-SANI), which use wastewater streams
385 from wet flue gas desulphurization, reveal even a greater performance potential (Qian et al.,
386 2015a, b, Jiang et al., 2013). The biological reduction of SO₃²⁻ in FGD-SANI and MD-SANI
387 provides more energy for bacterial growth, which is associated with a higher sludge efficiency

388 compared to the biological reduction of SO_4^{2-} (Jiang et al., 2013). Moreover, SO_3^{2-} is an
389 intermediate in SO_4^{2-} reduction, which may result in faster reduction by SRB.

390 Jiang et al. (2013) found that the removal rates of specific organics in the SO_3^{2-} and
391 SO_4^{2-} reducing reactors were similar. At the extremely low temperatures ($<10^\circ\text{C}$), incomplete
392 reduction of SO_3^{2-} in an anaerobic reactor (Figure 3c) resulted in accumulation of $\text{S}_2\text{O}_3^{2-}$ and
393 reduction in the removal rate of organics. However, the anoxic and aerobic reactors (Figure
394 3c) still provided a high removal efficiency of organics ($>94\%$), while $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$
395 were almost completely removed.

396 The MD reaction can lead to a much higher reduction of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$
397 compared to the S^{2-} based SANI process (Qian et al., 2015a). Qian et al. (2015b) reported that
398 the denitrification rate increased sevenfold in MD-SANI compared to SANI. Furthermore, in
399 comparison with SANI, FGD-SANI shows higher TN and COD removal efficiencies (98%
400 and 94%). The complete removal of SO_3^{2-} and TN was achieved in MD-SANI, while the
401 COD removal efficiency in that process was 81% (Table 4).

402 ***5.2. SRDAPN and PNASD - challenges resulting from the combination of aerobic and*** 403 ***anaerobic conditions***

404 The presence of S^{2-} in the influent wastewater imposes a significant risk of inhibition of the
405 AAOB responsible for anammox. Threshold levels of S^{2-} inhibiting AAOB were found in the
406 range of <1 to 64 mg S/l (Jin et al., 2013, Carvajal-Arroyo et al., 2013, Dapena-Mora et al.,
407 2007). The study by Wiśniewski et al. (2019) determined the half maximal inhibitory
408 concentration (IC_{50}) under two different S^{2-} conditions. The IC_{50} was 4.25 mg $\text{H}_2\text{S-S/L}$ at a
409 constant S^{2-} concentration of 11 mg TS-S/L and pH in the range $7-7.9$ vs. 4.67 mg $\text{H}_2\text{S-S/L}$ at
410 a varying concentration of S^{2-} ranging from 1 to 15 mg TS-S/L and a constant pH of 7 . The
411 decrease in AAOB activity was due to the pH-dependent non-ionized form of H_2S . In

412 addition, heterotrophic bacteria may coexistence with AAOB but also outcompete AAOB at
413 high influent C/N ratios (Chamchoi et al., 2008).

414 The PNASD process has been implemented in both one- and two-stage systems. The
415 two stage-systems are easier to maintain and allow to avoid the negative impact of S^{2-} on
416 AAOB and the competition between AOB and SOB for DO (Sahinkaya and Kilic, 2014a).

417 Zhang et al. (2020) used S^0 for denitrification and observed only a small effect, when
418 DO was kept at the level of 0.4-0.8 mg/L. When the DO concentration increased to 1.2 mg
419 O_2/L , the concentrations of NO_3^- -N and SO_4^{2-} also increased. This indicates excessive
420 oxidation of S^{2-} or its reduced compounds in aerobic systems. Under non-limited DO
421 conditions, autotrophic SOB can readily utilize oxygen, which leads to accumulation of SO_4^{2-} .
422 On the other hand, too low DO concentrations in the PNASD process can reduce the NO_2^- -N
423 production rate in PN.

424 **5.3. ASD, SPDA and sulfammox systems - coexistence of AAOB and denitrifiers**

425 Under anaerobic conditions, the combination of anammox process and S-dependent
426 autotrophic denitrification can work with high removal efficiencies of TN (88-96%) and S
427 (90-100%) (Table 4). AAOB and *T. denitrificans* can assist in the combined N and S removal
428 without inhibition by S^{2-} (Guo et al., 2016). In that study, most of S^{2-} was oxidized to S^0 at the
429 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
430 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
431 S/N ratio >1) (Cardoso et al., 2006).

432 When NO_2^- -N is fed to the anammox process, S-dependent autotrophic denitrification
433 may occur. When both NO_2^- -N (anammox substrate) and NO_3^- -N (anammox product) are
434 simultaneously present in the influent, the latter form is the preferred electron acceptor for

435 denitrification (Guo et al., 2016). However, a small portion of NO_2^- -N can also be used by T.
436 *denitrificans* and increase the overall efficiency of N and S removal.

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

442 The novel sulfamnox process has been applied in SO_4^{2-} and NH_4^+ -N-rich wastewater
443 treatment systems. One of the intermediates in the sulfamnox reaction is NO_2^- -N, which can
444 be used by either AAOB or S-dependent autotrophic denitrification along with the residual
445 NO_3^- -N from anammox. Wu et al. (2020) combined sulfamnox and anammox and obtained
446 high removal efficiencies of NH_4^+ -N (98.5%) and SO_4^{2-} (53%). Furthermore, the sulfamnox
447 and anammox processes can also be combined with S-dependent autotrophic denitrification
448 (Rios-Del Toro et al., 2018).

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

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

454 **6.1. Artificial neural networks (ANNs)**

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



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

464 **6.2. Mechanistic models**

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

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

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

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

483 **7.1. Processes application opportunities**

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

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

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



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

517 Other technologies that include anammox and SANI processes have discovered the
518 advantages of AAOB coexisting with SRB, SOB, and AOB. In addition, compared to the
519 SANI process, the combination of SO_4^{2-} reduction, denitrification/anammox and partial
520 nitrification will further reduce aeration energy consumption due to the lack of full
521 nitrification required for $\text{NO}_3\text{-N}$ production. The presence of anammox in the SRDAPN
522 process resulted in an increased $\text{NO}_2\text{-N}$ removal efficiency by over 30% (Kosugi et al.,
523 2019).

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

529 Instead of combining the heterotrophic SO_4^{2-} reduction with anammox, sulfammox
530 can replace or accompany both processes by using a SO_4^{2-} dependent AAOB. Recent studies



531 have proposed the use of sulfammox based on the combined reduction of $\text{NH}_4^+\text{-N}$ and SO_4^{2-} .
532 If SO_4^{2-} was reduced to S^{2-} or S^0 with organic compounds, this process would be replaced
533 with sulfammox, while eliminating the addition of external carbon. Another suggested
534 solution is to combine the sulfammox process with heterotrophic SO_4^{2-} reduction in order to
535 increase the reduction rate of SO_4^{2-} . Moreover, if sulfammox is used upstream of an S-
536 dependent autotrophic denitrification reactor, it contributes to oxidation of $\text{NH}_4^+\text{-N}$ to N_2
537 (which increases the overall efficiency of $\text{NH}_4^+\text{-N}$ removal) or $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ (which
538 can be used in S- dependent autotrophic denitrification). By combining sulfammox and
539 anammox, the efficiency of $\text{NH}_4^+\text{-N}$ removal and SO_4^{2-} reduction to S^0 can be simultaneously
540 increased (Liu et al., 2021, Grubba et al., 2021).

541 *7.2. Advantages and disadvantages of two cycles or three cycles in wastewater treatment*

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

544 **Advantages:**

- 545 1. Approximately 35% reduction in energy consumption and up to 90% reduction in sludge
546 production compared to full nitrification-denitrification.
- 547 2. Reduction or even no external carbon dosing for S-dependent autotrophic denitrification.
- 548 3. For the combined processes, almost complete N and S^{2-} removal and up to 75% efficiency
549 of SO_4^{2-} removal.
- 550 4. Products of one process used as the substrates for another process.
- 551 5. When replacing heterotrophic denitrification with S-dependent autotrophic denitrification,
552 carbon consumption is reduced by 100%. If heterotrophic SO_4^{2-} reduction is replaced by
553 sulfammox, carbon consumption is also reduced by 100%.
- 554 6. Removal of a few harmful compounds (NH_4^+ , NO_2^- , SO_4^{2-} , S^{2-}) in one system.
- 555 7. Approximately 30–40% reduction of volumes required for wastewater and sludge treatment
556 processes.
- 557 8. Reduction of GHG emissions by 36% compared to conventional nitrification-
558 denitrification.

559

560 **Disadvantages:**

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

568 **7.3. Processes application limitations**

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

572 An important factor that should be considered when implementing technologies
573 containing the S-dependent autotrophic denitrification process is the inhibition of this process
574 caused by S^{2-} (Cardoso et al., 2006) as well as NO_2^- -N, NO_3^- -N and free nitric acid (FNA)
575 (Cui et al., 2019). Even though S^0 is an inexpensive and non-toxic electron donor, but it
576 provides a low denitrification rate due to its low solubility. The use of smaller S granules with
577 a larger surface area improves the reaction efficiency, however it can cause low porosity and
578 clogging and fouling of the reactors due to small S grain size or cracking (Wu et al., 2021).
579 Moreover, as S^0 and H_2S reveal a much lower rate of NO_3^- -N reduction, mainly the use of
580 $S_2O_3^{2-}$ is recommended in the process. However, its natural content of wastewater is rather
581 limited due to its instability (Cui et al., 2019).

582 In the case of heterotrophic SO_4^{2-} reduction, the presence of DO, NO_3^- -N and NO_2^- -N
583 inhibits reduction of SO_4^{2-} and enhances oxidation of S^{2-} to S^0 or SO_4^{2-} (Mohanakrishnan et
584 al., 2009). Moreover, the activity of SRB is inhibited by heavy metals, including Pb and Cd

585 (Sinharoy and Pakshirajan, 2019b). The toxicity of heavy metals depends mainly on the type
586 of metal, responsible microorganisms, presence of other pollutants, and process conditions
587 (Mal et al., 2016). Therefore, the systems based on heterotrophic SO_4^{2-} reduction cannot be
588 used for wastewater rich in heavy metals. Moreover, a significant limitation is the limited
589 number of microorganisms that are able to carry out SO_4^{2-} reduction with the use of gaseous
590 substrates. Moreover, the low gas-liquid mass transfer also makes it difficult to scale-up the
591 process.

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

600 ***7.4. Processes application challenges***

601 Using specific N, S and C removal processes independently of each other is much easier to
602 maintain than the processes combining these cycles. To link those processes in the combined
603 technologies as presented in this review, it is important to recognize the effects of S^{2-} on N
604 removal processes, such as autotrophic/heterotrophic denitrification and anammox, as well as
605 the competition between AOB and SOB for DO. S^{2-} and organic matter, which are fed to an
606 anaerobic compartment, can inhibit AAOB in anammox-coupled systems (Kosugi et al.,
607 2019). Chen et al. (2018) showed that DO can react with S^{2-} while reducing the NO_3^- -N
608 removal rate. In addition, S^{2-} was reduced to S^0 and then converted to SO_4^{2-g} due to the

609 presence of DO. These findings highlight the challenges faced by single-stage integrated
610 systems.

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

619 In the case of sulfamox, more research is needed to identify potential applications
620 and integration with other systems. The key enzymes involved in the metabolism of $\text{NH}_4^+\text{-N}$
621 and SO_4^{2-} should also be investigated. For this purpose, it is important to develop appropriate
622 reactor configurations and create operational conditions that can enrich functional bacteria
623 and allow for simultaneous removal of $\text{NH}_4^+\text{-N}$ and SO_4^{2-} . Under non-limited $\text{NO}_3^-\text{-N}$
624 conditions, the SO_4^{2-} concentration may increase due to S-dependent autotrophic
625 denitrification. The role of organic matter also requires further investigation with regard to the
626 existence of the sulfamox process.

627 The combination of anammox, S-dependent autotrophic denitrification and sulfamox
628 processes is challenging due to the different requirements of the microorganisms responsible
629 for each process. The S-dependent autotrophic denitrification process may result in the
630 production of SO_4^{2-} from S^{2-} or S^0 , which negatively affects sulfamox, where SO_4^{2-} must be
631 reduced to S^0 (Liu et al., 2021). More focused research on the coexistence of sulfamox with
632 other bacteria and the development of a mechanistic model are needed to better understand
633 and predict N and S dynamics. Moreover, the S/N ratio also plays an important role in



634 determining the S-dependent autotrophic denitrification end products, requiring a closer look
635 at the N and S dynamics. On the other hand, in order to avoid fouling and clogging of the
636 reactors due to the presence of S^0 , it is important to search for the appropriate sulfur grain
637 size.

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

644 **8. Conclusions**

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

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

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

658 heterotrophic denitrifiers, development of a mechanistic model and appropriate control
659 strategy becomes challenging.

660 **Declaration of Competing Interest:** The authors declare that they have no known competing
661 financial interests or personal relationships that could have appeared to influence the work
662 reported in this paper.

663 **Funding:** This work was supported by the Narodowe Centrum Nauki (National Science
664 Center) under Grant [2020/37/N/ST8/02455].

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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	<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> 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



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^- -N 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

^akg/m³/d

^b g/g N_{removal}



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-}	SO_4^{2-} -removal efficiency or rate	References
		(°C)		concentration (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., 2019a
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
	Crab shell				721–738	
Batch	Potato	30	7	764–766	22–34 mg SO_4^{2-} /gVSS/d	Reyes-Alvarado et al., 2017
	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	Nevatalo et al., 2010
	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
	Ethanol, spent manure				961–1345 mg SO_4^{2-} /L/d	
Anaerobic filter	Methanol, spent manure	6	2.5–4.3	900	1057–1441 mg SO_4^{2-} /L/d	Tsukamoto et al., 2004
SULFAMMOX						
Upflow anaerobic sludge bed reactor	Ammonium nitrogen	35	7.9–8.3	80	8.18 mg S/L/d	Qin et al., 2021
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%	
				90	approx. 30%	
Self-designed circulating flow reactor	Ammonium nitrogen	30	8.1-8.6	170	approx. 30%	Zhang et al., 2019b
				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

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
SANI	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 desulfurization - 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 up-flow 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		~100% S ²⁻	84% (TN)	-	Yuan et al., 2020



	Single reactor under main-stream conditions				
Anammox and S-dependent autotrophic Denitrification (ASD)	Expanded granular sludge bed	90-100% $S_2O_3^{2-}$	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% $S_2O_3^{2-}$	>90% (TN)	-	Wu et al., 2019
SPDA	Up-flow anaerobic sludge blanket reactor	70% S^0	90% (NO_2^- -N)	-	Liu et al., 2017
Sulfamnox/Anammox (SA) with COD	Moving Bed Biofilm Reactor	10% SO_4^{2-}	30% (NH_4^+ -N)	-	Rikmann et al., 2016



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⁻ -S/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⁻ → S ₀ → 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.

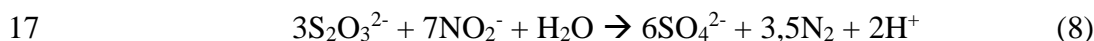
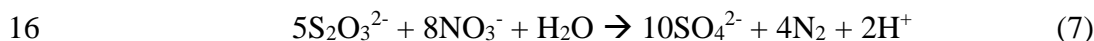
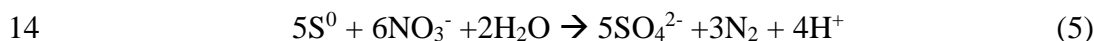
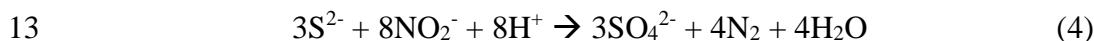
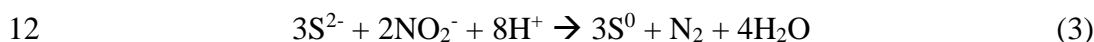
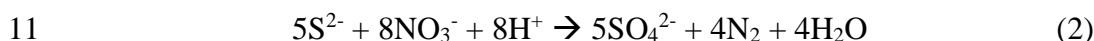


1 **Supporting information (SI)**

2 *Sulfur-dependent autotrophic denitrification (SI)*

3 *Mechanism of the process*

4 The process of autotrophic sulfur (S)-dependent denitrification is used for the treatment of
5 domestic and industrial wastewater (Shao et al., 2010), landfill leachate (Koenig and Liu,
6 1996), groundwater (Wan et al., 2009) and salt water (Wang et al., 2009). Process reactions
7 are illustrated by the equations 1-8 (Huang et al., 2019, Cui et al., 2019, Lin et al., 2018,
8 Pokorna and Zabranska, 2015, Sun and Nemati 2012):



18 Compared to heterotrophic denitrification, this process has many advantages, such as
19 no addition of organic substrate, reduction of biomass (the biomass yield coefficient is 0,15-
20 0,57 g for autotrophic biomass production, and 0,71–1,2 g for heterotrophic biomass
21 production per 1 g of denitrified NO_3^- -N and NO_2^- -N) and a decrease in N_2O emissions
22 (Huang et al., 2019, Sun and Nemati, 2012, Yang et al., 2016).

23 Thus, the process of autotrophic S-dependent denitrification can be used successfully
24 in major carbon-deficient wastewater treatment systems (Cui et al., 2019). This also explains
25 the use of this process for biogas desulfurization, cleaning of crude oil tanks before
26 acidification, and anti-corrosion treatment of sewage systems (Qian et al., 2015, Park et al.,
27 2014). To date, most of the research done on the S-dependent autotrophic denitrification
28 process has been carried out with flocculent sludge (Cui et al., 2019).

29 Zhou et al. (2016) indicated that $S_2O_3^{2-}$ is more efficient in S-dependent autotrophic
30 denitrification than S^0 or S^{2-} . Similarly, Park et al. (2015) obtained NO_3^- -N removal
31 efficiencies of 96.5% for $S_2O_3^{2-}$, 64.1% for S^{2-} , 58.1% for persulfide (pyrite) and 38.8% for
32 S^0 . Thus, it has frequently been shown that $S_2O_3^{2-}$ is a suitable S source for the described
33 process. Cardoso et al. (2006) found that the specific rate of nitrate reduction for $S_2O_3^{2-}$ was
34 4.6 and 9.5 times higher than for H_2S and S^0 , respectively.

35 When S compounds are transformed, intermediate compounds can also be involved in
36 the process. An example is participation of S^0 , resulting from oxidation of S^{2-} , in S-dependent
37 autotrophic denitrification (Xu et al., 2016). Fan et al. (2021) described the effect of
38 intermediates, such as S^{2-} , acid volatile sulfide (AVS), and S^0 , in autotrophic denitrification
39 with $S_2O_3^{2-}$ as an electron acceptor. When S^{2-} , $S_2O_3^{2-}$, AVS, and S^0 coexisted in the
40 autotrophic process of denitrification, their preferences were as follows: $S^{2-} > S_2O_3^{2-} > AVS \approx$
41 S^0 .

42 *Environmental factors influencing the process performance*

43 *Temperature*

44 The optimal temperature for most SOB remains under mesophilic conditions, i.e. in the range
45 of 25-35°C (Fajardo et al., 2014, Shao et al., 2010). When the temperature dropped from 20-
46 25°C to 5-10°C, the efficiency of N removal decreased from about 99% to 50% (Zhou et al.,
47 2011). However, there were also studies showing the denitrification rate of approximately 3.3



48 kg N/m³·d at 3°C in a fluidized-bed reactor with the HRT of 1h during 98 days (Di Capua et
49 al., 2017).

50 *pH*

51 The optimal pH for this process has been shown to be in the range of 6.8-8.2 and a decrease in
52 the activity has been reported at pH <6.5 and >9 (Chen et al., 2016a, Vidal et al., 2002). The
53 optimal pH values for reduction of NO₂-N and NO₃-N were 7.0 and 8.5, respectively, using S⁰
54 as an electron donor (Chen et al., 2018).

55 *Previous research*

56 Jing et al. (2010) performed the S-dependent autotrophic denitrification process with S²⁻ as an
57 electron donor in a 1.3 L up-flow continuous reactor fed with synthetic wastewater. When the
58 NO₃⁻-N concentration increased from 30.4 mg N/L to 169.6 mg N/L, the volumetric NO₃⁻-N
59 removal rate increased from 0.15 kg N/m³/d to 0.61 kg N/m³/d. However, when the NO₃⁻-N
60 concentration increased again to 189.7 mg N/L, the volumetric NO₃⁻-N removal rate
61 decreased to 0.59 kg N/m³/d. On the contrary, when the influent concentration of S²⁻
62 increased from 160 mg/L to 1000 mg/L, the volumetric S²⁻ removal rate increased from 0.78
63 kg/m³/d to 4.57 kg/m³/d and the efficiency of S²⁻ removal was >90%.

64 Similarly, in the experiments of Kim et al. (2004) with two FBRs, the effect of NO₃⁻-N
65 concentration on the process was explicitly observed. The feed of FBR-1 was prepared from
66 treated domestic wastewater, while pre-treated leachate from a municipal landfill was used in
67 FBR-2. FBR-1 was operated for 358 days with NO₃⁻-N of 20 mg N/L, and FBR-2 for 342
68 days with NO₃⁻-N of 700 mg N/L. The maximum denitrification rates in FBR-1 and FBR-2
69 were respectively 2.53 kg N/m³/d (denitrification efficiency 91.7%) and 3.37 kg N/m³/d
70 (denitrification efficiency 83%).

71 Zou et al. (2016) investigated the S-dependent autotrophic denitrification process in
72 two FBRs operated at different temperatures, i.e. 20 and 30°C, for 200 days. Oxidation of
73 $S_2O_3^{2-}$ was particularly unstable until day 54, and then $S_2O_3^{2-}$ remained below the detection
74 limit in both FBRs, and SO_4^{2-} concentration increased sharply to approximately 3700 and
75 3200 mg/L on days 38 and 33 in FBR-1 and FBR-2, respectively. The temperature had no
76 significant effect in the course of the process and the denitrification rate remained at the level
77 of 1.24-3.25 kg N/m³/d. Autotrophic denitrification and denitrification with $S_2O_3^{2-}$ as electron
78 donors were thus effectively maintained in the two FBRs.

79 In the study by Sahinkaya and Dursun (2015), the influent NO_3^- -N concentration
80 increased from 25 to 75 mg N/L in a FBR with S^0 as an electron donor. The start-up period
81 was relatively short as almost complete NO_3^- -N and NO_2^- -N reduction was achieved within
82 one week. However, the process efficiency decreased during the long-term operation, which
83 resulted in an increase in NO_3^- -N and NO_2^- -N concentrations. The denitrification rate ranged
84 from 0.07 to 0.2 kg N/m³/d. SO_4^{2-} was produced and reached the level of 100-600 mg S/L in
85 accordance with the process stoichiometry.

86 Moraes et al. (2012) investigated S-dependent autotrophic denitrification involving
87 two electron acceptors – NO_3^- -N and NO_2^- -N in the absence of S^{2-} , in excess of S^{2-} and
88 according to the stoichiometric equation of the S-dependent autotrophic denitrification
89 process in vertical FBRs. The results showed that sulfur intermediate compounds (S^0) were
90 mainly formed when excess of S^{2-} was used, especially for NO_3^- -N. Moreover, NO_2^- -N was
91 more readily consumed than NO_3^- -N, and higher concentrations of S^{2-} led to greater formation
92 of S intermediates. The observed NO_3^- -N removal efficiencies were 60-15%, 25.5-98.5% and
93 84%, respectively, in the absence of S^{2-} , in excess of S^{2-} and in concentration according to the
94 stoichiometric equation of the S-dependent autotrophic denitrification process.



95 In the study by Sahinkaya and Kilic (2014a), two parallel column bioreactors were
96 operated under autotrophic and heterotrophic conditions. For S-dependent autotrophic
97 denitrification, a packed bed reactor was used. The simultaneous removal of NO_3^- -N and
98 chromate (VI) was achieved under both autotrophic and heterotrophic conditions. Cr (VI)
99 concentrations up to 0.5 mg/L did not adversely affect the autotrophic denitrification
100 efficiency and higher concentrations reduced the denitrification potential of the column.
101 During the entire period, production of SO_4^{2-} ranged from 200 to 600 mg S/L and the
102 denitrification rate was 0.07-0.1 kg N/m³/d.

103 Sahinkaya et al. (2014b) performed S-dependent autotrophic denitrification under the
104 temperature falling from 26 to 10°C. Three identical pilot-scale column bioreactors were
105 installed, different S to limestone ratios (1/1–3/1) were used and the results were compared
106 under different loading conditions during the long-term operation. Complete denitrification
107 was achieved until the NO_3^- -N loading was 10 mg N/L/h. When the temperature dropped to
108 10°C in winter at the load of 18 mg N/L/h, the denitrification efficiency decreased to 60-70%
109 and the bioreactor with the S/L ratio of 1/1 showed slightly better performance. Throughout
110 the study, the denitrification rate was 0.03-0.24 kg N/m³/d.

111 Yang et al. (2016) conducted S-dependent autotrophic denitrification in an up-flow
112 anaerobic sludge blanket reactor operated continuously for 600 days. The nitrogen removal
113 efficiency of 94% and complete removal of S^{2-} were achieved. The denitrification rate was
114 0.09-0.31 kg N/m³/d with the HRT of 5 h, and the influent NO_3^- -N and S^{2-} loads were 0.33
115 kg-N/m³/d and 0.62 kg S/m³/d, respectively.



116 *Sulfate reduction (S2)*

117 *Mechanism of the process*

118 Due to the formation of wastewater streams rich in SO_4^{2-} resulting from various anthropogenic
119 activities, there is a growing interest in the SO_4^{2-} reduction process using SRB. Although high
120 concentrations of SO_4^{2-} do not pose a direct threat to the environment and health, they disrupt
121 the natural S cycle. This imbalance can lead to H_2S formation, metal corrosion, and SO_x
122 emissions (Abel et al., 2015). H_2S is often undesirable in wastewater treatment, but SO_4^{2-}
123 reduction may be beneficial due to the use of S^{2-} for heavy metal removal by precipitation,
124 autotrophic denitrification or autotrophic phosphorus removal (Rubio -Rincón et al., 2017).

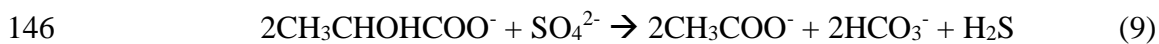
125 Although SO_4^{2-} is the main electron acceptor for SRB, they can also use S compounds
126 such as $\text{S}_2\text{O}_3^{2-}$, SO_3^{2-} , S^0 . When SO_4^{2-} is the electron acceptor, the process takes place in two
127 stages - first SO_4^{2-} is reduced to SO_3^{2-} and then to S^{2-} (Muyzer and Stams, 2008). When SO_4^{2-}
128 decomposes into S^{2-} , some of the decomposed S^{2-} leaves the reactor along with the biogas as
129 H_2S gas and the remaining H_2S present in the reactor as total dissolved sulfide (TDS). The
130 components of TDS in the aquatic environment are S^{2-} , HS^- and H_2S (aq) (Samarathunga and
131 Rathnasiri, 2019).

132 Organic electron donors are used for the biological reduction of SO_4^{2-} , which at the
133 same time provide a carbon source for the SRB, as well as inorganic ones that require
134 supplementation with a carbon source, e.g. CO_2 (Sinharoy et al., 2020a). Some SO_4^{2-} rich
135 wastewater also contains high concentrations of organic compounds that can be used by SRB
136 to reduce SO_4^{2-} . When they contain no organic compounds, compounds such as sugars
137 (glucose and sucrose) (Barber and Stuckey, 2000), alcohols (methanol and ethanol)
138 (Kaksonen et al., 2003), short-chain fatty acids (acetate, propionate, butyrate, lactate,
139 pyruvate, malate) (Kiran et al., 2017) and aromatics (benzoate, phenol) can support the
140 biological reduction of SO_4^{2-} (Liamleam and Annachatre, 2007).



141 Lactate, and especially sodium lactate, is the most common electron donor in the
142 biological reduction of SO_4^{2-} with SRB. It can be degraded by a wider range of SRB
143 compared to methanol. However, lactate is more expensive compared to methanol, ethanol or
144 acetate, making this solution on an industrial scale not economical.

145 Reduction of SO_4^{2-} with lactate (9) (Virpiranta et al., 2019):

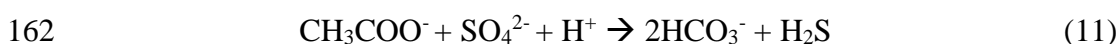
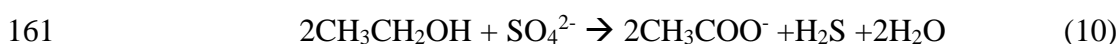


147 Alternative methanol is the cheapest carbon source and is therefore widely used as an
148 electron donor in biological processes. Compared to other carbon sources, its complete
149 oxidation to CO_2 is ensured, while e.g. lactate or ethanol are oxidized only to acetate (Rubio-
150 Rincón et al., 2017), which reduces treatment costs due to the smaller amount of carbon
151 source needed to reduce all SO_4^{2-} . However, it has been reported that methanogens compete
152 with SRB for methanol under mesophilic and thermophilic conditions (Kaksonen and
153 Puhakka, 2007). However, adjusting factors such as pH, temperature, S^{2-} concentration and
154 metal concentration can limit the growth of methanogens in the presence of methanol
155 (Tsukamoto et al., 2004).

156 Also, ethanol and succinate are widely used as a carbon and electron source as it is a
157 relatively economical option (Virpiranta et al., 2019).

158 Ethanol reduction occurs in two reactions (10-11), the latter of which is based on
159 acetate reduction (Virpiranta et al., 2019).

160 Reduction of SO_4^{2-} with ethanol (Virpiranta et al., 2019):

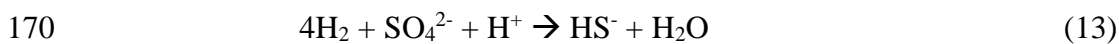


163 Reduction of SO_4^{2-} with succinate (12) (Virpiranta et al., 2019):



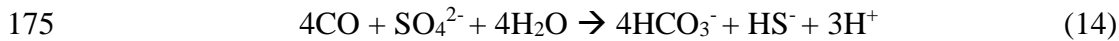
165 H_2 , CO and CH_4 can also be used as electron donors, as well as their mixtures, which
166 reduce the cost of the process. Van Houten et al. (1996) have indicated that H_2 is the best
167 electron donor for SO_4^{2-} reduction when working on a large scale. Often, however, CO_2 is
168 required to achieve high process efficiency.

169 Reduction of SO_4^{2-} with H_2 (13) (Sinharoy et al., 2020b):



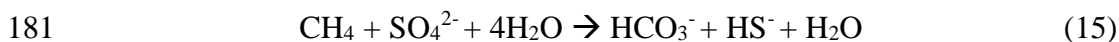
171 There are SRBs that use CO as a carbon and energy source, however, for most of
172 them, CO is toxic. However, this toxicity can be mitigated by the use of mixed culture
173 systems or the use of a layered structure of biomass (Sipma et al., 2006).

174 Reduction of SO_4^{2-} with CO (14) (Sinharoy et al., 2020b):



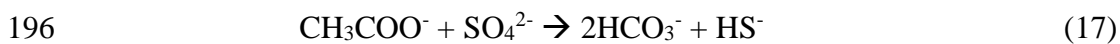
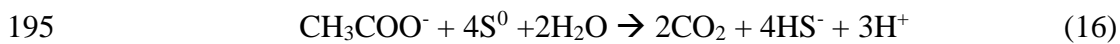
176 The biological reduction of SO_4^{2-} can also be combined with the anaerobic oxidation
177 of CH_4 . CH_4 can provide 4 electrons, which is twice as much as H_2 , so less gas volume is
178 required to achieve the same SO_4^{2-} reduction efficiency. However, CH_4 can only be used by a
179 limited number of microorganisms, which is a major disadvantage of this substrate.

180 Reduction of SO_4^{2-} with CH_4 (15) (Sinharoy et al., 2020b):



182 Electron donors were also investigated as manure, sawdust, wood shavings,
183 lignocellulosic materials (Reyes-Alvadro et al., 2018), agricultural residues (Chang et al.,
184 2000), sewage sludge, whey or dairy sewage (Wolicka, 2008) and organic sewage (Das et al.,
185 2013), which unfortunately cause secondary environmental pollution.

186 Sun et al. (2018) proposed a conceptual model for the reduction of SO_4^{2-} and S^0 in the
187 presence or absence of HS^- . When there is no HS^- in the system, the production of HS^- is
188 mainly through the reduction of SO_4^{2-} , while when HS^- is present, polysulfide is formed by
189 the reaction of S^0 with HS^- . As the concentration of HS^- increases, more polysulfide is
190 formed which stimulates indirect S^0 reduction. When HS^- exceeds a certain level, the indirect
191 reduction of S^0 with polysulfide plays a major role in the production of HS^- , and the reduction
192 of SO_4^{2-} is almost completely stopped. This is important from the point of view of the process
193 economy, as the reduction of S^0 requires $\frac{1}{4}$ of the organic matter needed to reduce SO_4^{2-} ,
194 according to the (16) and (17) reactions (Sun et al., 2018):



197 In a study by Sun et al. (2018), an effective S^0 reduction was observed in the
198 sulfidogenic bioreactor even at 1300 mg S/L of SO_4^{2-} in the inlet. 98.5% of HS^- was obtained
199 as a result of S^0 reduction, and SO_4^{2-} was barely consumed in the bioreactor.

200

201 *Environmental factors influencing the proces performance*

202 *Temperature*

203 SRBs are able to tolerate temperatures ranging from -5°C to 75°C and readily adapt to
204 temperature changes (Cocos et al., 2002). However, the optimal temperature for most SRBs is
205 in the narrow range of $28-30^\circ\text{C}$ (Virpiranta et al., 2019), although there are strains for which
206 the optimum temperature is below 20°C (Knoblauch et al., 1999). Most of the bacteria that
207 tolerate cold temperatures are mesophilic (rather than psychrophilic) strains that can grow
208 under such conditions, termed psychrotolerant bacteria or psychotrophs. For example, some
209 *Desulfobulbus* strains can grow at $6-10^\circ\text{C}$ (Kharrat et al., 2017, Virpiranta et al., 2019).

210 Studies have also been conducted in the range of even lower 4-8°C (Nielsen et al., 2018),
211 which, however, has extended the adaptation time to such extreme conditions. The presence
212 of SRB was revealed by the presence of the *Deltaproteobacteria* and *Clostridia*. The study
213 showed a change in the composition of SRB taxa over time - an increase in the relative
214 abundance of *Deltaproteobacteria* and a decrease in the relative abundance of members of the
215 *Clostridia*.

216 *pH*

217 Most of the studies on SO_4^{2-} reduction were carried out at pH in the range of 7-7.6, which is
218 close to the optimal value for SRB. Pagnanelli et al. (2012) found the optimal pH value of 7.6
219 for the SRB, while Bratkova et al. (2013) observed the maximum SO_4^{2-} reduction rate at pH
220 7.25.

221 *Previous research*

222 Sinharoy et al. (2019, 2020a) desulphurized wastewater containing SO_4^{2-} using carbon
223 monoxide in a moving bed biofilm reactor (2019) and in a gas lift reactor (2020a). The effect
224 of HRT on the process was investigated and it was found that at the HRT of 72, 48 and 24 h,
225 SO_4^{2-} removal was 93.5%, 91.9% and 80.1%, respectively, and CO use was 85% throughout
226 the study (Sinharoy et al., 2019). These results were improved in a subsequent study
227 (Sinharoy et al., 2020a). At the HRT of 72 h, the reduction of SO_4^{2-} and the use of CO were
228 97% and 89%, respectively (2020a). The best results in terms of SO_4^{2-} reduction (> 80%)
229 were obtained for low SO_4^{2-} loading and high CO loading conditions. The results for both
230 types of reactors were slightly better for the gas lift reactor.

231 Batch studies of Virpiranta et al. (2019) were performed in sealed vials and incubated
232 at 3 different temperatures of 6°C, 16°C and 22°C for cold acclimatization and
233 characterization of SRB consortia enriched from a sample of arctic sediments. Postgate

234 medium was supplemented with lactate, ethanol or succinic acid and the resulting consortia
235 grew with lactate and succinic acid, but not ethanol. The SO_4^{2-} reduction rates at 22°C were
236 169 mg/L/d , at 16°C - 98 mg/L/d , and for 6°C the rates ranged from 13 to 42 mg/L/d .
237 Temperature had a significant effect on the activity of SRB. However, it is useful to be able to
238 acclimatize SRB to low temperatures due to the versatile applicability of the process.

239 Nielsen et al. (2019) conducted batch tests to check various carbon sources for SO_4^{2-}
240 reduction. Simple organic carbon sources (methanol and ethylene glycol) and complex
241 organic carbon sources (potato oil, brewing residues, peat and straw) were used to support
242 SRB growth. After 162 days, all bioreactors showed a decrease in both total organic carbon
243 and SO_4^{2-} concentration at 5°C . However, a long acclimation period (98–112 days) was
244 required. The use of methanol and ethylene glycol resulted in SO_4^{2-} reduction by 71.2% and
245 36.9%, respectively. The decrease in SO_4^{2-} concentrations was limited to 13.8 and 5.3%,
246 respectively, when using peat and straw.

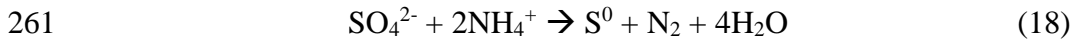
247 Reyes-Alvarado et al. (2017, 2018) used electron donors, such as potato, filter paper,
248 crab shell (2017) and natural scourer and cork (2018), in their research. Maintaining SO_4^{2-}
249 concentration close to $700\text{--}800 \text{ mg/L}$ in both studies, the following SO_4^{2-} removal rates were
250 obtained: 6-9, 22-34, 50-65, 34, $6.1 \text{ mg SO}_4^{2-}/\text{gVSS/d}$ for crab shell, potato, filter paper,
251 scourer, cork, respectively. It was shown that the natural properties of the carbohydrate-based
252 polymers limit the hydrolysis-fermentation step and thus the SO_4^{2-} reduction rate.

253 ***Sulfammox process (S3)***

254 *Mechanism of the process*

255 A novel sulfammox biological process has recently been described in which $\text{NH}_4^+\text{-N}$ is
256 oxidized to N_2 and SO_4^{2-} plays the role of an electron acceptor and is reduced to S^0 under
257 anaerobic conditions. Sulfammox was first reported by Fdz-Polanco et al. (2001b) in a

258 granular activated carbon anaerobic fluidized bed reactor treating vinasse from an ethanol
259 distillery of sugar beet molasses. The sulfammox process can be most generally represented
260 by the following equation (18):



262 In the follow-up studies, Liu et al. (2008) and Yang et al. (2009) identified SO_4^{2-} as a
263 potential electron acceptor as it was the feed component. The mechanism of the sulfammox
264 process is not fully understood yet - it turns out that there are exceptions to the described
265 process. In addition to N_2 and S^0 , sulfammox can lead to the formation of NO_2^- -N and NO_3^- -N
266 as well as S^{2-} . Moreover, there are two different assumptions about the actual course of the
267 sulfammox process. The first assumes that NH_4^+ -N is partially oxidized to NO_2^- -N with SO_4^{2-}
268 and some of the NO_2^- -N produced is reduced to N_2 by S^{2-} and then NO_2^- -N and NH_4^+ -N are
269 converted to N_2 . The second is that NH_4^+ -N is partially oxidized to NO_2^- -N by SO_4^{2-} and then
270 NH_4^+ -N is oxidized to N_2 by NO_2^- -N (Yang et al., 2009, Liu et al., 2008).

271 Even though COD is not required for the sulfammox process (Zhang et al., 2009), the
272 experiments were performed either without COD addition (Bi et al., 2020, Zhang et al.
273 2019a,b, Prachakittikul et al., 2016, Cai et al., 2010) or with COD addition (Wang et al.,
274 2017, Rikmann et al., 2016, Fdz-Polanco et al., 2001a,b). When COD is present in
275 wastewater, the sulfammox process can be coupled with subsequent heterotrophic
276 denitrification (Zhang et al. 2019b).

277 The differences in the NH_4^+ -N/ SO_4^{2-} ratio in the conducted studies with respect to the
278 stoichiometric ratio may result from the activity of ammonia oxidizing bacteria (AOB), which
279 oxidizes NH_4^+ -N, and the reduction of SO_4^{2-} by organic compounds, as described by Bi et al.
280 (2020). In this way, they questioned the presence of the sulfammox process, so it is important
281 to conduct more detailed research.



282 *Environmental factors influencing the process performance*

283 *Temperature*

284 The sulfammox process has been carried out at the temperatures in the range of 15-55°C
285 (Zhang et al., 2019a,b, Cai et al., 2010, Yang et al., 2009, Zhao et al., 2006) with the optimal
286 value in the range of 25-35°C (Cai et al., 2010). However, the sulfammox process could also
287 be maintained at lower temperatures, e.g. 20°C (Rikmann et al. 2016) and 14-15°C (Wu et al.
288 2020). In the latter case, the $\text{NH}_4^+\text{-N}$ and overall SO_4^{2-} removal efficiencies still remained
289 high, i.e. 98.5% and 52.8%, respectively.

290 *pH*

291 Sulfammox studies have mainly been carried out in the pH range of 7.5-8.6, specifically 7.5-
292 8.5 (Yang et al., 2009), 8-8.2 (Liu et al., 2008) 8.1-8.3 (Zhang et al., 2019a), 8.1-8.6 (Zhang et
293 al., 2019b) with the best value of 8.5 (Cai et al., 2010). The efficiency of $\text{NH}_4^+\text{-N}$ and SO_4^{2-}
294 removal decreased when the pH was < 7.5 and > 9.5 (Cai et al., 2010).

295 *Previous research*

296 Qin et al. (2021) conducted a sulfammox process in an upflow anaerobic sludge bed reactor
297 for more than one year. The influent $\text{NH}_4^+\text{-N}$ concentration was 70 mg N/L and the NO_2^- :
298 NO_3^- : SO_4^{2-} molar ratio were 1:0.2:0.2, 0.5: 0.1: 0.3 and 0: 0:0.5, respectively, in stages I, II
299 and III. The $\text{NH}_4^+\text{-N}$ and SO_4^{2-} removal rates were 31 mg N/L/d and 8.18 mg S/L/d,
300 respectively. The excessive conversion of $\text{NH}_4^+\text{-N}$ in stage III was mainly attributed to the
301 sulfammox reaction due to the high removal rate ratio of $\text{NH}_4^+\text{-N}$: SO_4^{2-} (8.67: 1),
302 accumulation of S^{2-} and decreased pH, as well as a changed structure of the microbial
303 communities.

304 The results of Zhang et al. (2019a) in a circulating flow reactor showed that the
305 efficiency of $\text{NH}_4^+\text{-N}$ oxidation and SO_4^{2-} reduction increased in the presence of NO_2^- -N and



306 NO_3^- -N. Nitrogen has been converted by nitrification, denitrification and conventional
307 anammox, simultaneously with the sulfammox process. The SO_4^{2-} removal efficiency reached
308 the maximum of 45%.

309 In the batch tests of Cai et al. (2010), reduction of SO_4^{2-} (40%) and NH_4^+ -N (44%)
310 was considered exclusively due to sulfammox. Similarly, Yang et al. (2009) successfully
311 performed sulfammox in an upflow anaerobic sludge blanket reactor. Only sulfammox was
312 assumed to be responsible for the reduction of NH_4^+ -N and SO_4^{2-} with the SO_4^{2-} removal
313 efficiency of 30%.

314 ***Modeling N, S and C transformations in wastewater treatment systems (S4)***

315 Koenig and Liu (2001) established a half-order kinetic model for biofilms to describe
316 autotrophic denitrification by *Thiobacillus denitrificans* in an upflow S packed-bed reactor
317 fed with synthetic wastewater. The half-order reaction rate constants for autotrophic
318 denitrification using S^0 were approximately one order of magnitude lower than those of
319 heterotrophic denitrification. However, the model was validated with pure substrate and pure
320 bacteria in a biofilm under autotrophic conditions, which largely limited its application to
321 other complex systems.

322 An et al. (2011) investigated the kinetics of two-step heterotrophic denitrification
323 (reduction of NO_3^- -N to NO_2^- -N, and subsequently to N oxides and N_2 gas) using an oil
324 reservoir culture, which was capable of functioning under both autotrophic and heterotrophic
325 conditions. The developed kinetic model predicted the experimental results of batch and
326 continuous systems in terms of simultaneous removal of S^{2-} , NO_3^- -N, NO_2^- -N and organic
327 compounds.

328 Wang et al (2010) proposed a kinetic model to monitor a denitrifying S^{2-} removal
329 (DSR) process with the ASM1 as a core model. By establishing inhibition and switch
330 functions, the competition between autotrophic and heterotrophic denitrifiers was described,



331 including the effect of S^{2-} inhibition on heterotrophic denitrification. The calibrated model
332 was used to quantify the impact of the influent C/S ratio and S^{2-} levels on the performance of
333 a bench-scale EGSB reactor. Model predictions indicated that the DSR reactor would operate
334 efficiently when the influent C/S ratio was kept in the range of 0.5-3.0, and the S^{2-}
335 concentration remained below 1000 mg S/L.

336 Xu et al (2014) developed a model to describe simultaneous removal of S^{2-} , NO_3^- -N
337 and acetate (sole organic substrate) under denitrifying S^{2-} removal conditions in a continuous
338 flow reactor. The kinetic parameters were estimated via data fitting while considering the
339 effects of initial S^{2-} concentration, S^{2-}/NO_3^- -N ratio and acetate/ NO_3^- -N ratio. The proposed
340 model accurately described the performance of DSR over wide ranges of the parameters.
341 Model predictions suggested that the adjustment of HRT would be an efficient way to
342 mitigate high S^{2-} loadings. Despite accurate predictions for a pure substrate (acetate), the
343 model might not be applicable for actual industrial wastewater with complex characteristics.

344 Xu et al (2016) developed an autotrophic denitrification kinetic model to describe S^{2-}
345 oxidation and NO_2^- -N removal in a bench-scale sequencing batch reactor (SBR). The model
346 parameters were estimated by data fitting from two studies with different combinations of S^{2-} ,
347 S^0 , SO_4^{2-} , NO_3^- -N and NO_2^- -N. The final products of S^{2-} oxidation (S^0 and SO_4^{2-}) and their
348 concentrations could be accurately predicted, providing a strategy to control the effluent
349 SO_4^{2-} concentration or recover S^0 as the main end product from S^{2-} oxidation.

350 Xu et al (2017) further developed a complex model for C-N-S removal by combining
351 the ASMs and ADM1 (Anaerobic Digestion Model No.1), extended with oxygen/ NO_3^- -N
352 driven S^{2-} oxidation processes. The proposed model was also capable of simulating S relevant
353 processes, such as SO_4^{2-} reduction, S^{2-} oxidation and denitrifying S^{2-} removal process. Due to
354 some simplifications in the model structure and parameter uncertainty, that model would not
355 yet serve as a precise and quantitative tool in various full-scale applications.



356 Mechanistic models may also be useful in understanding the mechanisms of
357 sulfammox and its interactions with co-existing biochemical processes. However, due to the
358 complexity of the interactions, it still lacks research reports about the model development for
359 the sulfammox process. Chen et al (2016b) established a S-involved anammox model to
360 simulate the coexistence of AOB, nitrate oxidizing bacteria (NOB), anaerobic ammonia
361 oxidizing bacteria (AAOB), denitrification anaerobic methane oxidation (DAMO) bacteria,
362 and SOB in a membrane biofilm reactor (MBfR). The model described removal of $\text{NH}_4^+\text{-N}$,
363 dissolved CH_4 , and S^{2-} from sidestream anaerobic sludge digestion liquors. However, other
364 potential processes, such as endogenous heterotrophic denitrification, S-dependent
365 autotrophic denitrification and $\text{SO}_4^{2-}/\text{S}$ reduction, were not incorporated in that model, and S^{2-}
366 inhibition was also neglected.

367 Azhar et al (2014) modeled the coupled N and S cycles in a coastal upwelling system.
368 In the S cycle, S^{2-} -driven denitrification, SO_4^{2-} reduction, and S^{2-} oxidation by $\text{NO}_3^-\text{-N}$
369 (chemolithoautotrophic $\text{NO}_3^-\text{-N}$ reduction) were considered. However, as the experimental
370 study was conducted in a natural costal water column and sediment, the reaction formulations,
371 process rates and kinetic parameters might not be directly applicable in wastewater treatment
372 systems. Moreover, some reactions, such as nitrogen fixation and remineralization, might be
373 neglected in those systems.

374

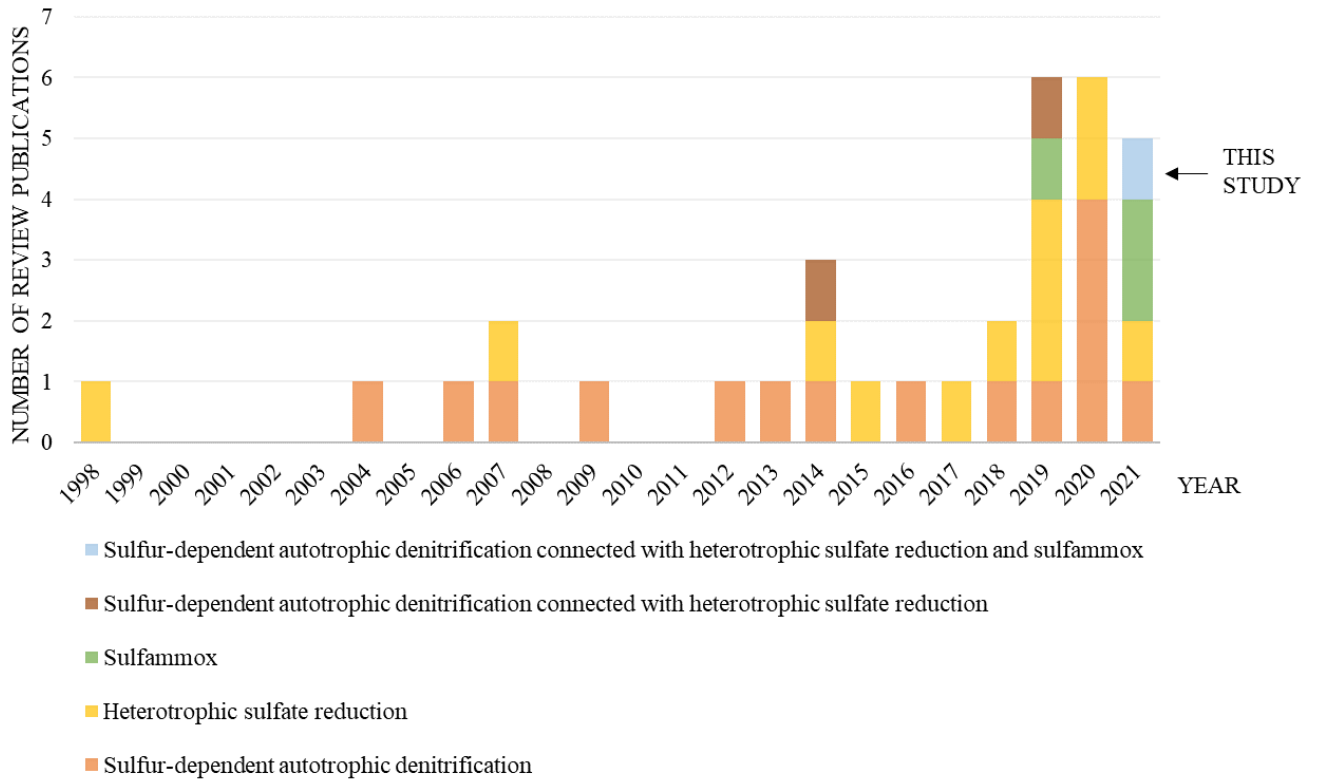
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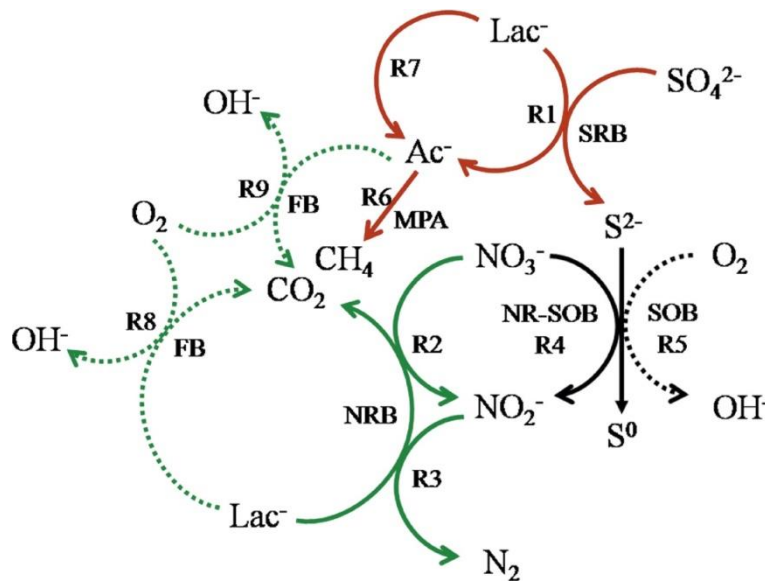




379

380 Figure S1. Number of review papers on S-dependent biochemical processes, including
 381 autotrophic denitrification, heterotrophic sulfate reduction and sulfamnox

382



383

384 Figure S2. Simplified representation of the biochemical process associated with C, N and S
 385 conversions (Xu et al., 2017).

386

Process/System	Issue	Cui et al., 2019	Tian and Yu, 2020	Hu et al., 2020	Ma et al., 2020	Wang et al., 2020	Park and Yoo, 2009	Lin et al., 2018	Wu et al., 2021	Show et al., 2013	Costa et al., 2020	Serrano et al., 2019	Lens et al., 1998	Xu and Chen, 2020	Kumar et al., 2021	Hao et al., 2014	van den Brand et al., 2015	Rios-Del Toro and Cervantes, 2021	Grubba et al., 2020	This study	
S - DEPENDENT AUTOTROPHIC DENITRIFICATION	recent advances in S-dependent autotrophic denitrification	✓	✓	✓	✓	✓	✓	✓	✓	✓										updated	
	potential use of S-dependent autotrophic denitrification	✓	✓	✓	✓	✓	✓	✓	✓	✓							✓	✓			updated
	microbiological reactions and microorganisms involved	✓		✓	✓	✓	✓	✓	✓	✓											✓
	factors influencing the operation of S-dependent autotrophic denitrification	✓		✓	✓	✓			✓	✓								✓			✓
	comparison of greenhouse gas emissions from autotrophic and heterotrophic denitrification	✓					✓		✓								✓				
	reactor configurations										✓										✓
	comparison of different electron donors (S compounds) for autotrophic denitrification	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓										✓
	cost comparison of reduced-S species and organic substrate as an electron donor	✓					✓	✓													
	recent works on the hydrogen and/or iron- and/or methane- and/or carbon- S-dependent autotrophic denitrification	✓	✓		✓	✓	✓	✓	✓												
	mixotrophic denitrification		✓					✓	✓	✓	✓										✓
natural occurrence of S-dependent autotrophic denitrification				✓																	

	characteristics and effects of water pollution by SO_4^{2-}				✓							
	treatment of acid mine drainage (AMD)	✓							✓			
	heavy metal removal	✓			✓	✓			✓			
	methods for enhancement of SRB activity				✓				✓			
	sulphide forms in aqueous medium at different pH values	✓		✓								
	prospects, limitations and future research needs	✓	✓	✓	✓	✓	✓		updated			
SULFAMMOX	recent advances in sulfammox process							✓	✓	✓	updated	
	characteristics of sulfammox							✓	✓	✓	✓	
	potential use of sulfammox process							✓	✓	✓	updated	
	characteristics of $\text{NH}_4\text{-N}$ anaerobic oxidation processes with different electron acceptors							✓				
	spontaneity and oxidation-reduction potential									✓		
	functional bacteria								✓	✓	✓	
	possible interactions with other bacteria								✓	✓	✓	
	bacteria responsible for the specific N and S transformations								✓	✓	✓	
	factors influencing the sulfammox process								✓	✓	✓	
	the occurrence of sulfammox in the environment								✓		✓	
	source of SO_4^{2-}									✓		
	reactors types								✓	✓	✓	✓
	co-existence of anammox, S-dependent autotrophic denitrification and sulfammox									✓	✓	✓

	prospects, limitations and future re- search needs			✓	✓	✓	✓
SANI PROCESS	SANI process characteristics	✓	✓				✓
	diagram of the SANI reactor sys- tem	✓	✓				✓
	SANI key-parameters		✓				✓
	recent advances in SANI process						✓
	FGD-SANI and MD-SANI charac- teristics						✓
	diagram of the FGD-SANI and MD-SANI reactors system						✓
	FGD-SANI and MD-SANI key-pa- rameters						✓
S CYCE WITH ANAMMOX	Sulfate Reduction, Denitrifica- tion/Anammox and Partial Nitrifi- cation (SRDAPN) characteristics						✓
	Partial Nitrification/Anammox and S-dependent autotrophic Denitrifi- cation (PNASD) characteristics						✓
	Anammox and S-dependent auto- trophic Denitrification (ASD) char- acteristics						✓
	S-dependent autotrophic Partial Denitrification/Anammox (SPDA) characteristics						✓
	diagram of SRDAPN, PNASD, ASD nad SPDA						✓
	recent advances in SRDAPN, PNASD, ASD nad SPDA						✓
	SRDAPN, PNASD, ASD nad SPDA key-parameters						✓

SYSTEMS WITH SULFAMMOX	Sulfamnox/Anammox (SA) characteristics	✓
	Sulfamnox - S-dependent autotrophic Denitrification (SSD) characteristics	✓
	Sulfamnox – Anammox - S-dependent autotrophic denitrification (SASD) characteristics	✓
	diagram of the SA, SSD and SASD	✓
	recent advances in SA, SSD and SASD	✓
	SA, SSD and SASD key parameters	✓

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