

## Sulfate reducing ammonium oxidation (SULFAMMOX) process under anaerobic conditions

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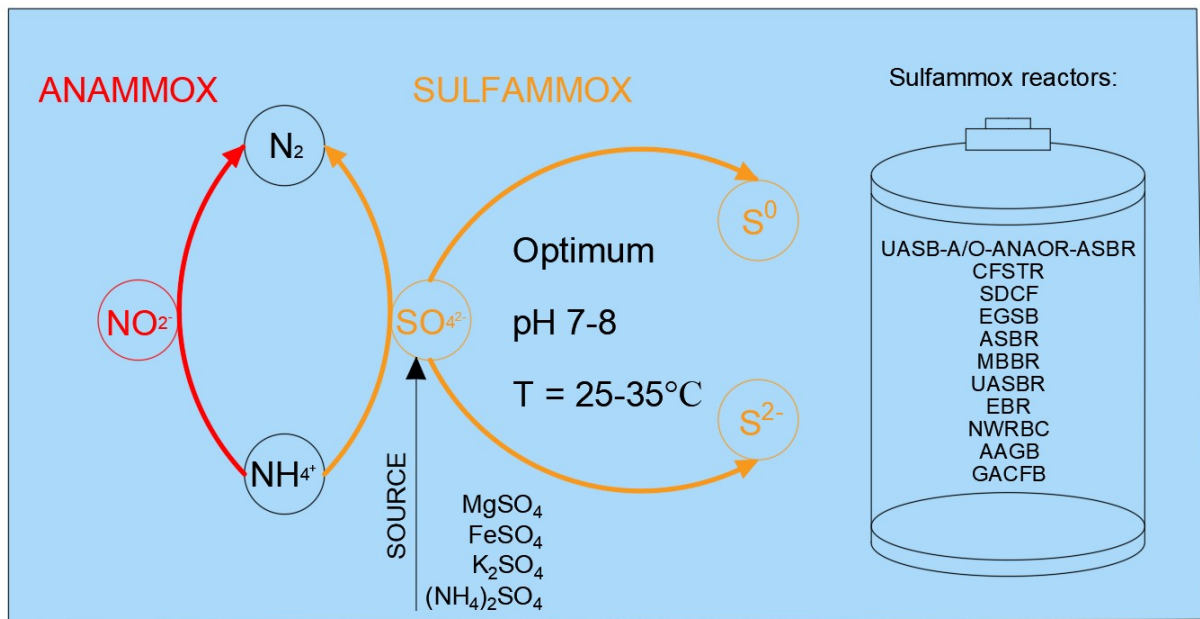
### Highlights

- Sulfate may be an additional electron acceptor in the anaerobic ammonium oxidation.
- *Bacillus Benzoevorans* and *Brocadia Anammoxoglobus Sulfate* can perform sulfammox.
- The optimal conditions for the sulfammox is 25°C -35°C and pH - 7.0 – 8.
- Sulfammox is a viable option for specific industrial wastewater with high NH<sub>4</sub><sup>+</sup> and SO<sub>4</sub><sup>2-</sup>.
- Sulfammox has been studied in suspended growth, biofilm, granular and hybrid reactors.

### Abstract

Sulfate (SO<sub>4</sub><sup>2-</sup>) can be an electron acceptor for ammonium nitrogen (NH<sub>4</sub><sup>+</sup>) oxidation under anaerobic conditions. The process is known as sulfammox and can be a viable alternative to conventional, nitrite (NO<sub>2</sub><sup>-</sup>) dependent, anammox. Two bacterial species, including *Bacillus Benzoevorans* and *Brocadia Anammoxoglobus Sulfate*, can perform that process. With sulfammox, an economically inefficient pre-nitration step (due to aeration) is not required. There are more than 10 different systems in which sulfammox has been studied, including suspended growth, biofilm, granular and hybrid reactors. A combination of anammox and sulfur related processes (sulfammox and autotrophic denitrification) would especially be appropriate for specific industrial wastewater with high content of nitrogen compounds and SO<sub>4</sub><sup>2-</sup>. The results of recent studies suggest that very high removal efficiencies could simultaneously be achieved with respect to both NH<sub>4</sub><sup>+</sup> (92-99%) and SO<sub>4</sub><sup>2-</sup> (53-60%).

27 Graphical abstract



28

29 **Keywords:** sulfamnox, anamnox, autotrophic denitrification, sulfate, sulfur cycle

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## 35 1. Introduction

36 Nitrification and denitrification are the most common processes responsible for nitrogen (N)  
37 conversions in wastewater treatment systems. A viable alternative to that pathway of nitrogen removal  
38 is the “anaerobic” ammonium oxidation (anammox) process. "Anaerobic" because it is actually an  
39 anoxic process due to the presence of nitrite ( $\text{NO}_2^-$ ). It is generally accepted that anaerobic ammonia  
40 oxidizing bacteria (AAOB) oxidize ammonia ( $\text{NH}_4^+$ ) to  $\text{N}_2$  with  $\text{NO}_2^-$  as an electron acceptor. In fact,  
41 however, AAOB have a more comprehensive metabolism than initially assumed and other phenomena  
42 of “anaerobic”  $\text{NH}_4^+$  oxidation have been discovered (Kartal et al. 2012; Liu et al. 2008). In addition to  
43  $\text{NO}_2^-$ , there may be other electron acceptors, including sulfate ( $\text{SO}_4^{2-}$ ), for  $\text{NH}_4^+$  oxidation under  
44 “anaerobic” conditions (Zandt et al. 2018). This process is known as sulfate reducing ammonium  
45 oxidation (SRAO) or sulfammox (Bi et al. 2020).

46 In addition to anaerobic sludge digester liquors, the sulfammox process may especially be appropriate  
47 for treatment of some industrial wastewater, containing high concentrations ( $>1000$  mg/l) of both  
48  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$ . Such characteristics are typical for the effluents from seafood, chemical, textile,  
49 paper, fermentation and sugar production (Rikmann et al. 2016).

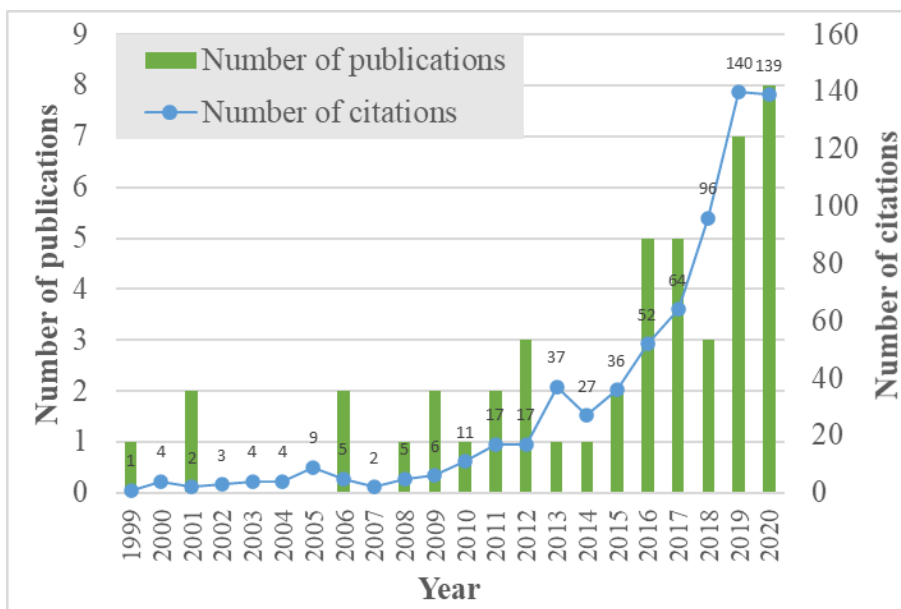
50 In comparison with the conventional anammox, sulfammox is easier to control as nitritation becomes  
51 unnecessary ( $\text{SO}_4^{2-}$  instead of  $\text{NO}_2^-$  serves as the electron acceptor) (Zhang et al. 2009). Besides, as a  
52 reducing process of  $\text{SO}_4^{2-}$ , it is also free of secondary pollution caused by sulphide ( $\text{S}^{2-}$ ), which is toxic  
53 and harmful to human health and aquatic ecosystems (Zhang et al. 2019a). Sulfammox can also  
54 prevent interference with the conventional anammox process caused by inhibition of  $\text{S}^{2-}$  (Xu et al.  
55 2020) or hydrogen sulfide ( $\text{H}_2\text{S}$ ) (Wiśniewski et al. 2019). Moreover, elemental sulfur ( $\text{S}^0$ ) is formed  
56 and its recovery provides a valuable by-product (Rios-Del Toro and Cervantes 2019). The recovered  
57  $\text{S}^0$  could be used as electron donor for autotrophic denitrification as reported by Ucar et al. (2020).  
58 Moreover, recovering  $\text{S}^0$  from wastewater is also essential to reuse it as fertilizer or to re-enter  
59 production lines in other industries. The combination of the technology based on the anammox process  
60 with the technology based on the sulfammox process would enable a balanced approach to the

61 problem of specific industrial wastewater with high content of nitrogen compounds and  $\text{SO}_4^{2-}$  through  
62 their co-treatment in combined processes.

63 The sulfammox process may occur either independently or in conjunction with the conventional  
64 anammox process. The combination of both processes can increase the overall nitrogen removal  
65 efficiency. Recent studies (Zhang et al. 2019a; Wu et al. 2020) have shown a high degree of  
66 simultaneous removal of  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$ , i.e. in the range 92-99% and 53-60%, respectively, with  
67  $\text{NO}_2^-$  and  $\text{SO}_4^{2-}$  as electron acceptors.

68 The sulfammox process has briefly been addressed in reviews on anammox in marine environments  
69 (Rios-Del Toro and Cervantes 2019) and in the state of anammox research in China (Ali et al. 2013).

70 There are still a few publications on this process and finding them is not straightforward, as  
71 sulfammox also appears as SRAO or sulfate-dependent anammox. Based on the Web of  
72 Science database, using the keywords "sulfate", "anammox" and "wastewater", a number of  
73 publications and their citations appearing in 1999-2020 years are presented in Fig. 1.



74  
75 **Fig 1** Number of publications based on keywords "sulfate", "anammox" and "wastewater" and their citations in  
76 the Web of Science database in 1999-2020

77 Based on the data in Fig. 1, it can be projected that the number of publications on sulfamox and their  
78 citations will be increasing fast over the next few years. Very recently, Liu et al. (2021) have  
79 published the first review paper especially dedicated to sulfamox. However, the paper does not  
80 incorporate a few studies, Zhang et al. (2019a) or Wang et al. (2017a), that have a significant effect on  
81 the process understanding.. In particular, the latter paper describes the  $\text{NH}_4^+/\text{SO}_4^{2-}$  ratio which plays a  
82 key role in the sulfamox process. Other issues, omitted or not sufficiently addressed in the study of  
83 Liu et a. (2021), comprised the spontaneity of the process, effect of COD on sulfamox, and feeding  
84 options ( $\text{NO}_2^-$  and  $\text{SO}_4^{2-}$  together and separately).

85 In the present study, the combination of several processes influencing removal of  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  from  
86 wastewater was addressed, including sulfamox, anammox, sulfide-dependent autotrophic  
87 denitrification, sulfur-dependent autotrophic denitrification, nitrification, denitrification, and  
88 heterotrophic sulfate reduction. A special attention was given to linking the sulfamox process with  
89 sulfur-dependent autotrophic denitrification. Moreover, a wide variety of sulfamox reactors was  
90 presented and discussed in terms of the operating conditions and performance efficiency.

91 Both soil, air and water are exposed to the influence of toxic sulfur compounds -  $\text{H}_2\text{S}$  and  $\text{S}^{2-}$ .  
92 Recognition of the sulfamox process may lead to the development of research on this process, and  
93 hence to environmental protection, thanks to the decomposition of these compounds into  $\text{S}^0$  and  
94 reduction of energy consumption by limiting two separate processes of removing  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  to  
95 one co-treatment. Therefore, the aim of this mini review is to characterize the sulfamox process,  
96 indicate the operational conditions in which it can be carried out, and compare the examined  
97 sulfamox reactors.

## 98 **2. The characteristics of the sulfamox process**

99 Sulfamox was first reported by Fdz-Polanco et al. (2001b) in a granular activated carbon anaerobic  
100 fluidized bed reactor treating vinasse from an ethanol distillery of sugar beet molasses. The authors  
101 observed that approximately 80% of  $\text{SO}_4^{2-}$  was converted to  $\text{S}^0$  with simultaneous oxidation of  $\text{NH}_4^+$  to  
102  $\text{N}_2$ . The combined process for removal of  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  was described as follows:



104 In the follow-up studies, Liu et al. (2008) and Yang et al. (2009) identified  $\text{SO}_4^{2-}$  as a potential electron  
105 acceptor as it was the feed component. They investigated the process of simultaneous removal of  
106  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  under anaerobic conditions. The ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  consumption was  
107 approximately 1.1 : 1 and 1 : 1.15, respectively, in a non-woven rotating biological contactor (NRBC)  
108 and upflow anaerobic sludge blanket reactor (UASBR) (see: Table 2). These values were significantly  
109 higher in comparison with 1 : 1.32, which is the theoretical ratio for the conventional,  $\text{NO}_2^-$ -dependent,  
110 anammox process (Xie et al. 2017).

111 Based on the literature (Strous et al. 2006; Zhang et al. 2009; Schrum et al. 2009), formation of  $\text{HS}^-$  in  
112 the sulfammox process may also be considered:



114 Alternatively, formation of  $\text{HS}^-$  may be associated with oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  (Schrum et al.  
115 2009):

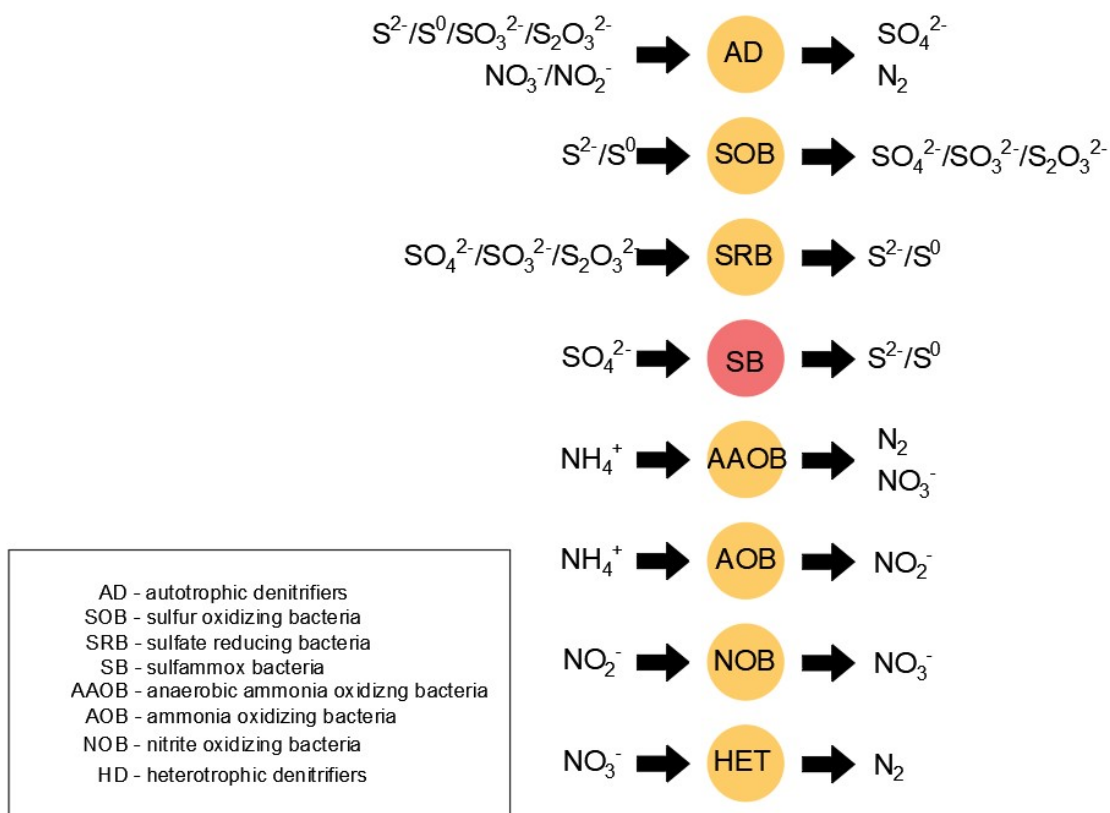


117 The consumption rate of  $\text{SO}_4^{2-}$  can be estimated based on the corresponding consumption rate of N-  
118  $\text{NH}_4^+$  and the theoretical stoichiometric consumption ratio (= 2) of  $\text{NH}_4^+$  to  $\text{SO}_4^{2-}$  in sulfammox (see:  
119 reaction 1). An inadequate influent ratio of  $\text{NH}_4^+/\text{SO}_4^{2-}$  - different than 2 (see: reaction 2,3), may result  
120 in the formation of  $\text{HS}^-$  in the sulfammox process according to reactions (2,3). The  $\text{NH}_4^+/\text{SO}_4^{2-}$  ratio of  
121 2 was indeed found in the studies of Zhang et al. (2009), Yang et al. (2009) and Cai et al. (2010). In  
122 other studies, the reported ratios were lower, i.e. 1.71 - 1.75 (Liu et al. 2008) and 1.65 (Bi et al. 2020).

123 Rikmann et al. (2012) noted that the stoichiometric ratio of  $\text{NH}_4^+$  moles consumed per mole of reduced  
124  $\text{SO}_4^{2-}$  was higher than could be expected from the amount of  $\text{SO}_4^{2-}$  reduced. This implicitly indicated  
125 the presence of additional electron acceptors, other than  $\text{SO}_4^{2-}$  (like humic matter) coupled with  $\text{NH}_4^+$   
126 oxidation or reoxidation of reduced sulfur compounds into  $\text{SO}_4^{2-}$ . The high efficiency of  $\text{NH}_4^+$  removal  
127 may result from complex interactions between organic compounds, nitrogen and sulfur like

128 sulfamnox, anammox, autotrophic denitrification, heterotrophic denitrification (Rikmann et al. 2012,  
 129 2014, 2016).

130 The newly discovered,  $\text{SO}_4^{2-}$  dependent, AAOB species have been found to be responsible for carrying  
 131 out the above reactions (2-4). The first one was *Brocadia Anammoxoglobus Sulfate* (Liu et al. 2008),  
 132 which was a functional microorganism in the simultaneous removal of  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  and ended the  
 133 conversion of  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  by producing  $\text{NO}_2^-$  as an intermediate. The second isolated species,  
 134 *Bacillus Benzoevorans* (Cai et al. 2010), was responsible for carrying out the entire sulfamnox  
 135 reaction. In the study of Liu et al. (2015b), the dominant bacteria changed from *Candidatus Brocadia*  
 136 to *Bacillus Benzoevorans* when the process transformed from the conventional anammox to  
 137 sulfamnox. Sulfamnox bacteria and AAOB combine the N and S cycles, increasing the range of N-S  
 138 transformations as shown in Fig. 2.



139  
 140 **Fig 2** Bacteria responsible for the specific N and S transformations

141

142 The  $\text{SO}_4^{2-}$  dependent AAOB are rodshaped with flagellum and spore, having a size of  $(0.7-1.0) \times (2.4-$   
143  $3.5) \mu\text{m}$ . The colony on the plate was round with a diameter of about 1 mm with a light yellow color,  
144 and its surface was smooth and wet. The cultivated biomass was dominated by chains of bacilli and  
145 cocci. Cocci generally had a diameter of  $0.9 \mu\text{m}$ , whereas bacilli varied around  $0.8 \mu\text{m}$  and  $1-1.2 \mu\text{m}$  in  
146 width and length, respectively (Zhang et al. 2009; Cai et al. 2010; Ali et al. 2013).

147 Some Proteobacteria, which may potentially perform sulfamox, include the following species: *Sulfu-*  
148 *rimonas*, *Desulfuromonadales*, *Desulfovibrio*, *Desulfuromonas*, *Desulfobulbus*, *norank Rhodobacter-*  
149 *aceae* and *Thiobacillus* (Rios-Del Toro et al. 2017; Wang et al. 2017a; Rios-Del Toro et al. 2018).

150 A syntrophic relationship between ammonia oxidizing bacteria (AOB), sulfate reducing bacteria  
151 (SRB) and AAOB could make thermodynamically not favorable oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  coupled  
152 with a possible reduction of  $\text{SO}_4^{2-}$  to  $\text{S}^0$  (Rikmann et al. 2014). A pure chemical reaction between  $\text{NH}_4^+$   
153 and  $\text{SO}_4^{2-}$  without microorganisms is not possible (Yang et al. 2009).

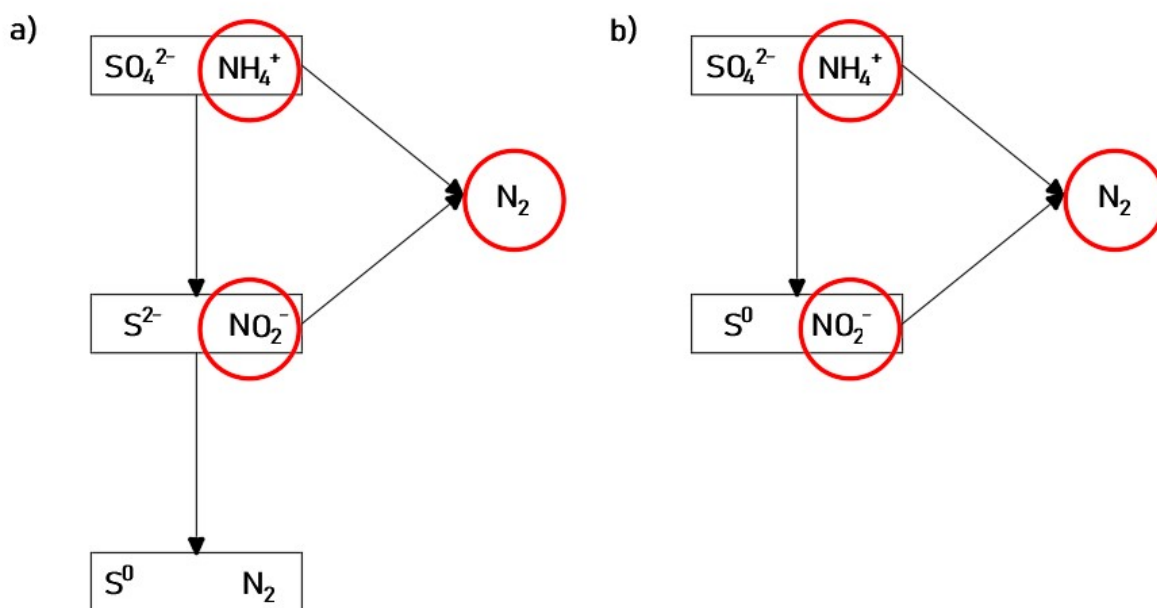
### 154 3. Relationship between sulfamox and sulfide-dependent autotrophic denitrification

155 The overall sulfamox reaction (reaction (1)) has been shown to occur in three consecutive  
156 biochemical reactions (reactions (4-6)) (FdZ-Polanco et al. 2001b; Zhang 2019a; Bi et al. 2020):



160 In reaction (4),  $\text{NH}_4^+$  reacts with  $\text{SO}_4^{2-}$  and is oxidized to  $\text{NO}_2^-$  (intermediate) inside the bacterial cell  
161 and  $\text{SO}_4^{2-}$  is simultaneously deoxygenated to  $\text{S}^{2-}$ . The  $\text{NO}_2^-$  produced diffuses outside of the bacterial  
162 cell. In reaction (5), part of  $\text{NO}_2^-$  is reduced with  $\text{S}^{2-}$ , which leads to production of  $\text{N}_2$  and  $\text{S}^0$ . Finally,  
163 reaction (6) is the conventional anammox process carried out by *Planctomyces* (Van der Star et al.  
164 2007). Yang et al. (2009) described reaction (5) as the denitrification process that occur through  
165 reduction of  $\text{NO}_2^-$  to  $\text{N}_2$  with simultaneous oxidation of  $\text{S}^{2-}$  by autotrophic denitrifiers, where the  
166 electron donor is  $\text{S}^{2-}$  and the electron acceptor is  $\text{NO}_2^-$ . For better understanding, reactions 4-6 are  
167 shown in Figure 3a.





168  
169 **Fig 3** Reactions involved in the sulfamnox process as proposed by Yang et al. (2009) (a), and Liu et al. (2008)

170 (b)

171 **It should be emphasized that sulfide-dependent autotrophic denitrification is one of the reactions**  
 172 **involved in the overall sulfamnox process, according to Fig 3a. Therefore, it is difficult to**  
 173 **distinguish a strict boundary between sulfamnox and sulfide-dependent autotrophic**  
 174 **denitrification. In fact, the denitrification reaction is one of the components of sulfamnox and**  
 175 **without it sulfamnox cannot occur, as shown in reactions (4-6).** Therefore, some researchers do  
 176 not distinguish the efficiency of  $\text{NH}_4^+$  removal in the sulfamnox process at all, but only report the total  
 177 efficiency of  $\text{NH}_4^+$  removal under anaerobic conditions in the presence of  $\text{SO}_4^{2-}$  (Wu et al 2020; Bi et  
 178 al. 2020; Zhang et al 2019a).

179 On the contrary, Liu et al. (2008) explained the sulfamnox process as a combination of two reactions  
 180 as shown earlier in Fig. 3b. According to that concept,  $\text{NH}_4^+$  would be partially converted to  $\text{NO}_2^-$  and  
 181 coupled with a conversion of  $\text{SO}_4^{2-}$  (electron acceptor) to  $\text{S}_0$ . Then  $\text{NH}_4^+$  would be oxidized to  $\text{N}_2$  by  
 182  $\text{NO}_2^-$  in the conventional anammox process. Currently, the exact pathway of sulfamnox remains  
 183 largely unknown. More detailed microbiological tests are needed to check which microorganisms and  
 184 genes are involved in that process.

185 It should be emphasized that  $S^{2-}$  in the sulfammox process (see: reaction 4) can be oxidized to either  $S^0$   
186 or  $SO_4^{2-}$ , depending on the initial  $S^{2-}$  to  $NO_2^-$  ratio. Therefore, that ratio must be strictly controlled to  
187 avoid re-oxidation to  $SO_4^{2-}$ . For sulfammox, it is important to reduce  $SO_4^{2-}$  to  $S^0$ . When  $S^{2-}$  is oxidized  
188 back to  $SO_4^{2-}$ , the total reduction of  $SO_4^{2-}$  in the sulfammox process decreases.

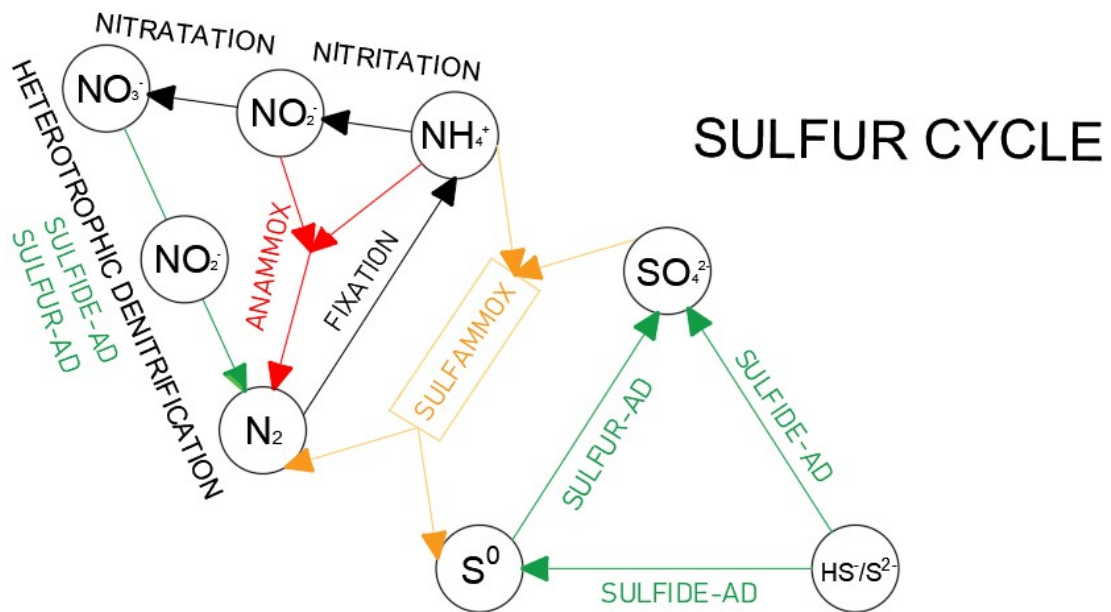
189 When the stoichiometric ratio of  $NH_4^+$  moles consumed per mole of reduced  $SO_4^{2-}$  is higher than  
190 might be expected from the degree of  $SO_4^{2-}$  reduction, this may also be due to the partial reoxidation  
191 of  $S^0$  or  $HS^-$  to  $SO_4^{2-}$  by sulfur-related autotrophic denitrification (Rikmann et al. 2012, 2014; Qin et al.  
192 2019; Wang et al. 2020). Some chemolithotrophic denitrifiers, such as *Thiobacillus denitrificans*, are  
193 capable of performing sulfur-related autotrophic denitrification.

194 The autotrophic denitrification reactions can occur with  $S^{2-}$ , sulphite ( $SO_3^{2-}$ ), thiosulphate ( $S_2O_3^{2-}$ ) or  
195  $S^0$  as electron donors, and either  $NO_3^-$  or  $NO_2^-$  as electron acceptors (Guo et al. 2013; Xu et al. 2013;  
196 Yu et al. 2013; Wang et al. 2017b; Di Capua et al. 2019). Then, either  $SO_4^{2-}$  or  $S^0$  is formed depending  
197 on the sulfur to nitrogen ratio (Kalyuzhnyi et al. 2006; Liu et al. 2015a). The following reactions  
198 describe those complex phenomena (Li i wsp. 2009; Wang et al. 2017b):



205 A simplified relationship of the sulfammox process with the nitrogen and sulfur cycles is presented in  
206 Fig. 4.

# NITROGEN CYCLE



207  
208 **Fig 4** Coupling the nitrogen and sulfur cycles in sulfamnox, sulfur-dependent autotrophic denitrification (sulfur-  
209 AD) and sulfide-dependent autotrophic denitrification (sulfide-AD)

## 4. Environmental factors and operational conditions affecting sulfamnox

### 4.1. Process medium and feeding options

212 Most of the sulfamnox studies have been carried out with synthetic wastewater or growth media  
213 (Zhao et al. 2006; Liu et al. 2008; Yang et al. 2009; Cai et al. 2010; Bi et al. 2020), but there have also  
214 been a few studies using real wastewater (Rikmann et al. 2012, 2014, 2016). Different compounds  
215 have been used as the  $\text{SO}_4^{2-}$  source in the medium (see: Table 1). The inoculum biomass originated  
216 from various sources (see: Table 2), including long-term operated anammox reactors and anaerobic  
217 digesters. The cultivation experiments have been carried out with three feeding options:

- 218 a) conventional anammox was run at the beginning, and then  $\text{NO}_2^-$  was replaced with  $\text{SO}_4^{2-}$  as a new  
219 electron acceptor (Yang et al. 2009; Rikmann et al. 2012, 2016; Zhang et al. 2019a, b; Bi et al.  
220 2020);
- 221 b)  $\text{SO}_4^{2-}$  was used since the beginning without any addition of  $\text{NO}_2^-$  (Zhang et al. 2009; Wang et al.  
222 2017a; Zhang et al. 2019b; Bi et al. 2020);

223 c)  $\text{SO}_4^{2-}$  and  $\text{NO}_2^-$  were simultaneously used as electron acceptors during the whole study period (Zhao  
224 et al. 2006; Liu et al. 2008; Zhang et al. 2019a; Wu et al. 2020).

#### 225 4.2. Temperature

226 The process temperature set point normally ranged from 30°C to 36°C as shown in Table 1. Cai et al.  
227 (2010) tested the sulfamox process efficiencies at the following series of temperatures: 15°C, 25°C,  
228 30°C, 35°C, 45°C and 55°C. The  $\text{NH}_4^+$  and overall  $\text{SO}_4^{2-}$  removal efficiencies were approximately  
229 37.5% and 35%, 36% and 30%, respectively, at  $T = 15^\circ\text{C}$  and  $T = 55^\circ\text{C}$ . The highest  $\text{NH}_4^+$  and overall  
230  $\text{SO}_4^{2-}$  removal efficiencies were observed at  $T = 30^\circ\text{C}$ , i.e. 44.4% and 40%, respectively. The removal  
231 rate of  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  at that temperature was 0.168 mg N/l/h ( $R^2 = 0.98$ ) and 0.191 mg S/l/h ( $R^2 =$   
232 0.95), respectively. The optimal temperature range for the sulfamox process is 25°C -35°C (Cai et  
233 al., 2010).

234 The sulfamox process was also studied at lower temperatures, e.g. 20°C (Rikmann et al. 2016) and  
235 14-15°C (Wu et al. 2020). In the latter case, despite such a low temperature, the  $\text{NH}_4^+$  and overall  
236  $\text{SO}_4^{2-}$  removal efficiencies remained at a high level, i.e. 98.5% and 52.8%, respectively (Wu et al.  
237 2020). Due to the combination of anaerobic-aerobic, continuous and batch processes adopted in this  
238 process, the anammox and sulfamox coupled to remove nitrogen. Rikmann et al. (2016) studied two  
239 reactors at different temperatures, i.e. MBBR (20°C) and UASBR (36°C). That approach was not  
240 clearly explained, but apparently resulted from the use of different sludges in both reactors. In the  
241 UASBR, the inoculum originated from an anaerobic reactor for treatment of industrial wastewater  
242 (yeast production), whereas the MBBR was inoculated with carriers with a well-deposited anammox  
243 biofilm developed in a conventional laboratory-scale anammox reactor. The TN removal efficiencies  
244 were in the range 5 - 72% for the MBBR and 10 - 75% for the UASBR, respectively. Despite the use  
245 of different temperatures, the TN removal rates were similar, i.e. 0.05 kg N/m<sup>3</sup>/d for the MBBR and  
246 0.04 kg N/m<sup>3</sup>/d for the UASBR.

#### 247 4.3. pH



248 The optimal pH value is 7.0 - 8.5 for both conventional anammox and sulfammox (Wu et al. 2020),  
 249 therefore, many studies on sulfammox have been carried out in that pH range (Yang et al. 2009; Zhang  
 250 et al. 2009; Zhang et al. 2019a,b; Bi et al. 2020; Wu et al. 2020). Cai et al. (2010) studied the effect of  
 251 pH on the efficiency of  $\text{NH}_4^+$  and overall  $\text{SO}_4^{2-}$  removal. The following pH values were considered:  
 252 6.5, 7.5, 8.5, 9.5 and 10.5, and the optimum pH was found at 8.5. On the contrary, Zhao et al. (2006)  
 253 found the optimum pH = 7.8.

254 **Tab. 1** Environmental factors and operational conditions in the sulfammox studies

Source of $\text{SO}_4^{2-}$	COD addition	pH [-]	Temperature [°C]	Reference
$\text{MgSO}_4 / \text{FeSO}_4$	No	7.8	35	Bi et al. [2020]
$(\text{NH}_4)_2\text{SO}_4$	No	8.1-8.3	35	Zhang et al. [2019a]
$(\text{NH}_4)_2\text{SO}_4$	No	8.1-8.6	30	Zhang et al. [2019b]
$\text{K}_2\text{SO}_4$	No	8.5	30	Cai et al. [2010]
$\text{Na}_2\text{SO}_4$	No	7.5-8.5	35	Yang et al. [2009]
$\text{Na}_2\text{SO}_4$	No	7.5	30	Zhang et al. [2009]
$(\text{NH}_4)_2\text{SO}_4$	No	8-8.2	35	Liu et al. [2008]
n.a.	Yes	7-8.5	14-15	Wu et al. [2020]
n.a.	Yes	6.9-8.1	36	Wang et al. [2017a]
$\text{K}_2\text{SO}_4$	Yes	8.4	20	Rikmann et al. [2016]
$\text{K}_2\text{SO}_4$	Yes	8.11	36	Rikmann et al. [2014]
n.a.	Yes	7.8-8.3	36	Fdz-Polanco et al. [2001b]

255 n.a.: not available

#### 256 4.4. COD addition

257 Even though COD is not required for the sulfammox process (Zhang et al. 2009), the experiments  
 258 were performed either without COD addition (Liu et al. 2008; Cai et al. 2010; Prachakittikul et al.  
 259 2016; Zhang et al. 2019a,b; Bi et al. 2020) or with COD addition (Fdz-Polanco et al. 2001a,b;  
 260 Rikmann et al. 2012, 2014, 2016; Wang et al. 2017a; Wu et al., 2020). When COD is present in  
 261 wastewater, the sulfammox process can be coupled with subsequent heterotrophic denitrification

262 (Zhang et al. 2019b). In the studies of Zhang et al. (2019b) sulfammox was mainly due to the high  
263 proportion of Proteobacteria, but approximately 12.4% of denitrifiers were also found in the sediment.  
264 This indicates that nitrification, denitrification and the traditional anammox with sulfammox may  
265 simultaneously occur in oxidation of  $\text{NH}_4^+$ . This allows for simultaneous removal of  $\text{NH}_4^+$ ,  $\text{SO}_4^{2-}$  and  
266 COD from wastewater (Wang et al. 2017a). Kosugi et al. (2019) proposed a combined  $\text{SO}_4^{2-}$  reduction,  
267 denitrification/anammox and partial nitrification process in an anaerobic-anoxic reactor. The authors  
268 confirmed the coexistence of heterotrophic denitrifying bacteria, sulfur denitrifying bacteria and  
269 anammox *Candidatus Brocadia* bacteria. They also recognized that heterotrophic and autotrophic  
270 denitrifying bacteria, competing for  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , can be used to oxidize  $\text{S}^{2-}$  to  $\text{S}^0$  prior to oxidation  
271 of organic carbon.

272 Yin et al. (2017) showed that sulfur-based autotrophic denitrification occurred with heterotrophic  
273 denitrification in an anaerobic baffled reactor. The authors also indicated that without addition of  $\text{S}^{2-}$  a  
274 significant amount of  $\text{NO}_3^-$  was reduced heterotrophically to  $\text{N}_2$  (76.6%). However, the addition of  $\text{S}^{2-}$   
275 stimulated autotrophic denitrification (from 19.7% to 40.8%) and inhibited heterotrophic  
276 denitrification (decreased to 46.9%), thereby resulting in a shift (8%) in the  $\text{NO}_3^-$  reduction pathway  
277 from denitrification to dissimilatory  $\text{NO}_3^-$  reduction to  $\text{NH}_4^+$ . The addition of  $\text{S}^{2-}$  caused a proportional  
278 increase in the population of sulfur-oxidizing nitrate-reducing bacteria (mainly *Paracoccus*) from  
279 18.6% to 27.2% and suppressed heterotrophic nitrate-reducing bacteria (mainly *Pseudoxanthomonas*  
280 and *Pseudomonas*), which caused a decrease (25.5%) in their population.

281 On the contrary, Zhao et al. (2006) found that more efficient removal of  $\text{NH}_4^+$  was obtained when the  
282 COD concentration was lower. In the studies of Wu et al. (2020), organic matter (300 mg COD/l in the  
283 influent) negatively affected conventional anammox, but sulfammox was not affected. As a  
284 consequence, the concentration of the dominant potential sulfammox bacteria (*Sulfurimonas*,  
285 *Desulfovibrio*, *Desulfuromonas*, *Desulfobulbus*, norank *Rhodobacteraceae* and *Thiobacillus*) was  
286 higher than the concentration of *Candidatus Kuenenia* performing conventional anammox.

287 4.5. Spontaneity and oxidation-reduction potential

288 Zhang et al. (2009) described the spontaneity of the sulfamnox reaction.  $\Delta G^0$  of the sulfamnox is  
289  $-45.35$  kJ/mol. The reaction is obviously more difficult to proceed than conventional anammox, which  
290 has  $\Delta G^0 = -357$  kJ/mol.

291 As the  $\text{SO}_4^{2-}$  dependent AAOB are obligate anaerobic bacteria, high substrate concentrations and a low  
292 oxidation-reduction potential (ORP) ( $< -100$  mV) can intensify the sulfamnox process (Zhang et al.  
293 2009; Ali et al. 2013). Fdz-Polanco et al. (2001a) found that the calculated values of redox potential  
294 for the half reactions of reduction of  $\text{N}_2$  to  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  to  $\text{S}^0$  at  $\text{pH} = 8$  was in the narrow range  
295 from  $-330$  to  $-360$  mV. Those results suggested that  $\text{SO}_4^{2-}$  reduction and  $\text{NH}_4^+$  oxidation could coexist  
296 together under anaerobic conditions. Similar to conventional anammox, hydrazine injections have also  
297 been reported to improve the sulfamnox activity (Rikmann et al. 2012, 2014, 2016).

#### 298 4.6. Other factors influencing the sulfamnox process

299 In contrast, there are also several factors that may negatively affect the sulfamnox process. Wu et al.  
300 (2020) found that DO levels  $>0.3 - 0.5$  mg/l could have a negative effect on sulfamnox, as this leads  
301 to partial nitrification and the production of  $\text{NO}_2^-$ . DO inhibits the enrichment of the dominant bacteria  
302 of both sulfamnox and anammox and leads to the growth of AOB, competing with AAOB for  $\text{NH}_4^+$ .  
303 High concentrations of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  also favor  $\text{SO}_4^{2-}$  resynthesis as a result of sulfur-related  
304 autotrophic denitrification. Rikmann et al. (2016) also pointed out that  $\text{NO}_2^-$  and  $\text{HCO}_3^-$  concentrations  
305 exceeding  $10$  mg N/l and  $1000$  mg/l, respectively, disrupted sulfamnox. - the latter because it  
306 affected TN removal efficiency.

307 Yang et al. (2009) noted that  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  removal efficiencies could negatively be affected by the  
308 presence of  $\text{H}_2\text{S}$  and  $\text{S}^{2-}$ . However, the authors did not provide the exact thresholds at which  
309 sulfamnox could be inhibited.

310 In the study of Zhao et al. (2006), the obtained efficiencies of  $\text{NH}_4^+ = 43\%$  (low) and  $\text{SO}_4^{2-} = 59\%$   
311 (high) implied a competition between SRB and not identified microorganisms responsible for  
312 simultaneous removal of  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$ . Therefore, it is worth of paying attention to the participation

313 of SRB in the sulfamox process, as they are responsible for the reduction of  $\text{SO}_4^{2-}$  to  $\text{S}^{2-}$  under  
314 anaerobic conditions. High COD concentration increases the growth of SRB.

### 315 **5. Sulfamox based reactors and reported efficiencies**

316 Until now, the sulfamox process has been studied in different reactors in terms of the flow  
317 conditions and biomass retention method (see: Table 2).

318



Tab. 2 Sulfamnox based reactors and efficiency of  $\text{NH}_4^+$ ,  $\text{SO}_4^{2-}$  removal

Reactor	Origin of biomass	Influent $\text{NH}_4^+$ [mg/l]	Influent $\text{SO}_4^{2-}$ [mg/l]	$\text{NH}_4^+$ removal efficiency [%]	$\text{SO}_4^{2-}$ removal efficiency [%]	Highlights of the study	Reference
<b>Combining system: Upflow Anaerobic Sludge Blanket (UASB), Anoxic/Oxic Reactor (A/O), Anammox and Sulfamnox Reactor (ANAOR), Anaerobic Sequencing Batch Reactor (ASBR)</b>	landfill leachate	610-700	1870-1920	ca. 98	ca. 53	Landfill leachate was used as a substrate. The tests were carried out at a low temperature (14-15°C). The relative abundances of dominant sulfamnox bacteria were 10-20 times higher than that of <i>Candidatus</i> Kuenenia (anammox). <b>Reduction of <math>\text{SO}_4^{2-}</math> and <math>\text{NH}_4^+</math> was considered as a combination of anammox, sulfamnox, nitrification and denitrification processes.</b>	Wu et al. [2020]
<b>Continuous Flow Stirred Tank Reactor (CFSTR)</b>	long-term operation anammox up-flow reactor	110 60 60	0-110 90 90	ca. 40 ca. 30 ca. 55	ca. 0 ca. 10 ca. 0	SRAO occurred only in the cases of high amounts of inoculum biomass at $\text{DO} = 0.2 - 0.5$ mg/L. When $\text{DO} < 0.2$ mg/L, the process was not observed. <b>SRAO was considered as a combination of aerobic ammonium oxidation, anammox, and heterotrophic sulfate reduction processes.</b>	Bi et al. [2020]
<b>Self-Designed Circulating Flowreactor (SDCF)</b>	n.a.	120 160 110 80 120 160 160 90	183 216 116 100 183 216 216 133	ca. 30 ca. 55 ca. 75 ca. 100 30 11 ca. 15 ca. 100	ca. 40 ca. 0 ca. 30 ca. 45 40 11 ca. 25 ca. 70	$\text{NH}_4^+$ oxidization and $\text{SO}_4^{2-}$ reduction efficiencies increased in the presence of $\text{NO}_2^-$ and $\text{NO}_3^-$ . <i>Proteobacteria</i> , <i>Chloroflexi</i> , <i>Bacteroidetes</i> , <i>Chlorobi</i> , <i>Acidobacteria</i> , <i>Planctomycetes</i> and <i>Nitrospirae</i> were detected. <i>Proteobacteria</i> were the dominant functional microorganisms removing nitrogen. <b>These results showed that nitrogen was converted by nitrification, denitrification, and conventional anammox, simultaneously with SRAO. The sulfur-based autotrophic denitrification and denitrification in the reactor were caused by the influent <math>\text{NO}_2^-</math> and <math>\text{NO}_3^-</math>.</b>	Zhang et al. [2019a]
<b>Self-Designed Circulating Flowreactor (SDCF)</b>	mixed sludge, which consisted anaerobic granular sludge from a municipal wastewater plant and denitrification sludge from a continuous stirred-tank reactor	50 120 180	90 170 360	ca. 40 ca. 90 ca. 20	ca. 30 ca. 30 ca. 5	The increasing ratio of N/S in the influent resulted in higher $\text{NO}_2^-$ concentrations in the effluent. The microbial community comprised <i>Proteobacteria</i> , <i>Chloroflexi</i> , <i>Bacteroidetes</i> , <i>Chlorobi</i> , <i>Acidobacteria</i> and <i>Planctomycetes</i> . SRAO was mainly due to the high performance of <i>Proteobacteria</i> (12.4% of denitrifying bacteria were found in the biomass). <b>Part of nitrogen was converted by nitrification-denitrification, and conventional anammox, simultaneously with SRAO.</b>	Zhang et al. [2019b]
<b>Expanded Granular Sludge Bed (EGSB)</b>	anaerobic hydrolysis acidification reactor	166-666 1000-2000 >3000	3600	40-58 40-70 10-25	64-71 66-82 28	The removal efficiency of $\text{SO}_4^{2-}$ gradually improved as the influent $\text{NH}_4^+$ concentrations increased from 166-666 mg N/l to 1000-2000 mg N/l. At the same time, 71% $\text{NH}_4^+$ was removed. After increasing the $\text{NH}_4^+$ concentration to > 3000 mg N/l, the $\text{SO}_4^{2-}$ reduction efficiency was reduced to 28%. <b>SRB and</b>	Wang et al. [2017a]





<b>Anaerobic Sequencing Batch Reactor (ASBR)</b>	activated sludge from the aerobic tank of digested liquor	97	261	ca. 88	ca. 19	<b>denitrifying bacteria were mainly responsible for SO<sub>4</sub><sup>2-</sup> and nitrogen removal.</b>		
<b>Moving Bed Biofilm Reactor (MBBR)</b>	well-established attached anammox biofilm withdrawn from a lab-scale conventional anammox reactor treating reject water	69	ca. 70	ca. 30	ca. 10	<b>The presence of Planctomycetes revealed that anammox was a highly involved pathway in NH<sub>4</sub><sup>+</sup> removal, even without NO<sub>2</sub><sup>-</sup> in the feed. Other autotrophic denitrifying bacteria, related to species the <i>Paracoccus Denitrificans</i>, were also present. These bacteria utilize S<sup>0</sup> as an electron donor and produce SO<sub>4</sub><sup>2-</sup>, and competitively use NO<sub>2</sub><sup>-</sup> with anammox.</b>	Pra-chakittikul et al.. [2016]	
<b>Upflow Anaerobic Sludge Blanket Reactor (UASBR)</b>	anaerobic sludge from a yeast factory wastewater treating facility (Salutaguse, Estonia)	69	ca. 70	ca. 25	ca. 10	<b>SRAO tests were performed in MBBR at 20°C and UASBR at 36°C. Very similar results of NH<sub>4</sub><sup>+</sup> and overall SO<sub>4</sub><sup>2-</sup> removal were obtained in both reactors. The SRAO process took place as one reaction of the multiple complex interactions between N-compounds, S-compounds, and organics (primarily humic matter) resulting in a significantly higher removal ratio of NH<sub>4</sub><sup>+</sup> than the SRAO stoichiometry predicts. It was postulated that the phylum Verrucomicrobia could also be involved in sulfamnox.</b>	Rikmann et al.. [2016]	
<b>Upflow Anaerobic Sludge Blanket Reactor (UASBR)</b>	reject water from anaerobic digestion of municipal wastewater sludge	221	193	ca. 30	ca. 20	<b>Sulfamnox and anammox tests were carried out at 36°C and 20°C, respectively. NO<sub>2</sub><sup>-</sup> was proved to be a more efficient electron acceptor than SO<sub>4</sub><sup>2-</sup>. The reduction of SO<sub>4</sub><sup>2-</sup> and NH<sub>4</sub><sup>+</sup> was considered as a combination of sulfamnox and denitrification processes.</b>	Rikmann et al.. [2014]	
<b>Expanded Bed Reactor (EBR)</b>	lab-scale reactor treating N-NH <sub>4</sub> <sup>+</sup> and SO <sub>4</sub> -S simultaneously for more than two years	229	163	ca. 44	40	<b><i>Bacillus Benzoevorans</i> was isolated. Its optimum pH and temperature were 8.5 and 30°C, respectively. The reduction of SO<sub>4</sub><sup>2-</sup> and NH<sub>4</sub><sup>+</sup> was considered as as sulfamnox only.</b>	Cai et al.. [2010]	
<b>Upflow Anaerobic Sludge Blanket Reactor (UASBR)</b>	nitrifying sludge in a municipal wastewater treatment plant	60	240	40	30	<b>Sulfamnox was successfully performed by changing NO<sub>2</sub><sup>-</sup> into SO<sub>4</sub><sup>2-</sup> as an electron acceptor. The reduction of SO<sub>4</sub><sup>2-</sup> and NH<sub>4</sub><sup>+</sup> was considered as sulfamnox only.</b>	Yang et al.. [2009]	
<b>Expanded Bed Reactor (EBR)</b>	anaerobic digester in a municipal wastewater treatment plant	84-270 30-90	450-740 80-200	ca. 40 ca. 55	ca. 10 ca. 43	<b>Sulfate-dependent anaerobic ammonium oxidation occurs with acclimated anaerobic digested sludge in the absence of organic matter. Anaerobic ammonium oxidation with sulfate does not tend to occur spontaneously due to its low ΔG<sup>o</sup> value. The experiment demonstrated that high substrate concentrations and low ORP may be favorable for sulfamnox. The reduction of SO<sub>4</sub><sup>2-</sup> and NH<sub>4</sub><sup>+</sup> was as a sulfamnox only.</b>	Zhang et al.. [2009]	
<b>Non-Woven Rotating Biological Contactor (NWRBC)</b>	long-term operation anammox up-flow reactor	ca. 198	ca. 528	ca. 100	ca. 70	<b>Bacteria belonging to <i>Planctomycetales</i>, especially the new species '<i>Anammoxoglobus Sulfate</i>, were identified as the functional community. The reduction of SO<sub>4</sub><sup>2-</sup> and NH<sub>4</sub><sup>+</sup> was considered as a sulfamnox only.</b>	Liu et al.. [2008]	
<b>Anaerobic Attached-Growth Bioreactor (AAGB)</b>	anaerobic activated sludge collected from an anaerobic continuous stirred tank reactor	50	57	ca. 43	ca. 59	<b>Low removal of NH<sub>4</sub><sup>+</sup> was obtained with high removal of SO<sub>4</sub><sup>2-</sup>, implying the existence of competition between SRB) and microorganisms responsible for using SO<sub>4</sub><sup>2-</sup> and NH<sub>4</sub><sup>+</sup>. Low COD, high SO<sub>4</sub><sup>2-</sup> and high NH<sub>4</sub><sup>+</sup> loadings at pH = 7.8 could</b>	Zhao et al.. [2006]	

						promoted sulfamox. <b>The reduction of <math>\text{SO}_4^{2-}</math> and <math>\text{NH}_4^+</math> was considered as a sulfamox only.</b>	
<b>Granular Activated Carbon Fluidized-Bed (GACFB)</b>	diluted vinasse originating from an ethanol distillery plant processing beet sugar molasses	<10	1000	50	80	The first report on the sulfamox process. The "anomalous" $\text{NH}_4^+$ removal was obtained in a granular activated carbon (GAC) anaerobic fluidized-bed reactor. The reactor treated vinasse from an ethanol distillery of sugar beet molasses. About 50% of the influent nitrogen load was removed from the liquid phase appearing as $\text{N}_2$ in the gas phase. Simultaneously, only 20% of the $\text{SO}_4^{2-}$ initially present in the influent appears as $\text{S}^{2-}$ in the effluent or $\text{H}_2\text{S}$ in the biogas, indicating that 80% of the sulfur was removed in sulfamox. <b>The reduction of <math>\text{SO}_4^{2-}</math> and <math>\text{NH}_4^+</math> was considered as a combination of sulfamox and denitrification processes.</b>	Fdz-Polanco et al.. [2001b]

268 Zhang et al. (2019a) studied the effects of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  on sulfamox and found that the removal  
269 efficiencies of both  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  increased from 30% to 100% and from 40% to 70%, respectively,  
270 while increasing  $\text{NO}_x$  concentrations. Autotrophic denitrification had a large share in the removal.  
271 With the influent  $\text{NH}_4^+$  concentration of 80 mg N/l,  $\text{SO}_4^{2-}$  of 100 mg S/l and  $\text{NO}_2^-$  of 28 mg N/l, the  
272  $\text{NH}_4^+$  removal efficiency reached almost 100%, while the overall  $\text{SO}_4^{2-}$  removal efficiency was only  
273 45%. Similarly, with the influent  $\text{NH}_4^+$  concentration of 90 mg N/l and  $\text{SO}_4^{2-}$  of 133 mg S/l and  $\text{NO}_3^-$   
274 of 90 mg N/l, the  $\text{NH}_4^+$  and overall  $\text{SO}_4^{2-}$  removal efficiencies were approximately 100% and 70%,  
275 respectively.

276 On the contrary, there have been studies indicating a lower efficiency of sulfamox for reject water,  
277 i.e. approximately 30% and 10% for  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$ , respectively (Rikmann et al. 2016). The influent  
278 ratio of  $\text{NH}_4^+/\text{SO}_4^{2-}$  was implicitly a key factor as studied by Wang et al. (2017a). When the  $\text{SO}_4^{2-}$   
279  $/\text{NH}_4^+$  ratio was close to 2, the process efficiency was highest, while too low or too high ratios resulted  
280 in lower efficiencies.

281 In an Expanded Granular Sludge Bed Reactor (EGSBR) performing sulfamox (Wang et al. 2017a),  
282 the removal efficiency of  $\text{SO}_4^{2-}$  and organic compounds gradually improved from 64% to 71% and  
283 66% to 82%, respectively, as the influent  $\text{NH}_4^+$  concentrations increased from 166-666 mg N/l  
284 ( $\text{NH}_4^+/\text{SO}_4^{2-} = 0.25-0.99$ ) to 1000-2000 mg N/l ( $\text{NH}_4^+/\text{SO}_4^{2-} = 1.48-2.96$ ). At the same time,  
285 approximately 71%  $\text{NH}_4^+$  was removed. However, after increasing the  $\text{NH}_4^+$  concentration to >3000  
286 mg N/l ( $\text{NH}_4^+/\text{SO}_4^{2-} > 4.44$ ), the  $\text{SO}_4^{2-}$  reduction efficiency was reduced to approximately 28%. Zhao  
287 et al. (2006) also reported that the volumetric  $\text{NH}_4^+$  removal rates could reach the highest level when  
288 the concentration of  $\text{NH}_4^+$  was 450 mg N/l (37.5 g N/m<sup>3</sup>/d), compared to 50 mg N/l (4.17 g N/m<sup>3</sup>/d)  
289 and 250 mg N/l (20.8 g N/m<sup>3</sup>/d).

290 Wu et al. (2020) investigated the sulfamox process in a system consisting of four types of reactors  
291 connected in series, including a UASBR, an anoxic/oxic reactor (A/O), an Anammox and Sulfamox  
292 reactor (ANAOR), and an ASBR. In the first reactor (UASBR), the  $\text{NH}_4^+$  concentration decreased  
293 mainly due to dilution, while  $\text{NO}_2^-$  and  $\text{NO}_3^-$  (from nitrification solution recycle) were reduced by  
294 denitrification. Partial nitrification was carried out at the A/O reactor, while anammox and sulfamox



295 were performed in the ANAOR and ASBR. In the ANAOR,  $\text{NH}_4^+$  was removed by anammox (38 mg  
296  $\text{NH}_4^+$ /l) and sulfammox (148 mg  $\text{NH}_4^+$ /l). Those results indicated that the sulfammox share in the  
297  $\text{NH}_4^+$  removal was more than 3 times higher than conventional anammox. Moreover, relatively high  
298 amounts of  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  were removed in the ANAOR compared to other reactors. These amounts  
299 were 187 mg N/l, 52 mg N/l and 35 mg N/l of  $\text{NH}_4^+$  in the ANAOR, A/O and ASBR respectively. The  
300 corresponding amounts for  $\text{SO}_4^{2-}$  were 393 mg S/l, 73.5 mg S/l and 42.3 mg S/l. The mass balance  
301 calculations revealed that the combined system allowed to achieve the  $\text{NH}_4^+$  removal efficiency at  
302 98.5%, including 44.2% removed by sulfammox, whereas the overall  $\text{SO}_4^{2-}$  removal efficiency was  
303 52.8%.

304 Rikmann et al. (2012) found that changing the electron acceptor from  $\text{NO}_2^-$  to  $\text{SO}_4^{2-}$  resulted in  
305 reduction of the anammox efficiency. The efficiency of TN removal with  $\text{NO}_2^-$  was 85%, whereas after  
306 changing to  $\text{SO}_4^{2-}$ , the average TN removal efficiency was only 23-24% in two different reactors  
307 (MBBR and UASBR).

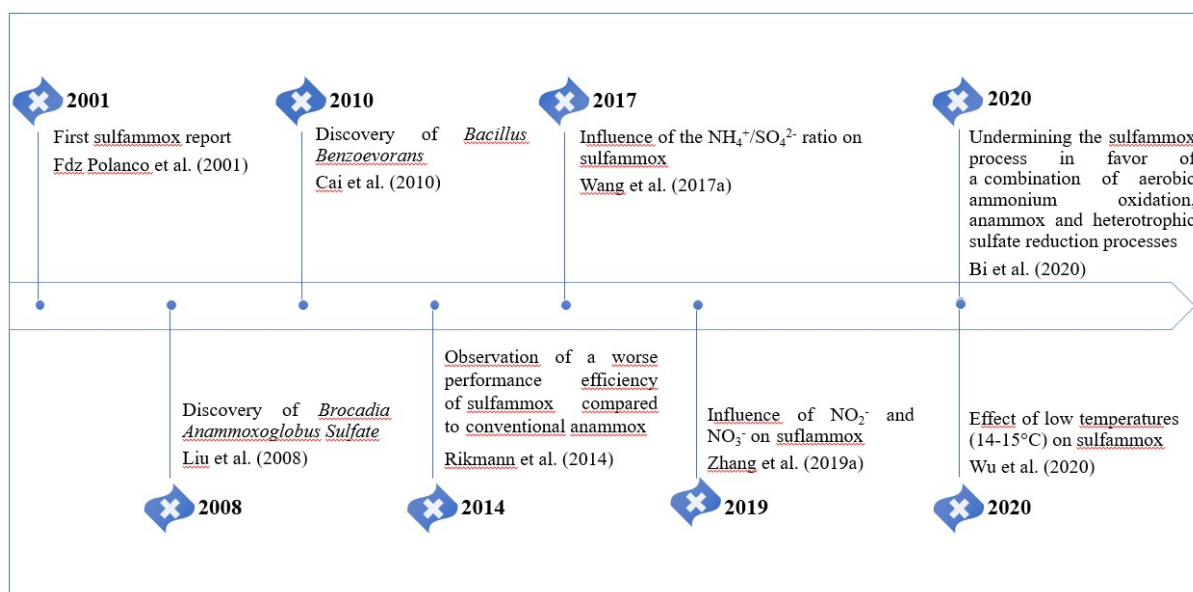
308 In order to compare sulfammox and conventional anammox, these processes were run in two parallel  
309 UASBRs (Rikmann et al. 2014). It was assumed that a higher temperature could promote sulfammox,  
310 partially compensating for its thermodynamic deficiency. Therefore, sulfammox and anammox  
311 reactors were carried out at 36°C and 20°C, respectively. The use of  $\text{NO}_2^-$  as an electron acceptor was  
312 still much more efficient than  $\text{SO}_4^{2-}$  as evidenced by the TN removal efficiency, i.e. 75% (conventional  
313 anammox) and 17% (sulfammox), despite the significant temperature difference.

314 In the most recent study of Rikmann et al. (2016), sulfammox was carried out in a MBBR at 20°C and  
315 a UASBR at 36°C. Very similar  $\text{NH}_4^+$  and overall  $\text{SO}_4^{2-}$  removal efficiencies were obtained in both  
316 reactors, i.e. 30% and 25% for  $\text{NH}_4^+$  in the MBBR and UASBR, respectively, and 10% for  $\text{SO}_4^{2-}$  in  
317 both reactors.

318 One of the principal drawbacks of sulfammox is the start-up time of the process. The sulfammox  
319 reactor start-up takes even more time than conventional anammox due to the fact that the growth rate  
320 of  $\text{SO}_4^{2-}$  dependent AAOB is very slow (Ali et al. 2013). For example, Zhang et al. (2009) found that  
321 the cultivated sludge became capable of sulfammox reaction after 3 years of the operation under



322 anaerobic conditions. This makes sulfamnox impossible to implement in the mainstream reactor. In  
 323 addition, when undesirable process disturbances occur, slow growth causes a long period of bacterial  
 324 regeneration. However, this disadvantage (slow start-up) can be partially overcome by enriching the  
 325 reactor by sulfamnox consortia from marine sediments (Ali et al. 2013). Figure 5 summarizes all the  
 326 major research and discoveries related to the development of the sulfamnox process.



327  
 328 **Fig 5** Sulfamnox process development timeline

### 329 6. Perspectives and conclusions

330 The conventional anammox process appears to be more advantageous than sulfamnox for treatment of  
 331 nitrogen rich wastewater. With sulfamnox, however, an economically inefficient pre-nitration step  
 332 (due to aeration) is not required and formation of toxic sulphide ( $\text{S}^{2-}$ ) could be avoided. The main  
 333 disadvantage of  $\text{SO}_4^{2-}$  dependent AAOB, which is a very slow doubling time, could partially be  
 334 overcome by enriching inoculum biomass with marine sediments.

335 A combination of anammox and sulfur related processes (sulfamnox and autotrophic denitrification)  
 336 would be a viable option for specific industrial wastewater with high content of nitrogen compounds  
 337 and  $\text{SO}_4^{2-}$ . There are more than 10 different novel systems in which sulfamnox has been studied,  
 338 including suspended growth, biofilm, granular and hybrid reactors. Evidence suggests that high  
 339 removal efficiencies could be achieved with respect to both  $\text{NH}_4^+$  (>90%) and  $\text{SO}_4^{2-}$  (>50%).

340

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