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Sulfate reducing ammonium oxidation (SULFAMMOX) process under anaerobic conditions

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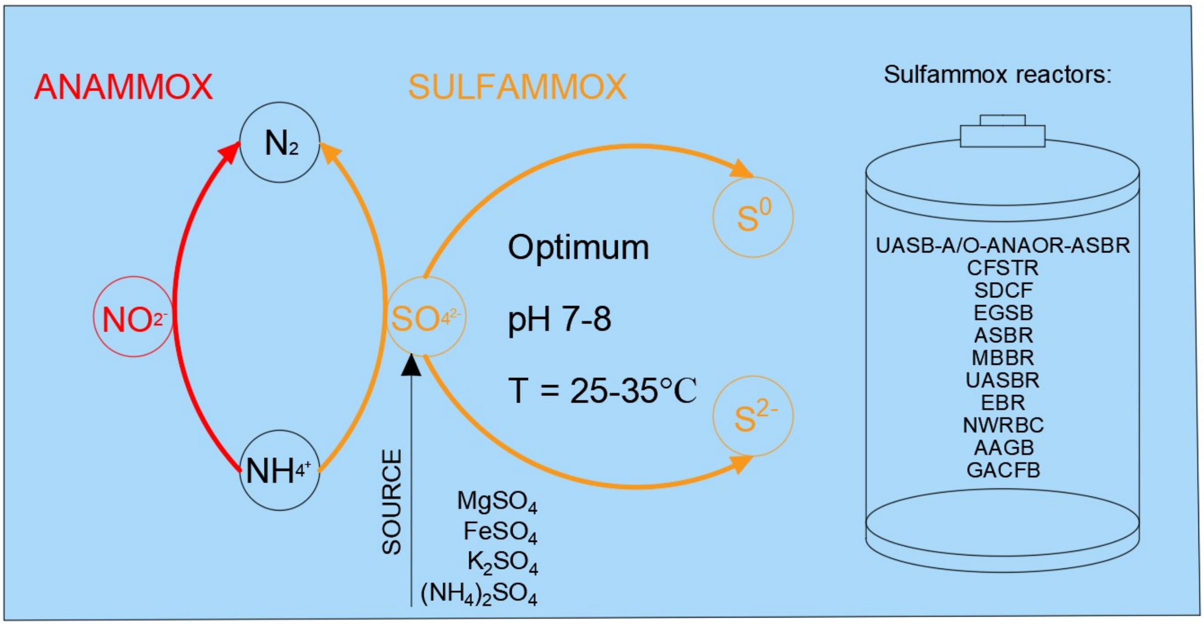
Highlights

- 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_4^+ and SO_4^{2-}.
- Sulfammox has been studied in suspended growth, biofilm, granular and hybrid reactors.

Abstract

Sulfate (SO_4^{2-}) can be an electron acceptor for ammonium nitrogen (NH_4^+) oxidation under anaerobic conditions. The process is known as sulfammox and can be a viable alternative to conventional, nitrite (NO_2^-) 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_4^{2-}. The results of recent studies suggest that very high removal efficiencies could simultaneously be achieved with respect to both NH_4^+ (92-99%) and SO_4^{2-} (53-60%).

Graphical abstract



Keywords: sulfammox, anammox, autotrophic denitrification, sulfate, sulfur cycle

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1. Introduction

Nitrification and denitrification are the most common processes responsible for nitrogen (N) conversions in wastewater treatment systems. A viable alternative to that pathway of nitrogen removal is the “anaerobic” ammonium oxidation (anammox) process. "Anaerobic" because it is actually an anoxic process due to the presence of nitrite (NO_2^-). It is generally accepted that anaerobic ammonia oxidizing bacteria (AAOB) oxidize ammonia (NH_4^+) to N_2 with NO_2^- as an electron acceptor. In fact, however, AAOB have a more comprehensive metabolism than initially assumed and other phenomena of “anaerobic” NH_4^+ oxidation have been discovered (Kartal et al. 2012; Liu et al. 2008). In addition to NO_2^-, there may be other electron acceptors, including sulfate (SO_4^{2-}), for NH_4^+ oxidation under “anaerobic” conditions (Zandt et al. 2018). This process is known as sulfate reducing ammonium oxidation (SRAO) or sulfammox (Bi et al. 2020).

In addition to anaerobic sludge digester liquors, the sulfammox process may especially be appropriate for treatment of some industrial wastewater, containing high concentrations (>1000 mg/l) of both NH_4^+ and SO_4^{2-}. Such characteristics are typical for the effluents from seafood, chemical, textile, paper, fermentation and sugar production (Rikmann et al. 2016).

In comparison with the conventional anammox, sulfammox is easier to control as nitritation becomes unnecessary (SO_4^{2-} instead of NO_2^- serves as the electron acceptor) (Zhang et al. 2009). Besides, as a reducing process of SO_4^{2-}, it is also free of secondary pollution caused by sulphide (S^{2-}), which is toxic and harmful to human health and aquatic ecosystems (Zhang et al. 2019a). Sulfammox can also prevent interference with the conventional anammox process caused by inhibition of S^{2-} (Xu et al. 2020) or hydrogen sulfide (H_2S) (Wiśniewski et al. 2019). Moreover, elemental sulfur (S^0) is formed and its recovery provides a valuable by-product (Rios-Del Toro and Cervantes 2019). The recovered S^0 could be used as electron donor for autotrophic denitrification as reported by Ucar et al. (2020). Moreover, recovering S^0 from wastewater is also essential to reuse it as fertilizer or to re-enter production lines in other industries. The combination of the technology based on the anammox process with the technology based on the sulfammox process would enable a balanced approach to the problem of specific industrial wastewater with high content of nitrogen compounds and SO_4^{2-} through their co-treatment in combined processes.

The sulfammox process may occur either independently or in conjunction with the conventional annamox process. The combination of both processes can increase the overall nitrogen removal efficiency. Recent studies (Zhang et al. 2019a; Wu et al. 2020) have shown a high degree of simultaneous removal of NH_4^+ and SO_4^{2-}, i.e. in the range 92-99% and 53-60%, respectively, with NO_2^- and SO_4^{2-} as electron acceptors.

The sulfammox process has briefly been addressed in reviews on anammox in marine environments (Rios-Del Toro and Cervantes 2019) and in the state of anammox research in China (Ali et al. 2013). There are still a few publications on this process and finding them is not straightforward, as sulfammox also appears as SRAO or sulfate-dependent anammox. Based on the Web of Science database, using the keywords "sulfate", "anammox" and "wastewater", a number of publications and their citations appearing in 1999-2020 years are presented in Fig. 1.

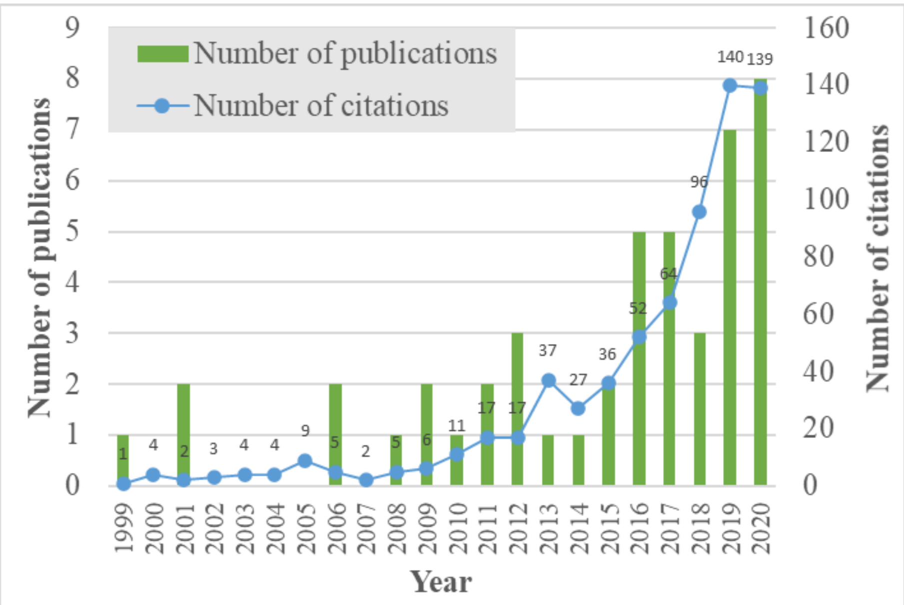

Fig 1 Number of publications based on keywords "sulfate", "anammox" and "wastewater" and their citations in the Web of Science database in 1999-2020

Based on the data in Fig. 1, it can be projected that the number of publications on sulfammox and their citations will be increasing fast over the next few years. Very recently, Liu et al. (2021) have published the first review paper especially dedicated to sulfammox. However, the paper does not incorporate a few studies, Zhang et al. (2019a) or Wang et al. (2017a), that have a significant effect on the process understanding.. In particular, the latter paper describes the NH_4^+/SO_4^{2-} ratio which plays a key role in the sulfammox process. Other issues, omitted or not sufficiently addressed in the study of Liu et a. (2021), comprised the spontaneity of the process, effect of COD on sulfammox, and feeding options (NO_2^- and SO_4^{2-} together and separately).

In the present study, the combination of several processes influencing removal of NH_4^+ and SO_4^{2-} from wastewater was addressed, including sulfammox, anammox, sulfide-dependent autotrophic denitrification, sulfur-dependent autrotrophic denitrification, nitrification, denitrification, and heterotrophic sulfate reduction. A special attention was given to linking the sulfammox process with sulfur-dependent autotrophic denitrification. Moreover, a wide variety of sulfammox reactors was presented and discussed in terms of the operating conditions and performance efficiency.

Both soil, air and water are exposed to the influence of toxic sulfur compounds - H_2S and S^{2-}. Recognition of the sulfammox process may lead to the development of research on this process, and hence to environmental protection, thanks to the decomposition of these compounds into S^0 and reduction of energy consumption by limiting two separate processes of removing NH_4^+ and SO_4^{2-} to one co-treatment. Therefore, the aim of this mini review is to characterize the sulfammox process, indicate the operational conditions in which it can be carried out, and compare the examined sulfammox reactors.

2. The characteristics of the sulfammox process

Sulfammox was first reported by Fdz-Polanco et al. (2001b) in a granular activated carbon anaerobic fluidized bed reactor treating vinasse from an ethanol distillery of sugar beet molasses. The authors observed that approximately 80% of SO_4^{2-} was converted to S^0 with simultaneous oxidation of NH_4^+ to N_2. The combined process for removal of NH_4^+ and SO_4^{2-} was described as follows:

$$SO_4^{2-} + 2NH_4^+ \rightarrow S^0 + N_2 + 4H_2O \qquad (1)$$

In the follow-up studies, Liu et al. (2008) and Yang et al. (2009) identified SO_4^{2-} as a potential electron acceptor as it was the feed component. They investigated the process of simultaneous removal of $NH4^+$ and SO_4^{2-} under anaerobic conditions. The ratio of NH_4^+ to NO_2^- consumption was approximately 1.1 : 1 and 1 : 1.15, respectively, in a non-woven rotating biological contactor (NRBC) and upflow anaerobic sludge blanket reactor (UASBR) (see: Table 2). These values were significantly higher in comparison with 1 : 1.32, which is the theoretical ratio for the conventional, NO_2^--dependent, anammox process (Xie et al. 2017).

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

$$8NH_4^+ + 3SO_4^{2-} \rightarrow 4N_2 + 3HS^- + 12H_2O + 5H^+ \qquad (2)$$

Alternatively, formation of HS^- may be associated with oxidation of NH_4^+ to NO_3^- (Schrum et al. 2009):

$$NH_4^+ + SO_4^{2-} \rightarrow NO_3^- + HS^- + H_2O + H^+ \qquad (3)$$

The consumption rate of SO_4^{2-} can be estimated based on the corresponding consumption rate of N-NH_4^+ and the theoretical stoichiometric consumption ratio (= 2) of NH_4^+ to SO_4^{2-} in sulfammox (see: reaction 1). An inadequate influent ratio of NH_4^+/SO_4^{2-} - different than 2 (see: reaction 2,3), may result in the formation of HS^- in the sulfammox process according to reactions (2,3). The NH_4^+/SO_4^{2-} ratio of 2 was indeed found in the studies of Zhang et al. (2009), Yang et al. (2009) and Cai et al. (2010). In other studies, the reported ratios were lower, i.e. 1.71 - 1.75 (Liu et al. 2008) and 1.65 (Bi et al. 2020).

Rikmann et al. (2012) noted that the stoichiometric ratio of NH_4^+ moles consumed per mole of reduced SO_4^{2-} was higher than could be expected from the amount of SO_4^{2-} reduced. This implicitly indicated the presence of additional electron acceptors, other than SO_4^{2-} (like humic matter) coupled with NH_4^+ oxidation or reoxidation of reduced sulfur compounds into SO_4^{2-}. The high efficiency of NH_4^+ removal may result from complex interactions between organic compounds, nitrogen and sulfur like sulfammox, anammox, autotrophic denitrification, heterotrophic denitrification (Rikmann et al. 2012, 2014, 2016).

The newly discovered, SO_4^{2-} dependent, AAOB species have been found to be responsible for carrying out the above reactions (2-4). The first one was *Brocadia Anammoxoglobus Sulfate* (Liu et al. 2008), which was a functional microorganism in the simultaneous removal of NH_4^+ and SO_4^{2-} and ended the conversion of NH_4^+ and SO_4^{2-} by producing NO_2^- as an intermediate. The second isolated species, *Bacillus Benzoevorans* (Cai et al. 2010), was responsible for carrying out the entire sulfammox reaction. In the study of Liu et al. (2015b), the dominant bacteria changed from *Candidatus* Brocadia to *Bacillus Benzoevorans* when the process transformed from the conventional anammox to sulfammox. Sulfammox bacteria and AAOB combine the N and S cycles, increasing the range of N-S transformations as shown in Fig. 2.

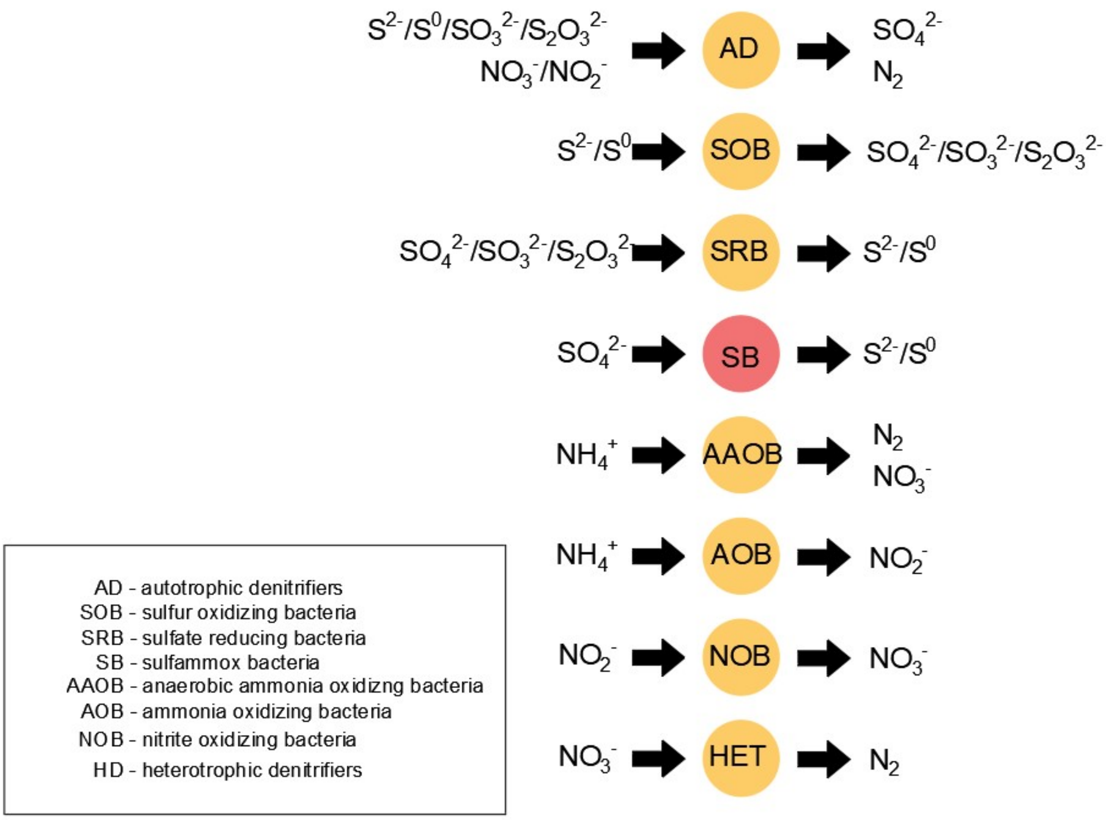

Fig 2 Bacteria responsible for the specific N and S transformations

The SO_4^{2-} dependent AAOB are rodshaped with flagellum and spore, having a size of (0.7-1.0) × (2.4-3.5) *μ*m. The colony on the plate was round with a diameter of about 1 mm with a light yellow color, and its surface was smooth and wet. The cultivated biomass was dominated by chains of bacilli and cocci. Cocci generally had a diameter of 0.9 μm, whereas bacilli varied around 0.8 μm and 1-1.2 μm in width and length, respectively (Zhang et al. 2009; Cai et al. 2010; Ali et al. 2013).

Some Proteobacteria, which may potentially perform sulfammox, include the following species: *Sulfurimonas, Desulfuromonadales, Desulfovibrio, Desulfuromonas, Desulfobulbus, norank Rhodobacteraceae* and *Thiobacillus* (Rios-Del Toro et al. 2017; Wang et al. 2017a; Rios-Del Toro et al. 2018). A syntrophic relationship between ammonia oxidizing bacteria (AOB), sulfate reducing bacteria (SRB) and AAOB could make thermodynamically not favorable oxidation of NH_4^+ to NO_2^- coupled with a possible reduction of SO_4^{2-} to S^0 (Rikmann et al. 2014). A pure chemical reaction between NH_4^+ and SO_4^{2-} without microorganisms is not possible (Yang et al. 2009).

3. Relationship between sulfammox and sulfide-dependent autotrophic denitrification

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

$$3SO_4^{2-} + 4NH_4^+ \rightarrow 4NO_2^- + 3S^{2-} + 4H_2O + 8H^+ \quad (4)$$

$$3S^{2-} + 2NO_2^- + 8H^+ \rightarrow N_2 + 3S^0 + 4H_2O \quad (5)$$

$$2NO_2^- + 2NH_4^+ \rightarrow 2N_2 + 4H_2O \quad (6)$$

In reaction (4), NH_4^+ reacts with SO_4^{2-} and is oxidized to NO_2^- (intermediate) inside the bacterial cell and SO_4^{2-} is simultaneously deoxygenated to S^{2-}. The NO_2^- produced diffuses outside of the bacterial cell. In reaction (5), part of NO_2^- is reduced with S^{2-}, which leads to production of N_2 and S^0. Finally, reaction (6) is the conventional anammox process carried out by *Planctomycetes* (Van der Star et al. 2007). Yang et al. (2009) described reaction (5) as the denitrification process that occur through reduction of NO_2^- to N_2 with simultaneous oxidation of S^{2-} by autotrophic denitrifiers, where the electron donor is S^{2-} and the electron acceptor is NO_2^-. For better understanding, reactions 4-6 are shown in Figure 3a.

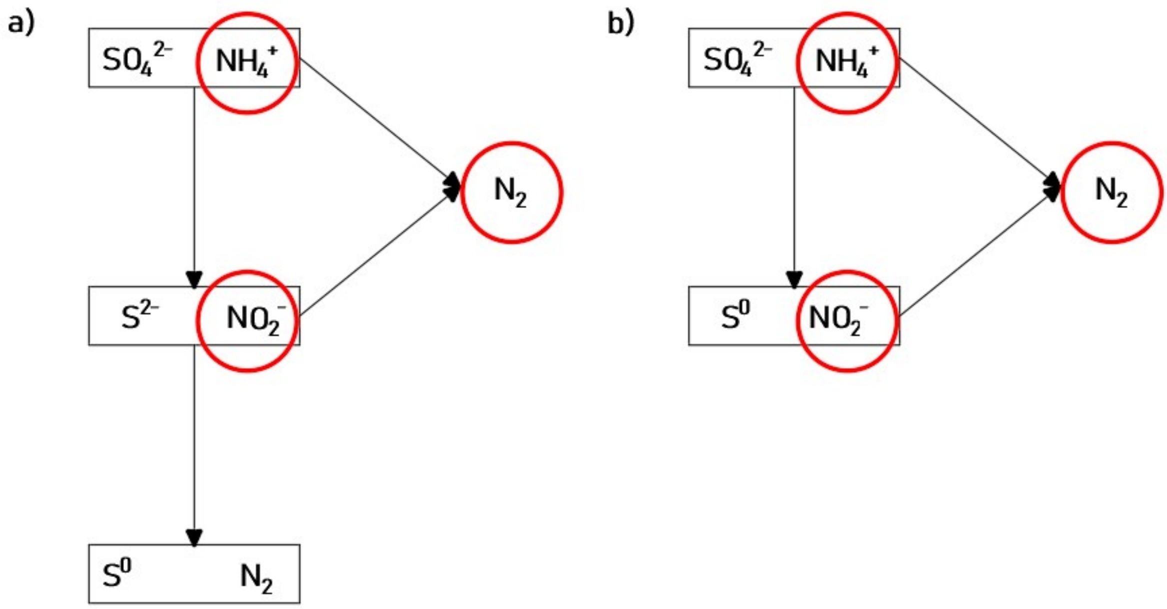

Fig 3 Reactions involved in the sulfammox process as proposed by Yang et al. (2009) (a), and Liu et al. (2008) (b)

It should be emphasized that sulfide-dependent autotrophic denitrification is one of the reactions involved in the overall sulfammox process, according to Fig 3a. Therefore, it is difficult to distinguish a strict boundary between sulfammox and sulfide-dependent autotrophic denitrification. In fact, the denitrification reaction is one of the components of sulfammox and without it sulfammox cannot occur, as shown in reactions (4-6). Therefore, some researchers do not distinguish the efficiency of NH_4^+ removal in the sulfammox process at all, but only report the total efficiency of NH_4^+ removal under anaerobic conditions in the presence of SO_4^{2-} (Wu et al 2020; Bi et al. 2020; Zhang et al 2019a).

On the contrary, Liu et al. (2008) explained the sulfammox process as a combination of two reactions as shown earlier in Fig. 3b. According to that concept, NH_4^+ would be partially converted to NO_2^- and coupled with a conversion of SO_4^{2-} (electron acceptor) to S_0. Then NH_4^+ would be oxidized to N_2 by NO_2^- in the conventional anammox process. Currently, the exact pathway of sulfammox remains largely unknown. More detailed microbiological tests are needed to check which microorganisms and genes are involved in that process.

It should be emphasized that S^{2-} in the sulfammox process (see: reaction 4) can be oxidized to either S^0 or SO_4^{2-}, depending on the initial S^{2-} to NO_2^- ratio. Therefore, that ratio must be strictly controlled to 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 back to SO_4^{2-}, the total reduction of SO_4^{2-} in the sulfammox process decreases.

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

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

$$5S^{2-} + 2NO_3^- + 12H^+ \rightarrow 5S + N_2 + 6H_2O \quad (7)$$

$$5S^0 + 6NO_3^- + 2H_2O \rightarrow 5SO_4^{2-} + 3N_2 + 4H^+ \quad (8)$$

$$5S^{2-} + 8NO_3^- + 8H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O \quad (9)$$

$$3S^{2-} + 2NO_2^- + 8H^+ \rightarrow 3S^0 + N_2 + 4H_2O \quad (10)$$

$$3S^0 + 6NO_2^- \rightarrow 3SO_4^{2-} + 3N_2 \quad (11)$$

$$3S^{2-} + 8NO_2^- + 8H^+ \rightarrow 3SO_4^{2-} + 4N_2 + 4H_2O \quad (12)$$

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

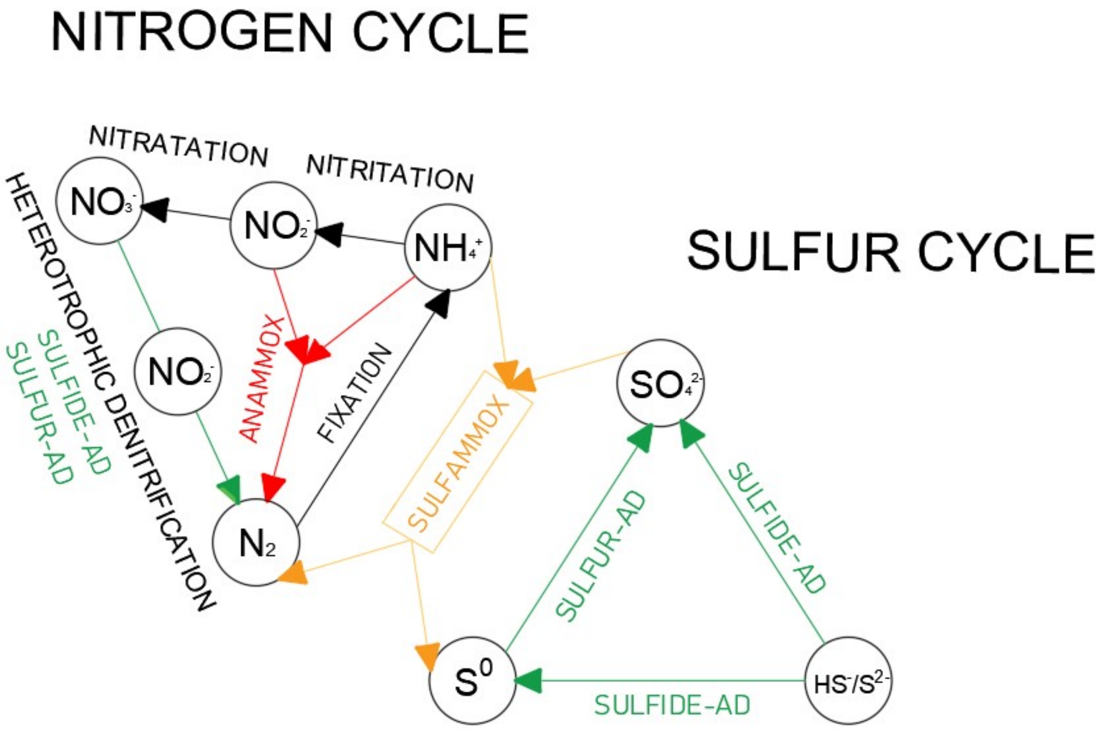

Fig 4 Coupling the nitrogen and sulfur cycles in sulfammox, sulfur-dependent autotrophic denitrification (sulfur-AD) and sulfide-dependent autotrophic denitrification (sulfide-AD)

4. Environmental factors and operational conditions affecting sulfammox

4.1. Process medium and feeding options

Most of the sulfammox studies have been carried out with synthetic wastewater or growth media (Zhao et al. 2006; Liu et al. 2008; Yang et al. 2009; Cai et al. 2010; Bi et al. 2020), but there have also been a few studies using real wastewater (Rikmann et al. 2012, 2014, 2016). Different compounds have been used as the SO_4^{2-} source in the medium (see: Table 1). The inoculum biomass originated from various sources (see: Table 2), including long-term operated anammox reactors and anaerobic digesters. The cultivation experiments have been carried out with three feeding options:

a) conventional anammox was run at the beginning, and then NO_2^- was replaced with SO_4^{2-} as a new electron acceptor (Yang et al. 2009; Rikmann et al. 2012, 2016; Zhang et al. 2019a, b; Bi et al. 2020);

b) SO_4^{2-} was used since the beginning without any addition of NO_2^- (Zhang et al. 2009; Wang et al. 2017a; Zhang et al. 2019b; Bi et al. 2020);

c) SO_4^{2-} and NO_2^- were simultaneously used as electron acceptors during the whole study period (Zhao et al. 2006; Liu et al. 2008; Zhang et al. 2019a; Wu et al. 2020).

4.2. Temperature

The process temperature set point normally ranged from 30°C to 36°C as shown in Table 1. Cai et al. (2010) tested the sulfammox process efficiencies at the following series of temperatures: 15°C, 25°C, 30°C, 35°C, 45°C and 55°C. The NH_4^+ and overall SO_4^{2-} removal efficiencies were approximately 37.5% and 35%, 36% and 30%, respectively, at T = 15°C and T = 55°C. The highest NH_4^+ and overall SO_4^{2-} removal efficiencies were observed at T = 30°C, i.e. 44.4% and 40%, respectively. The removal rate of NH_4^+ and 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 = 0.95), respectively. The optimal temperature range for the sulfammox process is 25°C -35°C (Cai et al., 2010).

The sulfammox process was also studied at lower temperatures, e.g. 20°C (Rikmann et al. 2016) and 14-15°C (Wu et al. 2020). In the latter case, despite such a low temperature, the NH_4^+ and overall SO_4^{2-} removal efficiencies remained at a high level, i.e. 98.5% and 52.8%, respectively (Wu et al. 2020). Due to the combination of anaerobic-aerobic, continuous and batch processes adopted in this process, the anammox and sulfammox coupled to remove nitrogen. Rikmann et al. (2016) studied two reactors at different temperatures, i.e. MBBR (20ºC) and UASBR (36ºC). That approach was not clearly explained, but apparently resulted from the use of different sludges in both reactors. In the UASBR, the inoculum originated from an anaerobic reactor for treatment of industrial wastewater (yeast production), whereas the MBBR was inoculated with carriers with a well-deposited anammox biofilm developed in a conventional laboratory-scale anammox reactor. The TN removal efficiencies were in the range 5 - 72% for the MBBR and 10 - 75% for the UASBR, respectively. Despite the use of different temperatures, the TN removal rates were similar, i.e. 0.05 kg N/m^3/d for the MBBR and 0.04 kg N/m^3/d for the UASBR.

4.3. pH

The optimal pH value is 7.0 - 8.5 for both conventional anammox and sulfammox (Wu et al. 2020), therefore, many studies on sulfammox have been carried out in that pH range (Yang et al. 2009; Zhang et al. 2009; Zhang et al. 2019a,b; Bi et al. 2020; Wu et al. 2020). Cai et al. (2010) studied the effect of pH on the efficiency of NH_4^+ and overall SO_4^{2-} removal. The following pH values were considered: 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) found the optimum pH = 7.8.

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

Source of SO_4^{2-}	COD addition	pH [-]	Temperature [°C]	Reference
$MgSO_4$ / $FeSO_4$	No	7.8	35	Bi et al. [2020]
$(NH_4)_2SO_4$	No	8.1-8.3	35	Zhang et al. [2019a]
$(NH_4)_2SO_4$	No	8.1-8.6	30	Zhang et al. [2019b]
K_2SO_4	No	8.5	30	Cai et al. [2010]
Na_2SO_4	No	7.5-8.5	35	Yang et al. [2009]
Na_2SO_4	No	7.5	30	Zhang et al. [2009]
$(NH_4)_2SO_4$	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]
K_2SO_4	Yes	8.4	20	Rikmann et al. [2016]
K_2SO_4	Yes	8.11	36	Rikmann et al. [2014]
n.a.	Yes	7.8-8.3	36	Fdz-Polanco et al. [2001b]

n.a.: not available

4.4. COD addition

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

(Zhang et al. 2019b). In the studies of Zhang et al. (2019b) sulfammox was mainly due to the high proportion of Proteobacteria, but approximately 12.4% of denitrifiers were also found in the sediment. This indicates that nitrification, denitrification and the traditional anammox with sulfammox may simultaneously occur in oxidation of NH_4^+. This allows for simultaneous removal of NH_4^+, SO_4^{2-} and COD from wastewater (Wang et al. 2017a). Kosugi et al. (2019) proposed a combined SO_4^{2-} reduction, denitrification/anammox and partial nitrification process in an anaerobic-anoxic reactor. The authors confirmed the coexistence of heterotrophic denitrifying bacteria, sulfur denitrifying bacteria and anammox Candidatus Brocadia bacteria. They also recognized that heterotrophic and autotrophic denitrifying bacteria, competing for NO_3^- and NO_2^-, can be used to oxidize S^{2-} to S^0 prior to oxidation of organic carbon.

Yin et al. (2017) showed that sulfur-based autotrophic denitrification occurred with heterotrophic denitrification in an anaerobic baffled reactor. The authors also indicated that without addition of S^{2-} a significant amount of NO_3^- was reduced heterotrophically to N_2 (76.6%). However, the addition of S^{2-} stimulated autotrophic denitrification (from 19.7% to 40.8%) and inhibited heterotrophic denitrification (decreased to 46.9%), thereby resulting in a shift (8%) in the NO_3^- reduction pathway from denitrification to dissimilatory NO_3^- reduction to NH_4^+. The addition of S^{2-} caused a proportional increase in the population of sulfur-oxidizing nitrate-reducing bacteria (mainly Paracoccus) from 18.6% to 27.2% and suppressed heterotrophic nitrate-reducing bacteria (mainly Pseudoxanthomonas and Pseudomonas), which caused a decrease (25.5%) in their population.

On the contrary, Zhao et al. (2006) found that more efficient removal of NH_4^+ was obtained when the COD concentration was lower. In the studies of Wu et al. (2020), organic matter (300 mg COD/l in the influent) negatively affected conventional anammox, but sulfammox was not affected. As a consequence, the concentration of the dominant potential sulfammox bacteria (*Sulfurimonas, Desulfovibrio, Desulfuromonas, Desulfobulbus, norank Rhodobacteraceae* and *Thiobacillus*) was higher than the concentration of *Candidatus* Kuenenia performing conventional anammox.

4.5. Spontaneity and oxidation-reduction potential

Zhang et al. (2009) described the spontaneity of the sulfammox reaction. ΔG^{θ} of the sulfammox is −45.35 kJ/mol. The reaction is obviously more difficult to proceed than conventional anammox, which has ΔG^{θ} = -357 kJ/mol.

As the SO_4^{2-} dependent AAOB are obligate anaerobic bacteria, high substrate concentrations and a low oxidation-reduction potential (ORP) (< -100 mV) can intensify the sulfammox process (Zhang et al. 2009; Ali et al. 2013). Fdz-Polanco et al. (2001a) found that the calculated values of redox potential for the half reactions of reduction of N_2 to NH_4^+ and SO_4^{2-} to S^0 at pH = 8 was in the narrow range from -330 to -360 mV. Those results suggested that SO_4^{2-} reduction and NH_4^+ oxidation could coexist together under anaerobic conditions. Similar to conventional anammox, hydrazine injections have also been reported to improve the sulfammox activity (Rikmann et al. 2012, 2014, 2016).

4.6. Other factors influencing the sulfammox process

In contrast, there are also several factors that may negatively affect the sulfammox process. Wu et al. (2020) found that DO levels >0.3 - 0.5 mg/l could have a negative effect on sulfammox, as this leads to partial nitritation and the production of NO_2^-. DO inhibits the enrichment of the dominant bacteria of both sulfammox and anammox and leads to the growth of AOB, competing with AAOB for NH_4^+. High concentrations of NO_2^- and NO_3^- also favor SO_4^{2-} resynthesis as a result of sulfur-related autotrophic denitrification. Rikmann et al. (2016) also pointed out that NO_2^- and HCO_3^- concentrations exceeding 10 mg N/l and 1000 mg/l, respectively, disrupted sulfammox. - the latter because it affected TN removal efficiency.

Yang et al. (2009) noted that NH_4^+ and SO_4^{2-} removal efficiencies could negatively be affected by the presence of H_2S and S^{2-}. However, the authors did not provide the exact thresholds at which sulfammox could be inhibited.

In the study of Zhao et al. (2006), the obtained efficiencies of NH_4^+ = 43% (low) and SO_4^{2-} = 59% (high) implied a competition between SRB and not identified microorganisms responsible for simultaneous removal of NH_4^+ and SO_4^{2-}. Therefore, it is worth of paying attention to the participation of SRB in the sulfammox process, as they are responsible for the reduction of SO_4^{2-} to S^{2-} under anaerobic conditions. High COD concentration increases the growth of SRB.

5. Sulfammox based reactors and reported efficiencies

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

Tab. 2 Sulfammox based reactors and efficiency of NH_4^+, SO_4^{2-} removal

Reactor	Origin of biomass	Influent NH_4^+ [mg/l]	Influent SO_4^{2-} [mg/l]	NH_4^+ removal efficiency [%]	SO_4^{2-} removal efficiency [%]	Highlights of the study	Reference
Combining system: Upflow Anaerobic Sludge Blanket (UASB), Anoxic/Oxic Reactor (A/O), Anammox and Sulfammox Reactor (ANAOR), Anaerobic Sequencing Batch Reactor (ASBR)	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 sulfammox bacteria were 10-20 times higher that of *Candidatus* Kuenenia (anammox). **Reduction of SO_4^{2-} and NH_4^+ was considered as a combination of anammox, sulfammox, nitrification and denitrification processes.**	Wu et al. [2020]
Continuous Flow Stirred Tank Reactor (CFSTR)	long-term operation anammox up-flow reactor	110	0-110	ca. 40	ca. 0	SRAO occurred only in the cases of high amounts of inoculum biomass at DO = 0.2 - 0.5 mg/L). When DO<0.2 mg/L, the process was not observed. **SRAO was considered as a combination of aerobic ammonium oxidation, anammox, and heterotrophic sulfate reduction processes.**	Bi et al.. [2020]
		60	90	ca. 30	ca. 10		
		60	90	ca. 55	ca. 0		
Self-Designed Circulating Flowreactor (SDCF)	n.a.	120	183	ca. 30	ca. 40	NH_4^+ oxidization and SO_4^{2-} reduction efficiencies increased in the presence of NO_2^- and NO_3^-. *Proteobacteria, Chloroflexi, Bacteroidetes, Chlorobi, Acidobacteria, Planctomycetes* and *Nitrospirae* were detected. *Proteobacteria* were the dominant functional microorganisms removing nitrogen. **These results showed that nitrogen was converted by nitrification, denitrification, and conventional anammox, simultaneously with SRAO. The sulfur-based autotrophic denitration and denitrification in the reactor were caused by the influent NO_2^- and** NO_3^-.	Zhang et al.. [2019a]
		160	216	ca. 55	ca. 0		
		110	116	ca. 75	ca. 30		
		80	100	ca. 100	ca. 45		
		120	183	30	40		
		160	216	11	11		
		160	216	ca. 15	ca. 25		
		90	133	ca. 100	ca. 70		
Self-Designed Circulating Flowreactor (SDCF)	mixed sludge, which consisted anaerobic granular sludge from a municipal wastewater plant and denitrification sludge from a continuous stirred-tank reactor	50	90	ca. 40	ca. 30	The increasing ratio of N/S in the influent resulted in higher NO_2^- concentrations in the effluent. The microbial community comprised *Proteobacteria, Chloroflexi, Bacteroidetes, Chlorobi, Acidobacteria* and *Planctomycetes*. SRAO was mainly due to the high performance of Proteobacteria (12.4% of denitrifying bacteria were found in the biomass). **Part of nitrogen was converted by nitrification-denitrification, and conventional anammox, simultaneously with SRAO.**	Zhang et al.. [2019b]
		120	170	ca. 90	ca. 30		
		180	360	ca. 20	ca. 5		
Expanded Granular Sludge Bed (EGSB)	anaerobic hydrolysis acidification reactor	166-666	3600	40-58	64-71	The removal efficiency of SO_4^{2-} gradually improved as the influent NH_4^+ concentrations increased from 166-666 mg N/l to 1000-2000 mg N/l. At the same time, 71% NH_4^+ was removed. After increasing the NH_4^+ concentration to > 3000 mg N/l, the SO_4^{2-} reduction efficiency was reduced to 28%. **SRB and**	Wang et al. [2017a]
		1000-2000		40-70	66-82		
		>3000		10-25	28		

						denitrifying bacteria were mainly responsible for SO_4^{2-} and nitrogen removal.	
Anaerobic Sequencing Batch Reactor (ASBR)	activated sludge from the aerobic tank of digested liquor	97	261	ca. 88	ca. 19	**The presence of Planctomycetes revealed that anammox was a highly involved pathway in NH_4^+ removal, even without NO_2^- in the feed. Other autotrophic denitrifying bacteria, related to species the *Paracoccus Denitrificans***, were also present**.** These bacteria utilize S^0 as an electron donor and produce SO_4^{2-}, and competitively use NO_2^- with anammox.	Prachakittikul et al.. [2016]
Moving Bed Biofilm Reactor (MBBR)	well-established attached anammox biofilm withdrawn from a lab-scale conventional anammox reactor treating reject water	69	ca. 70	ca. 30	ca. 10	SRAO tests were performed in MBBR at 20°C and UASBR at 36°C. Very similar results of NH_4^+ and overall SO_4^{2-} 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_4^+ than the SRAO stoichiometry predicts. It was postulated that the phylum Verrucomicrobia could also be involved in sulfammox.**	Rikmann et al.. [2016]
Upflow Anaerobic Sludge Blanket Reactor (UASBR)	anaerobic sludge from a yeast factory wastewater treating facility (Salutaguse, Estonia)	69	ca. 70	ca. 25	ca. 10		
Upflow Anaerobic Sludge Blanket Reactor (UASBR)	reject water from anaerobic digestion of municipal wastewater sludge	221	193	ca. 30	ca. 20	Sulfammox and anammox tests were carried out at 36°C and 20°C, respectively. NO_2^- was proved to be a more efficient electron acceptor than SO_4^{2-}. **The reduction of SO_4^{2-} and NH_4^+ was considered as a combination of sulfammox and denitrification processes.**	Rikmann et al.. [2014]
Expanded Bed Reactor (EBR)	lab-scale reactor treating N-NH_4^+ and SO_4-S simultaneously for more than two years	229	163	ca. 44	40	*Bacillus Benzoevorans* was isolated. Its optimum pH and temperature were 8.5 and 30°C, respectively. **The reduction of SO_4^{2-} and NH_4^+ was considered as as sulfammox only.**	Cai et al.. [2010]
Upflow Anaerobic Sludge Blanket Reactor (UASBR)	nitrifying sludge in a municipal wastewater treatment plant	60	240	40	30	Sulfammox was successfully performed by changing NO_2^- into SO_4^{2-} as an electron acceptor. **The reduction of SO_4^{2-} and NH_4^+ was considered as sulfammox only.**	Yang et al.. [2009]
Expanded Bed Reactor (EBR)	anaerobic digester in a municipal wastewater treatment plant	84-270 30-90	450-740 80-200	ca. 40 ca. 55	ca. 10 ca. 43	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^o value. The experiment demonstrated that high substrate concentrations and low ORP may be favorable for sulfammox. **The reduction of SO_4^{2-} and NH_4^+ was as a sulfammox only.**	Zhang et al.. [2009]
Non-Woven Rotating Biological Contactor (NWRBC)	long-term operation anammox up-flow reactor	ca. 198	ca. 528	ca. 100	ca. 70	Bacteria belonging to *Planctomycetales*, especially the new species '*Anammoxoglobus Sulfate*, were identified as the functional community. **The reduction of SO_4^{2-} and NH_4^+ was considered as a sulfammox only.**	Liu et al.. [2008]
Anaerobic Attached-Growth Bioreactor (AAGB)	anaerobic activated sludge collected from an anaerobic continuous stirred tank reactor	50	57	ca. 43	ca. 59	Low removal of NH_4^+ was obtained with high removal of SO_4^{2-}, implying the existence of competition between SRB) and microorganisms responsible for using SO_4^{2-} and NH_4^+. Low COD, high SO_4^{2-} and high NH_4^+ loadings at pH = 7.8 could	Zhao et al.. [2006]

						promoted sulfammox. **The reduction of SO_4^{2-} and NH_4^+ was considered as a sulfammox only.**	
Granular Activated Carbon Fluidized-Bed (GACFB)	diluted vinasse originating from an ethanol distillery plant processing beet sugar molasses	<10	1000	50	80	The first report on the sulfammox process. The "anomalous" 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 N_2 in the gas phase. Simultaneously, only 20% of the SO_4^{2-} initially present in the influent appears as S^{2-} in the effluent or H_2S in the biogas, indicating that 80% of the sulfur was removed in sulfammox. **The reduction of SO_4^{2-} and NH_4^+ was considered as a combination of sulfammox and denitrification processes.**	Fdz-Polanco et al.. [2001b]

Zhang et al. (2019a) studied the effects of NO_2^- and NO_3^- on sulfammox and found that the removal efficiencies of both NH_4^+ and SO_4^{2-} increased from 30% to 100% and from 40% to 70%, respectively, while increasing NOx concentrations. Autotrophic denitrification had a large share in the removal. With the influent NH_4^+ concentration of 80 mg N/l, SO_4^{2-} of 100 mg S/l and NO_2^- of 28 mg N/l, the NH_4^+ removal efficiency reached almost 100%, while the overall SO_4^{2-} removal efficiency was only 45%. Similarly, with the influent NH_4^+ concentration of 90 mg N/l and SO_4^{2-} of 133 mg S/l and NO_3^- of 90 mg N/l, the NH_4^+ and overall SO_4^{2-} removal efficiencies were approximately 100% and 70%, respectively.

On the contrary, there have been studies indicating a lower efficiency of sulfammox for reject water, i.e. approximately 30% and 10% for NH_4^+ and SO_4^{2-}, respectively (Rikmann et al. 2016). The influent ratio of NH_4^+/SO_4^{2-} was implicitly a key factor as studied by Wang et al. (2017a). When the SO_4^{2-}/NH_4^+ ratio was close to 2, the process efficiency was highest, while too low or too high ratios resulted in lower efficiencies.

In an Expanded Granular Sludge Bed Reactor (EGSBR) performing sulfammox (Wang et al. 2017a), the removal efficiency of SO_4^{2-} and organic compounds gradually improved from 64% to 71% and 66% to 82%, respectively, as the influent NH_4^+ concentrations increased from 166-666 mg N/l (NH_4^+/SO_4^{2-} = 0.25-0.99) to 1000-2000 mg N/l (NH_4^+/SO_4^{2-} = 1.48-2.96). At the same time, approximately 71% NH_4^+ was removed. However, after increasing the NH_4^+ concentration to >3000 mg N/l (NH_4^+/SO_4^{2-} > 4.44), the SO_4^{2-} reduction efficiency was reduced to approximately 28%. Zhao et al. (2006) also reported that the volumetric NH_4^+ removal rates could reach the highest level when the concentration of NH_4^+ was 450 mg N/l (37.5 g N/m^3/d), compared to 50 mg N/l (4.17 g N/m^3/d) and 250 mg N/L (20.8 g N/m^3/d).

Wu et al. (2020) investigated the sulfammox process in a system consisting of four types of reactors connected in series, including a UASBR, an anoxic/oxic reactor (A/O), an Anammox and Sulfammox reactor (ANAOR), and an ASBR. In the first reactor (UASBR), the NH_4^+ concentration decreased mainly due to dilution, while NO_2^- and NO_3^- (from nitrification solution recycle) were reduced by denitrification. Partial nitrification was carried out at the A/O reactor, while anammox and sulfammox were performed in the ANAOR and ASBR. In the ANAOR, NH_4^+ was removed by anammox (38 mg NH_4^+/l) and sulfammox (148 mg NH_4^+/l). Those results indicated that the sulfammox share in the NH_4^+ removal was more than 3 times higher than conventional anammox. Moreover, relatively high amounts of NH_4^+ and SO_4^{2-} were removed in the ANAOR compared to other reactors. These amounts were 187 mg N/l, 52 mg N/l and 35 mg N/l of NH_4^+ in the ANAOR, A/O and ASBR respectively. The corresponding amounts for SO_4^{2-} were 393 mg S/1, 73.5 mg S/l and 42.3 mg S/l. The mass balance calculations revealed that the combined system allowed to achieve the NH_4^+ removal efficiency at 98.5%, including 44.2% removed by sulfammox, whereas the overall SO_4^{2-} removal efficiency was 52.8%.

Rikmann et al. (2012) found that changing the electron acceptor from NO_2^- to SO_4^{2-} resulted in reduction of the anammox efficiency. The efficiency of TN removal with NO_2^- was 85%, whereas after changing to SO_4^{2-}, the average TN removal efficiency was only 23-24% in two different reactors (MBBR and UASBR).

In order to compare sulfammox and conventional anammox, these processes were run in two parallel UASBRs (Rikmann et al. 2014). It was assumed that a higher temperature could promote sulfammox, partially compensating for its thermodynamic deficiency. Therefore, sulfammox and anammox reactors were carried out at 36°C and 20°C, respectively. The use of NO_2^- as an electron acceptor was still much more efficient than SO_4^{2-} as evidenced by the TN removal efficiency, i.e. 75% (conventional anammox) and 17% (sulfammox), despite the significant temperature difference.

In the most recent study of Rikmann et al. (2016), sulfammox was carried out in a MBBR at 20°C and a UASBR at 36°C. Very similar NH_4^+ and overall SO_4^{2-} removal efficiencies were obtained in both reactors, i.e. 30% and 25% for NH_4^+ in the MBBR and UASBR, respectively, and 10% for SO_4^{2-} in both reactors.

One of the principal drawbacks of sulfammox is the start-up time of the process. The sulfammox reactor start-up takes even more time than conventional anammox due to the fact that the growth rate of SO_4^{2-} dependent AAOB is very slow (Ali et al. 2013). For example, Zhang et al. (2009) found that the cultivated sludge became capable of sulfammox reaction after 3 years of the operation under anaerobic conditions. This makes sulfammox impossible to implement in the mainstream reactor. In addition, when undesirable process disturbances occur, slow growth causes a long period of bacterial regeneration. However, this disadvantage (slow start-up) can be partially overcome by enriching the reactor by sulfammox consortia from marine sediments (Ali et al. 2013). Figure 5 summarizes all the major research and discoveries related to the development of the sulfammox process.

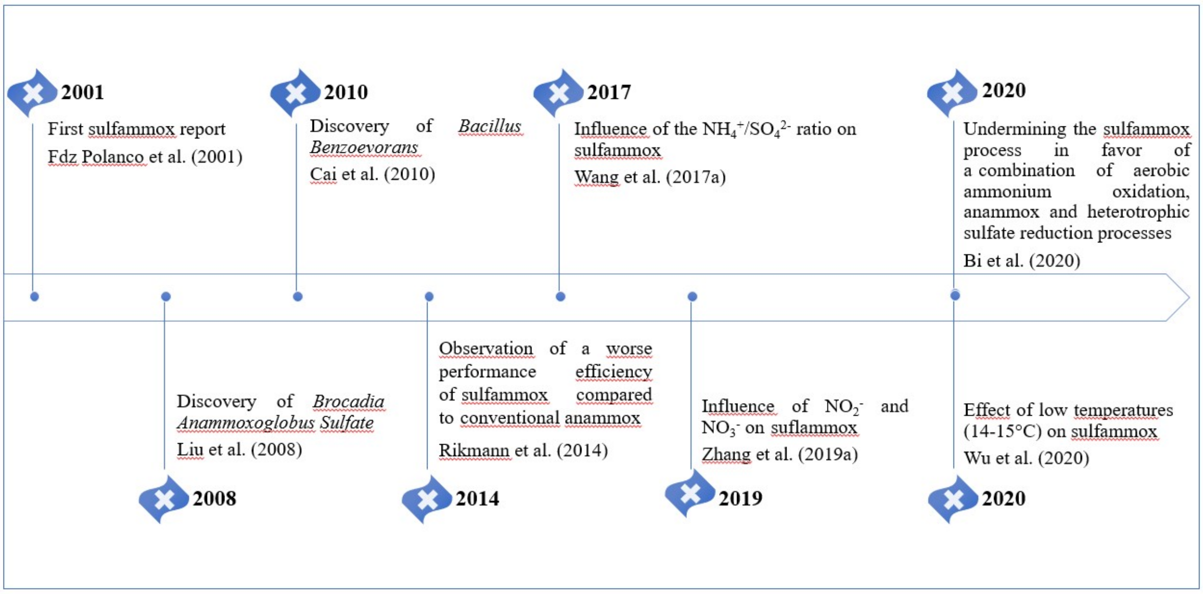

Fig 5 Sulfammox process development timeline

6. Perspectives and conclusions

The conventional anammox process appears to be more advantageous than sulfammox for treatment of nitrogen rich wastewater. With sulfammox, however, an economically inefficient pre-nitration step (due to aeration) is not required and formation of toxic sulphide (S^{2-}) could be avoided. The main disadvantage of SO_4^{2-} dependent AAOB, which is a very slow doubling time, could partially be overcome by enriching inoculum biomass with marine sediments.

A combination of anammox and sulfur related processes (sulfammox and autotrophic denitrification) would be a viable option for specific industrial wastewater with high content of nitrogen compounds and SO_4^{2-}. There are more than 10 different novel systems in which sulfammox has been studied, including suspended growth, biofilm, granular and hybrid reactors. Evidence suggests that high removal efficiencies could be achieved with respect to both NH_4^+ (>90%) and SO_4^{2-} (>50%).

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