The Influence of Sulfate on Anaerobic Ammonium Oxidation in a Sequencing Batch Reactor

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Abstract: Anaerobic ammonia-oxidizing bacteria have a more comprehensive metabolism than expected - there may be other electron acceptors that oxidize ammonium nitrogen under anaerobic conditions, in addition to the well-known nitrite nitrogen, one of which is sulfate in the sulfammox process. Sulfate-containing compounds are part of the medium for the anammox process, but their concentrations are not particularly high (0.2 g MgSO₄·7H₂O/dm³ and 0.00625 g FeSO₄/dm³). They can react to some extent with influent ammonium nitrogen. In this work, tests were carried out in two sequencing batch reactors with granular sludge. The first reactor (R1) operated in a 6 h cycle, and the concentration of the inflowing sulfate was kept at 44 mg/dm³·d. The second reactor (R2) was operated until the 36th day in a 6 h cycle; the influencing concentration was 180 mg SO₄²⁻/dm³·d from the 37th to 64th day in a 3 h cycle, with an influencing concentration of 360 mg SO₄²⁻/dm³·d; and from the 65th to 90th day, the reactor was operated again in a 6 h cycle with an influencing concentration of 180 mg SO₄²⁻/dm³·d. Along with the increased share of sulfate, both the ammonium utilization rate and specific anammox activity showed an increasing trend. As soon as the sulfate dosage was reduced, the ammonium utilization rate and specific anammox activity values dropped. Therefore, it can be concluded that sulfate-containing compounds contribute to the efficiency and rate of the anammox process.

Keywords: sulfammox; anammox; sulfate; ammonium utilization rate; specific anammox activity

1. Introduction

Several industrial processes such as fermentation, tanning, landfill leachate production, paper production, pharmaceutical production and food processes produce wastewater containing high concentrations of sulfate (SO₄²⁻) and ammonium nitrogen (NH₄-N) [1]. Such sewage requires treatment before discharge to the environment, as it is harmful to human life [2].

SO₄²⁻ is conventionally removed by anaerobic processes by sulfate-reducing bacteria (SRB) [3,4], where SO₄²⁻ is the final electron acceptor and organic carbon is the electron donor [5]. In contrast, the combined nitrification–denitrification processes are the main pathway responsible for the transformation of nitrogen (N) compounds in wastewater treatment systems in which ammonia-oxidizing bacteria (AOB), nitrogen-oxidizing bacteria (NOB) and heterotrophic bacteria are involved. The discovery of the anammox process shed new light on the nitrogen cycle. This biological process involves oxidizing ammonium nitrogen (NH₄-N) under anoxic conditions to gaseous nitrogen (N₂), using nitrite nitrogen (NO₂-N) as the electron acceptor, via anaerobic ammonia-oxidizing bacteria (AAOB). Accordingly, the removal of SO₄²⁻ and NH₄-N generally takes place in separate processes, as each purification step requires different bacterial groups and environmental conditions. This is associated with high costs due to the necessity of aeration, external carbon sources and excess sludge.
At the beginning of the research, SO\(_4^{2-}\) with granular sludge: one operates under a constant load of SO\(_4^{2-}\) (SBR) with granular sludge: one operates under a constant load of SO\(_4^{2-}\) (SBR) and constant duration of the disposal [6]. However, to date, little is known about the ability of AAOB to use SO\(_4^{2-}\) as an electron acceptor [6].

Fdz-Polanco et al. [7] described the reaction of the autotrophic anaerobic oxidation of NH\(_4^-\)N and deoxidation of SO\(_4^{2-}\) in three Equations (1)–(3):

\[
\begin{align*}
3\text{SO}_4^{2-} + 4\text{NH}_4^+ & \rightarrow 4\text{NO}_2^- + 3\text{S}^{2-} + 4\text{H}_2\text{O} + 8\text{H}^+ \quad (1) \\
3\text{S}^{2-} + 2\text{NO}_2^- + 8\text{H}^+ & \rightarrow \text{N}_2 + 3\text{S}^0 + 4\text{H}_2\text{O} \quad (2) \\
2\text{NO}_2^- + 2\text{NH}_4^+ & \rightarrow 2\text{N}_2 + 4\text{H}_2\text{O} \quad (3)
\end{align*}
\]

At first, NH\(_4^-\)N is partially oxidized and deoxygenated by SO\(_4^{2-}\) to produce NO\(_2^-\)N and sulfides (S\(^{2-}\)) (see reaction 1). Then, some of the NO\(_2^-\)N is reduced by S\(^{2-}\) in the sulfur-dependent autotrophic denitrification process and converted into N\(_2\) and elemental sulfur (S\(^0\)) (see reaction 2). Ultimately, the conventional anammox process follows (see reaction 3).

It turns out that AAOB’s metabolism is more comprehensive than expected [8,9] and, in addition to the commonly known electron acceptor in the form of NO\(_2^-\)N, there may be other electron acceptors that oxidize NH\(_4^-\)N under anaerobic conditions [10]. The process described in reactions 1–3 is called the sulfammox process (i.e., sulfate-reducing ammonium oxidation (SRAO)) [11]. The sulfammox process is a promising resource for wastewater treatment systems, because wastewater contains high amounts of sulfur compounds [12]. It can be represented in one reaction as follows [13] (4):

\[
\text{SO}_4^{2-} + 2\text{NH}_4^+ \rightarrow \text{S}^0 + \text{N}_2 + 4\text{H}_2\text{O} \quad (4)
\]

Producing N\(_2\) and elemental sulfur (S\(^0\)) is desirable in wastewater treatment and for the recovery of resources. Moreover, the simultaneous removal of SO\(_4^{2-}\) and NH\(_4^-\)N is more beneficial in terms of reducing costs than the separate removal of these pollutants [14]. The discovery of the sulfammox process suggests that the interrelationships between the N and S biochemical cycles is far more complex than previously assumed.

It is worth noting that the process of sulfur-dependent autotrophic denitrification has been described as a component of sulfammox. It is an autotrophic process in which chemotrophic sulfur-oxidizing bacteria (SOB) oxidize reduced sulfur compounds such as S\(^{2-}\), S\(^0\), sulfite (SO\(_3^{2-}\)) or thiosulfate (S\(_2\)O\(_3^{2-}\)) as electron donors with NO\(_3^-\)N or NO\(_2^-\)N as electron acceptors [15–18]. Then, SO\(_4^{2-}\) or S\(^0\) is formed depending on the sulfur-to-nitrogen ratio [2]. S\(^{2-}\) produced by sulfate-reducing bacteria can also be used as an electron donor for sulfur denitrification [19].

Due to the complex transformations of sulfur and nitrogen in anaerobic conditions, it is worth considering the effect of SO\(_4^{2-}\) on anaerobic NH\(_4^-\)N oxidation. The sulfammox process can run independently without the addition of NO\(_2^-\)N or in combination with the conventional (NO\(_2^-\)N based) anammox process. Research on the sulfammox process was carried out in various configurations. At the beginning of the research, SO\(_4^{2-}\) was used as an electron acceptor without the addition of NO\(_2^-\)N [9,11,20–23]. Other studies started with a conventional anammox, with NO\(_2^-\)N as an electron acceptor, and replaced NO\(_2^-\)N with a new SO\(_4^{2-}\) electron acceptor [11,12,24]. There are also reports in which SO\(_4^{2-}\) was used simultaneously with NO\(_2^-\)N as an electron acceptor [25,26]. For example, Zhang et al. [25] and Wu et al. [26] showed a high degree of simultaneous removal of NH\(_4^-\)N and SO\(_4^{2-}\), in the range of 92–99% and 53–60%, respectively, when anammox and sulfammox reactions occurred simultaneously. Therefore, the research shows that combining the two processes can achieve an increase in the overall nitrogen removal efficiency.

To date, research work has focused mainly on the effect of increased proportions of NH\(_4^-\)N and N/S ratio in relation to the sulfammox process [10,20,21]. The influence of increased proportions of SO\(_4^{2-}\) on anaerobic NH\(_4^-\)N oxidation in the presence of NO\(_2^-\)N due to the reduced cycle time has yet to be described. The purpose of this study is to compare the operation of two sequencing batch reactors (SBR) with granular sludge: one operates under a constant load of SO\(_4^{2-}\) and constant duration of the
process cycle, and the other operates with an increased and variable load of SO$_4^{2−}$ in a variable cycle time. The process efficiency was compared by calculating the ammonia utilization rate (AUR) and the specific anammox activity (SAA). It is suspected that SO$_4^{2−}$ will increase the AUR and SAA as it will act as an additional electron acceptor in the anaerobic oxidation of NH$_4$-N.

2. Materials and Methods

2.1. Laboratory-Scale Bioreactor

The inoculated biomass originated from a full-scale side-stream deammonification system in Plettenberg, Germany.

The laboratory scale system used in this study consisted of two 4 dm$^3$ sequencing batch reactors (SBRs) laid out according to the scheme in Figure 1. The system was equipped with a thermostatic jacket maintaining a constant temperature in the range of −35 to +200 °C, with an accuracy of ± 0.1 °C. Each reactor was equipped with an electric stirrer with variable speed. In the main reactor, probes were placed to measure pH (Endress + Hauser EH CPS 471D-7211, Switzerland) and to measure dissolved oxygen (DO) (Endress + Hauser COS22D-10P3/O, Germany).

All measured data were transmitted to the programmable logic controller (PLC) and used for control and regulation. Measurement data for archival and further use were sent to an application called Intouch’a.

2.2. Operational Conditions of the Laboratory-Scale SBRs

The tests were carried out continuously for 90 days. During the entire test period, the SBRs operated at a constant temperature of 30 (± 1) °C. The pH was maintained in the range of 7.5–7.8 through the automatic addition of 4 M sodium hydroxide (NaOH). The DO concentration in unventilated SBRs did not exceed 0.2 mg/dm$^3$, and SBRs were fed with synthetic substrate according to the method of Dapena-Mora et al. [27] and Table 1.
Table 1. Number of cycles and concentrations of compounds in R1 and R2.

| Reactor | Day   | Number of Cycles per Day | Time of One Cycle (h) | SO$_4^{2-}$ Concentration in the Reactor per Cycle (mg/dm$^3$) | NH$_4^+$-N Concentration in the Reactor per Cycle (mg/dm$^3$) | NO$_2^-$-N Concentration in the Reactor per Cycle (mg/dm$^3$) | NO$_3^-$-N Concentration in the Reactor per Cycle (mg/dm$^3$) |
|---------|-------|--------------------------|-----------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| R1      | 0–90  | 4                        | 6                     | 11                                                            | 44                                                            | 38                                                            | 152                                                           |
| R2      | 0–36  | 4                        | 6                     | 180                                                           | 38                                                            | 152                                                           | 50                                                            |
|         | 37–44 | 8                        | 3                     | 45                                                            | 360                                                           | 304                                                           | 408                                                           |
|         | 65–90 | 4                        | 6                     | 180                                                           | 38                                                            | 152                                                           | 50                                                            |

In each cycle, 2 dm$^3$ of supernatant water was withdrawn from both reactors and replaced with a new portion of the synthetic substrate. The most important ingredients—i.e., nitrite, ammonium and sulfate—were supplied in the form of NH$_4$Cl, NaNO$_2$, and MgSO$_4$, respectively.

2.3. Analytical Methods

The concentration of NO$_3^-$-N, NO$_2^-$-N and NH$_4^+$-N compounds was determined using a DR 3900 spectrophotometer using cuvette tests from Hach Lange GmbH (Dusseldorf, Germany) for analysis. The biomass concentrations were determined as a volatile suspended solids (VSS) fraction of the total suspended solids (TSS) in accordance with the standard methods [28]. The biomass-specific AUR, SAA and nitrate production rate (NPR) were determined based on the maximum slope of NH$_4^+$-N consumption, NH$_4^+$-N combined with NO$_2^-$-N consumption and NO$_3^-$-N production in the reaction phase divided into mixed liquor volatile suspended solids (MLVSS) concentrations, respectively. Throughout the operation period, the MLVSS value was 1750 (±50) mg/dm$^3$ in R1 and 1900 (±50) mg/dm$^3$ in R2. AUR, SAA and NPR are given in units of mg N/g VSS·h to represent these rates in relation to the indicated MLVSS.

3. Results and Discussion

The efficiency of NH$_4^+$-N oxidation in anaerobic conditions is influenced by anammox, sulfammox, heterotrophic and autotrophic (full and partial) denitrification processes. On the other hand, under aerobic conditions, the oxidation of NH$_4^+$-N takes place in the process of nitrification or partial nitrification. In our studies, SBR controlled DO at a low level (<0.2 mg/dm$^3$), and the lack of an added external carbon source prevented the occurrence of heterotrophic conditions. Accordingly, the only possible pathways for NH$_4^+$-N oxidation were through anammox, sulfammox and sulfur-dependent autotrophic denitrification.

Previous studies describe the complete efficiency of NH$_4^+$-N and SO$_4^{2-}$ removal as a combination of anammox, sulfammox, nitrification and denitrification [10,11,20,26] or a result of anaerobic processes only [21,29] or of the sulfammox reaction only [30,31] (see Table 2). Moreover, it is worth noting that a few studies on the anaerobic oxidation of NH$_4$-N in the presence of SO$_4^{2-}$ have been carried out with NO$_2$-N [10,26]. Some of them consisted of only replacing NO$_2$-N with a new electron acceptor in the form of SO$_4^{2-}$ [11,31], yet the vast majority of the oxidation took place without NO$_2$-N [9,11,20,21,23,29,30].
Table 2. Concentrations of influent NH$_4$-$\cdot$N and SO$_4^{2-}$ and the efficiency of their removal under anaerobic conditions. SRAO: sulfate-reducing ammonium oxidation; SRB: sulfate-reducing bacteria.

| Reactor Type                                      | Influent NH$_4$-$\cdot$N $\text{ (mg dm}^{-3}\text{)}$ | Influent SO$_4^{2-}$ $\text{ (mg dm}^{-3}\text{)}$ | NH$_4$-$\cdot$N Removal Efficiency (%) | SO$_4^{2-}$ Removal Efficiency (%) | Brief Characteristics                                                                 | Reference |
|--------------------------------------------------|-------------------------------------------------------|--------------------------------------------------|----------------------------------------|-----------------------------------|----------------------------------------------------------------------------------------|-----------|
| Combining system: Upflow Anaerobic Sludge Blanket (UASB), Anoxic/Oxic Reactor (A/O), Anammox and Sulfammox Reactor (ANAOR), Anaerobic Sequencing Batch Reactor (ASBR) | 610–700                                               | 1870–1920                                        | ca. 98                                 | ca. 53                             | Reduction of SO$_4^{2-}$ and NH$_4$-$\cdot$N was considered as a combination of anammox, sulfammox, nitrification and denitrification processes. | [26]      |
| Continuous Flow Stirred Tank Reactor (CFSTR)     | 110                                                   | 180                                              | ca. 40                                 | ca. 0                              | SRAO was considered as a combination of aerobic ammonium oxidation, anammox and heterotrophic sulfate reduction processes. | [11]      |
| Self-Designed Circulating Flowreactor (SDCF)      | 120                                                   | 183                                              | ca. 30                                 | ca. 40                             | 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 NO$_2$-$\cdot$N. | [10]      |
| Self-Designed Circulating Flowreactor (SDCF)      | 160                                                   | 216                                              | ca. 30                                 | ca. 0                              | Part of nitrogen was converted by nitrification–denitrification and conventional anammox, simultaneously with SRAO. | [20]      |
| Expanded Granular Sludge Bed (EGSB)               | 166–666                                               | 360                                              | 40–58                                  | 64–71                             | SBR and denitrifying bacteria were mainly responsible for SO$_4^{2-}$ and nitrogen removal. | [21]      |
| ESB (EGSB)                                        | 1000–2000                                             | 600                                              | 40–70                                  | 66–82                             |                                                                                       |           |
|                                                   | >3000                                                 | 360                                              | 10–25                                  | 28                                |                                                                                       |           |
| Anaerobic Sequencing Batch Reactor (ASBR)         | 97                                                    | 261                                              | ca. 88                                 | ca. 19                             | The presence of Planctomycetes revealed that anammox was highly involved in NH$_4$-$\cdot$N removal, even without NO$_2$-$\cdot$N in the feed. Other autotrophic denitrifying bacteria, related to the species Paracoccus Denitrificans, were also present. These bacteria utilize S$^0$ as an electron donor, produce SO$_4^{2-}$ and competitively use NO$_2$-$\cdot$N with anammox. | [29]      |
| Expanded Bed Reactor (EBR)                       | 229                                                   | 163                                              | ca. 44                                 | 40                                | The reduction of SO$_4^{2-}$ and NH$_4$-$\cdot$N was considered as sulfammox only. | [30]      |
| Upflow Anaerobic Sludge Blanket Reactor (UASBR)   | 50–60                                                 | 210–240                                          | 40                                     | 30                                | The reduction of SO$_4^{2-}$ and NH$_4$-$\cdot$N was considered as sulfammox only. | [31]      |
| Non-Woven Rotating Biological Contactor (NWRBC)    | 198                                                   | 528                                              | 100                                    | 70                                | The reduction of SO$_4^{2-}$ and NH$_4$-$\cdot$N was considered as a sulfammox only. | [9]       |
| Anaerobic Attached-Growth Bioreactor (AAGB)       | 50                                                    | 57                                               | ca. 43                                 | ca. 59                             | The reduction of SO$_4^{2-}$ and NH$_4$-$\cdot$N was considered as a sulfammox only. | [23]      |
A study by Zhang et al. [10] investigated the effect of NO$_2$-N on the anaerobic oxidation of NH$_4$-N. They showed that, with a combined decrease in concentration of SO$_4^{2-}$ from 216 to 100 mg/dm$^3$, NH$_4$-N from 183 to 80 mg/dm$^3$ and NO$_2$-N from 34 to 28 mg/dm$^3$, the efficiency of NH$_4$-N removal increased from 55% to 100%. However, this study does not clearly show the influence of SO$_4^{2-}$ itself on the process. In our study, we decided to keep the NH$_4$-N and NO$_2$-N inflow to the reactors unchanged in order to determine the influence of SO$_4^{2-}$ on the process.

In R1, where the influent SO$_4^{2-}$ concentration was constant at 22 mg SO$_4^{2-}$/dm$^3$, a gradual increase in the rates of AUR and SAA could be observed as well as their stabilization from day 49, as shown in Figure 2a. Comparing these values with the values in R2 in Figure 2b, it can be seen that, despite the approximately four-fold higher SO$_4^{2-}$ concentration in the effluents in R2 (90 mg SO$_4^{2-}$/dm$^3$ for R2), the AUR and SAA showed similar values from the beginning of the test to day 29. The AUR increased from 1.3 mg N/g VSS-h to 2.1 mg N/g VSS-h (R1) and from 1.1 mg N/g VSS-h to 2.1 mg N/g VSS-h (R2), while the SAA increased from 4 mg N/g VSS-h to 5.6 mg N/g VSS-h (R1) and 3.7 mg N/g VSS-h to 5.3 mg N/g VSS-h (R2).

![Figure 2](image_url)

**Figure 2.** Ammonia utilization rate (AUR), specific annamox activity (SAA) and nitrate production rate (NPR) in R1 (a) and in R2 (b).

On day 37, there was a clear increase in AUR, SAA and NPR in R1. This showed that the efficiency of the anammox process was greatly improved as more NH$_4$-N was oxidized with NO$_2$-N. The increase in NPR also confirmed that more NH$_4$-N was oxidized as approximately 11% was converted to NO$_3$-N in this process.

Near the end of the study, there was a stabilization of AUR values, SAA and a decrease in NPR in R1. AUR increased to a maximum of 4.4 mg N/g VSS-h, and SAA increased to 8.1 mg N/g VSS-h.

In R2, on day 37, the cycle time was reduced from 6 h to 3 h, which resulted in the concentration of SO$_4^{2-}$ being twice as high as in the previous period: −360 mg/dm$^3$-d and 180 mg/dm$^3$-d for phases II and I, respectively. This affected the AUR and SAA significantly, as can be seen in Figure 2b. This increase was evident throughout phase II. The AUR value at the end of this phase was 9.7 mg N/g VSS-h, while SAA was 22.5 mg N/g VSS-h. This confirmed the positive influence of SO$_4^{2-}$ on the course of the NH$_4$-N oxidation process. SO$_4^{2-}$ seems to be an additional acceptor that improves the rate and efficiency of the process, increasing the efficiency of NH$_4$-N removal as shown in Figure 3.
There are reports in the literature confirming that SO$_4^{2-}$ can increase the total removal of NH$_4$-N. Liu et al. [9] noted in his research that the NH$_4$-N removal rate was always higher than expected and the NH$_4$-N/NO$_2$-N consumption ratio was about 1.1:1, which was much higher than previously reported [32]. It was then concluded that, due to large amounts of (NH$_4$)$_2$SO$_4$ in the feed, SO$_4^{2-}$ could be the source of the additional electron acceptor.

Moreover, Yang et al. [31] noted that as the concentration of NH$_4$-N and SO$_4^{2-}$ increased, incrementally more of both were removed in their batch tests. When the NH$_4$-N and SO$_4^{2-}$ concentrations in the inflow were approximately 28 and 76 mg/dm$^3$, respectively, the removal efficiency was close to 0%. However, when the average NH$_4$-N and SO$_4^{2-}$ concentrations in the inflow increased to 92 and 307 mg/dm$^3$, the removed amount decreased to 40 and 130 mg/dm$^3$, respectively. Thus, high concentrations of NH$_4$-N and SO$_4^{2-}$ may promote the simultaneous removal of these compounds, as shown in our research.

Phase III in R2 showed a downward trend in AUR and anammox rates from 9.7 mg N/g VSS-h to 7.1 mg N/g VSS-h and from 22.5 mg N/g VSS-h to 18.7 mg N/g VSS-h, respectively. This was due to the reduction of the SO$_4^{2-}$ concentration flowing into the reactor. Again, fewer electron acceptors, in the form of SO$_4^{2-}$, were present in the environment; therefore, the rate of NH$_4$-N oxidation decreased because half as much SO$_4^{2-}$ flowed in per day. The tests were performed until the process stabilized, and constant values of AUR, SAA and NPR were achieved by the 90th day.

Moreover, Zhang et al. [20] noticed that, as the concentration of SO$_4^{2-}$ increased from about 90 mg/dm$^3$ to about 170 mg/dm$^3$ and NH$_4$-N from about 50 mg/dm$^3$ to about 120 mg/dm$^3$, the efficiency of NH$_4$-N removal increased from 40% to 90%. However, a further increase in the concentration of SO$_4^{2-}$ to about 360 mg/dm$^3$ and NH$_4$-N to about 180 mg/dm$^3$ resulted in a decrease in NH$_4$-N removal up to roughly 20%. Similarly, in an Expanded Granular Sludge Bed Reactor (EGSBR) [21] under chemical oxygen demand (COD) conditions, the NH$_4$-N removal efficiency gradually improved from 40–58% to 40–70% when the inflow NH$_4$-N concentrations increased from 166–666 mg N/dm$^3$ to 1000–2000 mg N/dm$^3$. Comparatively, after increasing the NH$_4$-N concentration to >3000 mg N/dm$^3$, the efficiency of NH$_4$-N reduction decreased to approximately 10–25%. This was due to the inhibition of the anammox process with free ammonia. This proves that an increase in NH$_4$-N and SO$_4^{2-}$ concentrations improves the process of anaerobic NH$_4$-N oxidation only to a certain extent. In our study, there was no inhibition of the process due to excessively high concentrations of these compounds.

Wu et al. [26] noted that they had achieved an NH$_4$-N removal efficiency of 98%, including 44% removed through sulfammosx. Compounds containing SO$_4^{2-}$ can therefore effectively improve the efficiency of the anaerobic oxidation of NH$_4$-N, but at the same time, anaerobic conditions favor the decomposition of SO$_4^{2-}$ to S$^0$, which is less toxic to the environment. The sulfammosx process has so far been studied mainly as an independent process (without NO$_2$-N addition). Moreover, there has been more interest in the influence of NH$_4$-N concentration on the sulfammosx process [21] and the N/S ratio [20] rather than directly considering the effect of SO$_4^{2-}$ itself.

There are reports in the literature confirming that SO$_4^{2-}$ can increase the total removal of NH$_4$-N. Liu et al. [9] noted in his research that the NH$_4$-N removal rate was always higher than expected and the NH$_4$-N/NO$_2$-N consumption ratio was about 1.1:1, which was much higher than previously reported [32]. It was then concluded that, due to large amounts of (NH$_4$)$_2$SO$_4$ in the feed, SO$_4^{2-}$ could be the source of the additional electron acceptor.

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Moreover, Zhang et al. [20] noticed that, as the concentration of SO$_4^{2-}$ increased from about 90 mg/dm$^3$ to about 170 mg/dm$^3$ and NH$_4$-N from about 50 mg/dm$^3$ to about 120 mg/dm$^3$, the efficiency of NH$_4$-N removal increased from 40% to 90%. However, a further increase in the concentration of SO$_4^{2-}$ to about 360 mg/dm$^3$ and NH$_4$-N to about 180 mg/dm$^3$ resulted in a decrease in NH$_4$-N removal up to roughly 20%. Similarly, in an Expanded Granular Sludge Bed Reactor (EGSBR) [21] under chemical oxygen demand (COD) conditions, the NH$_4$-N removal efficiency gradually improved from 40–58% to 40–70% when the inflow NH$_4$-N concentrations increased from 166–666 mg N/dm$^3$ to 1000–2000 mg N/dm$^3$. Comparatively, after increasing the NH$_4$-N concentration to >3000 mg N/dm$^3$, the efficiency of NH$_4$-N reduction decreased to approximately 10–25%. This was due to the inhibition of the anammox process with free ammonia. This proves that an increase in NH$_4$-N and SO$_4^{2-}$ concentrations improves the process of anaerobic NH$_4$-N oxidation only to a certain extent. In our study, there was no inhibition of the process due to excessively high concentrations of these compounds.

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Bi et al. [11] challenged the sulfammox process and postulated that AAOBs did not have the ability to oxidize NH$_4^+$-N using SO$_4^{2-}$ as an electron acceptor and that SRAO was a combination of aerobic ammonium oxidation, anammox and heterotrophic sulfate reduction processes. Moreover, the specification of the efficiency of NH$_4^+$-N and SO$_4^{2-}$ removal in the sulfammox process does not reflect the course of the process as thoroughly as the AUR and the SAA, which the authors do not provide in their research.

4. Conclusions

In this study, it was shown that SO$_4^{2-}$ could be used as an additional electron acceptor in the anaerobic oxidation of NH$_4^+$-N. Along with the increased share of SO$_4^{2-}$, both AUR and SAA showed an increasing trend. In R1, where the concentration of SO$_4^{2-}$ in the inflow was constant at the level of 22 mg SO$_4^{2-}$/dm$^3$, there was a gradual increase in the AUR and SAA indicators from 1.2 mg N/g VSS·h to 4.4 mg N/g VSS·h and from 3.9 mg N/g VSS·h to 8.2 mg N/g VSS·h, respectively. In R2 in phase I, over a 6 h cycle, AUR and SAA increased from 1 mg N/g VSS·h to 2.1 mg N/g VSS·h and from 3.6 mg N/g VSS·h to 5.3 mg N/g VSS·h; in phase II, over a 3 h cycle, they increased to 9.7 mg N/g VSS·h and 22.5 mg N/g VSS·h; and in phase III, over a 3 h cycle, they dropped to 7.1 mg N/g VSS·h and 18.8 mg N/g VSS·h, respectively. It can therefore be concluded that SO$_4^{2-}$ contributes to the rate and efficiency of the anammox process. Further studies on the influence of the NH$_4^+$-N/SO$_4^{2-}$ ratio on the process and identification of the bacteria responsible for sulfammox are suggested.

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