

Article

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

Dominika Grubba * and Joanna Majtacz

Faculty of Civil and Environmental Engineering, Gdansk University of Technology, Narutowicza Street 11/12, 80-233 Gdansk, Poland; joamajta@pg.edu.pl

* Correspondence: dominika.grubba@pg.edu.pl

Received: 15 September 2020; Accepted: 25 October 2020; Published: 26 October 2020

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 sulfamox process. Sulfate-containing compounds are part of the medium for the anammox process, but their concentrations are not particularly high ($0.2 \text{ g MgSO}_4 \cdot 7\text{H}_2\text{O}/\text{dm}^3$ and $0.00625 \text{ g FeSO}_4/\text{dm}^3$). 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 hour cycle, and the concentration of the inflowing sulfate was kept at $44 \text{ mg}/\text{dm}^3\cdot\text{d}$. The second reactor (R2) was operated until the 36th day in a 6 hour cycle; the influencing concentration was $180 \text{ mg SO}_4^{2-}/\text{dm}^3\cdot\text{d}$ from the 37th to 64th day in a 3 hour cycle, with an influencing concentration of $360 \text{ mg SO}_4^{2-}/\text{dm}^3\cdot\text{d}$; and from the 65th to 90th day, the reactor was operated again in a 6 hour cycle with an influencing concentration of $180 \text{ mg SO}_4^{2-}/\text{dm}^3\cdot\text{d}$. 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: sulfamox; anammox; sulfate; ammonium utilization rate; specific anammox activity

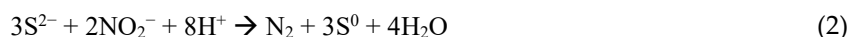
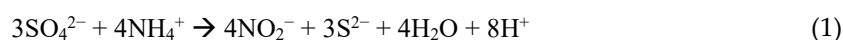
1. Introduction

Several industrial processes such as fermentation, tanning, landfill leachate production, paper production, pharmaceutical production and food processes produce wastewater containing high concentrations of sulfate (SO_4^{2-}) and ammonium nitrogen ($\text{NH}_4\text{-N}$) [1]. Such sewage requires treatment before discharge to the environment, as it is harmful to human life [2].

SO_4^{2-} is conventionally removed by anaerobic processes by sulfate-reducing bacteria (SRB) [3,4], where SO_4^{2-} 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 ($\text{NH}_4\text{-N}$) under anoxic conditions to gaseous nitrogen (N_2), using nitrite nitrogen ($\text{NO}_2\text{-N}$) as the electron acceptor, via anaerobic ammonia-oxidizing bacteria (AAOB). Accordingly, the removal of SO_4^{2-} and $\text{NH}_4\text{-N}$ 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

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 $\text{NH}_4\text{-N}$ and deoxidation of SO_4^{2-} in three equations (1–3):



At first, $\text{NH}_4\text{-N}$ is partially oxidized and deoxygenated by SO_4^{2-} to produce $\text{NO}_2\text{-N}$ and sulfides (S^{2-}) (see reaction 1). Then, some of the $\text{NO}_2\text{-N}$ 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 $\text{NO}_2\text{-N}$, there may be other electron acceptors that oxidize $\text{NH}_4\text{-N}$ under anaerobic conditions [10]. The process described in reactions 1–3 is called the sulfamox process (i.e., sulfate-reducing ammonium oxidation (SRAO)) [11]. The sulfamox 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):



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 $\text{NH}_4\text{-N}$ is more beneficial in terms of reducing costs than the separate removal of these pollutants [14]. The discovery of the sulfamox 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 sulfamox. 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 ($\text{S}_2\text{O}_3^{2-}$) as electron donors with $\text{NO}_3\text{-N}$ or $\text{NO}_2\text{-N}$ 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 $\text{NH}_4\text{-N}$ oxidation. The sulfamox process can run independently without the addition of $\text{NO}_2\text{-N}$ or in combination with the conventional ($\text{NO}_2\text{-N}$ based) anammox process. Research on the sulfamox 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 $\text{NO}_2\text{-N}$ [9,11,20–23]. Other studies started with a conventional anammox, with $\text{NO}_2\text{-N}$ as an electron acceptor, and replaced $\text{NO}_2\text{-N}$ with a new SO_4^{2-} electron acceptor [11,12,24]. There are also reports in which SO_4^{2-} was used simultaneously with $\text{NO}_2\text{-N}$ 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 $\text{NH}_4\text{-N}$ and SO_4^{2-} , in the range of 92–99% and 53–60%, respectively, when anammox and sulfamox 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 $\text{NH}_4\text{-N}$ and N/S ratio in relation to the sulfamox process [10,20,21]. The influence of increased proportions of SO_4^{2-} on anaerobic $\text{NH}_4\text{-N}$ oxidation in the presence of $\text{NO}_2\text{-N}$ 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 $\text{NH}_4\text{-N}$.

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 dm³ 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.

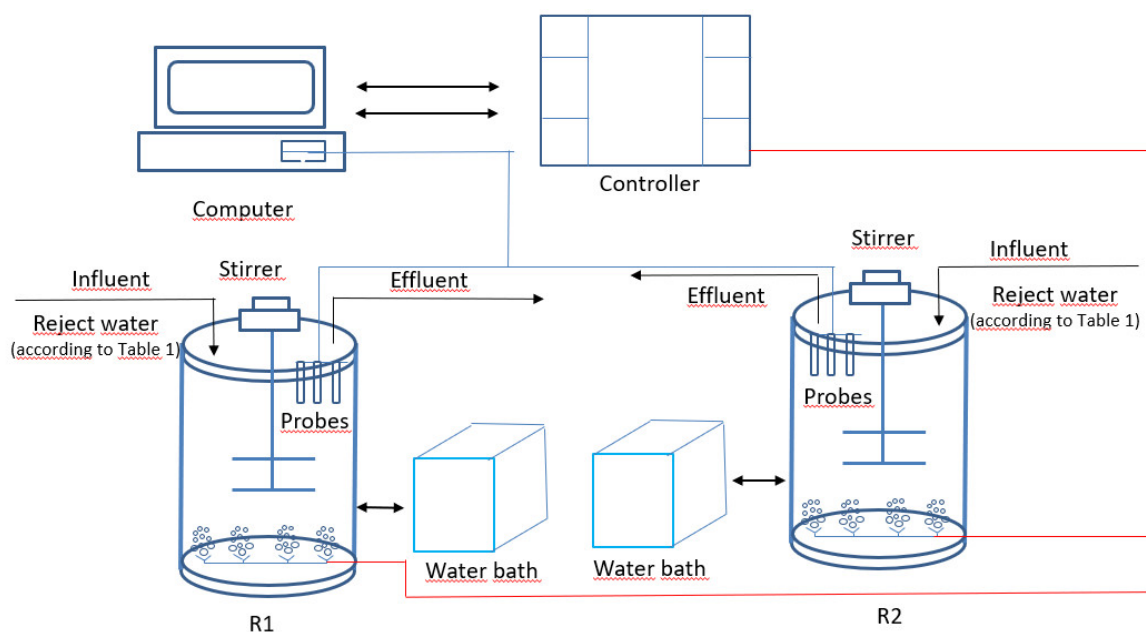


Figure 1. Laboratory-scale system for the anammox process.

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 $30 (\pm 1)$ °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/dm³, and SBRs were fed with synthetic substrate according to the method of Dapena-Mora et al. [27] and Table 1.

In each cycle, 2 dm³ 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_4Cl , NaNO_2 and MgSO_4 , respectively.

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		NH ₄ -N Concentration in the Reactor		NO ₂ -N Concentration in the Reactor	
				per Cycle (mg/dm ³)	per Day (mg/(dm ³ -d))	per Cycle (mg/dm ³)	per Day (mg/(dm ³ -d))	per Cycle (mg/dm ³)	per Day (mg/(dm ³ -d))
				R1	0–90	4	6	11	44
	0–36	4	6		180		152		200
R2	37–64	8	3		360	38	304	50	400
	65–90	4	6	45	180		152		200

2.3. Analytical Methods

The concentration of NO₃-N, NO₂-N and NH₄-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 NH₄-N consumption, NH₄-N combined with NO₂-N consumption and NO₃-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/dm³ in R1 and 1900 (±50) mg/dm³ 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 NH₄-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 NH₄-N takes place in the process of nitrification or partial nitrification. In our studies, SBR controlled DO at a low level (<0.2 mg/dm³), and the lack of an added external carbon source prevented the occurrence of heterotrophic conditions. Accordingly, the only possible pathways for NH₄-N oxidation were through anammox, sulfammox and sulfur-dependent autotrophic denitrification.

Previous studies describe the complete efficiency of NH₄-N and SO₄²⁻ 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 NH₄-N in the presence of SO₄²⁻ have been carried out with NO₂-N [10,26]. Some of them consisted of only replacing NO₂-N with a new electron acceptor in the form of SO₄²⁻ [11,31], yet the vast majority of the oxidation took place without NO₂-N [9,11,20,21,23,29,30].

A study by Zhang et al. [10] investigated the effect of NO₂-N on the anaerobic oxidation of NH₄-N. They showed that, with a combined decrease in concentration of SO₄²⁻ from 216 to 100 mg/dm³, NH₄-N from 183 to 80 mg/dm³ and NO₂-N from 34 to 28 mg/dm³, the efficiency of NH₄-N removal increased from 55% to 100%. However, this study does not clearly show the influence of SO₄²⁻ itself on the process. In our study, we decided to keep the NH₄-N and NO₂-N inflow to the reactors unchanged in order to determine the influence of SO₄²⁻ on the process.

In R1, where the influent SO₄²⁻ concentration was constant at 22 mg SO₄²⁻/dm³, 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 SO₄²⁻ concentration in the effluents in R2 (90 mg SO₄²⁻/dm³ 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).

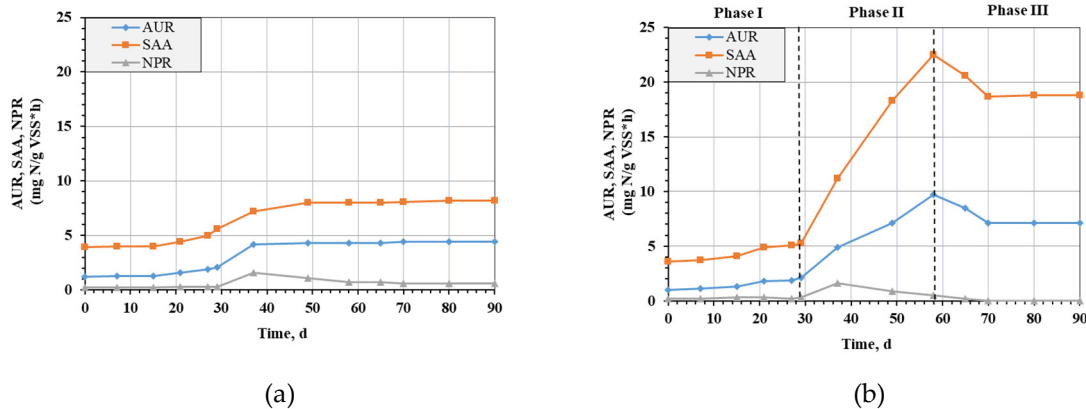


Figure 2. Ammonia utilization rate (AUR), specific annamox activity (SAA) and nitrate production rate (NPR) in R1 (a) and in R2 (b).

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 $\text{NH}_4\text{-N}$ was oxidized with $\text{NO}_2\text{-N}$. The increase in NPR also confirmed that more $\text{NH}_4\text{-N}$ was oxidized as approximately 11% was converted to $\text{NO}_3\text{-N}$ 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: $-360 \text{ mg/dm}^3\cdot\text{d}$ and $180 \text{ mg/dm}^3\cdot\text{d}$ 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 $\text{NH}_4\text{-N}$ oxidation process. SO_4^{2-} seems to be an additional acceptor that improves the rate and efficiency of the process, increasing the efficiency of $\text{NH}_4\text{-N}$ removal as shown in Figure 3.

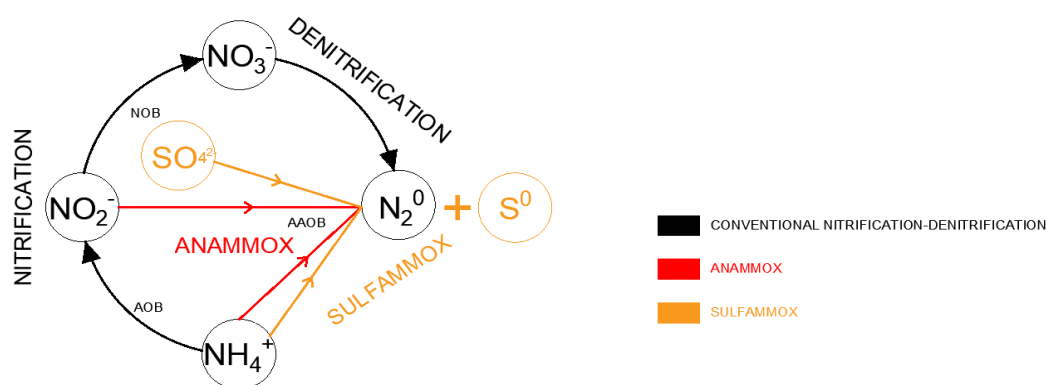


Figure 3. Diagram showing the coexistence of anammox and sulfamnox processes.

There are reports in the literature confirming that SO_4^{2-} can increase the total removal of $\text{NH}_4\text{-N}$. Liu et al. [9] noted in his research that the $\text{NH}_4\text{-N}$ removal rate was always higher than expected and the $\text{NH}_4\text{-N}/\text{NO}_2\text{-N}$ 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 $(\text{NH}_4)_2\text{SO}_4$ 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 $\text{NH}_4\text{-N}$ and SO_4^{2-} increased, incrementally more of both were removed in their batch tests. When the $\text{NH}_4\text{-N}$ 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 $\text{NH}_4\text{-N}$ 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 $\text{NH}_4\text{-N}$ 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 $\text{mg N}/\text{g VSS}\cdot\text{h}$ to 7.1 $\text{mg N}/\text{g VSS}\cdot\text{h}$ and from 22.5 $\text{mg N}/\text{g VSS}\cdot\text{h}$ to 18.7 $\text{mg N}/\text{g VSS}\cdot\text{h}$, 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 $\text{NH}_4\text{-N}$ 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 $\text{NH}_4\text{-N}$ from about 50 mg/dm^3 to about 120 mg/dm^3 , the efficiency of $\text{NH}_4\text{-N}$ removal increased from 40% to 90%. However, a further increase in the concentration of SO_4^{2-} to about 360 mg/dm^3 and $\text{NH}_4\text{-N}$ to about 180 mg/dm^3 resulted in a decrease in $\text{NH}_4\text{-N}$ removal up to roughly 20%. Similarly, in an Expanded Granular Sludge Bed Reactor (EGSBR) [21] under chemical oxygen demand (COD) conditions, the $\text{NH}_4\text{-N}$ removal efficiency gradually improved from 40–58% to 40–70% when the inflow $\text{NH}_4\text{-N}$ concentrations increased from 166–666 $\text{mg N}/\text{dm}^3$ to 1000–2000 $\text{mg N}/\text{dm}^3$. Comparatively, after increasing the $\text{NH}_4\text{-N}$ concentration to >3000 $\text{mg N}/\text{dm}^3$, the efficiency of $\text{NH}_4\text{-N}$ 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 $\text{NH}_4\text{-N}$ and SO_4^{2-} concentrations improves the process of anaerobic $\text{NH}_4\text{-N}$ 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 $\text{NH}_4\text{-N}$ removal efficiency of 98%, including 44% removed through sulfammox. Compounds containing SO_4^{2-} can therefore effectively improve the efficiency of the anaerobic oxidation of $\text{NH}_4\text{-N}$, 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 sulfammox process has so far been studied mainly as an independent process (without $\text{NO}_2\text{-N}$ addition). Moreover, there has been more interest in the influence of $\text{NH}_4\text{-N}$ concentration on the sulfammox process [21] and the N/S ratio [20] rather than directly considering the effect of SO_4^{2-} itself.

Bi et al. [11] challenged the sulfammox process and postulated that AAOBs did not have the ability to oxidize $\text{NH}_4\text{-N}$ 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 $\text{NH}_4\text{-N}$ 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.

Table 2. Concentrations of influent $\text{NH}_4\text{-N}$ and SO_4^{2-} and the efficiency of their removal under anaerobic conditions. SRAO: sulfate-reducing ammonium oxidation; SRB: sulfate-reducing bacteria.

Reactor	Influent $\text{NH}_4\text{-N}$ (mg/dm ³)	Influent SO_4^{2-} (mg/dm ³)	$\text{NH}_4\text{-N}$ Removal Efficiency (%)	SO_4^{2-} Removal Efficiency (%)	Brief Characteristics	Reference
Combining system: Upflow Anaerobic Sludge Blanket (UASB), Anoxic/Oxic Reactor (A/O), Anammox and Sulfamox Reactor (ANAOR), Anaerobic Sequencing Batch Reactor (ASBR)	610–700	1870–1920	ca. 98	ca. 53	Reduction of SO_4^{2-} and $\text{NH}_4\text{-N}$ was considered as a combination of anammox, sulfamox, nitrification and denitrification processes.	[26]
Continuous Flow Stirred Tank Reactor (CFSTR)	110	0–110	ca. 40	ca. 0	SRAO was considered as a combination of aerobic ammonium oxidation, anammox and heterotrophic sulfate reduction processes.	[11]
	60	90	ca. 30	ca. 10		
	60	90	ca. 55	ca. 0		
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 $\text{NO}_2\text{-N}$.	[10]
	160	216	ca. 55	ca. 0		
	110	116	ca. 75	ca. 30		
	80	100	ca. 100	ca. 45		
Self-Designed Circulating Flowreactor (SDCF)	50	90	ca. 40	ca. 30	Part of nitrogen was converted by nitrification–denitrification and conventional anammox, simultaneously with SRAO.	[20]
	120	170	ca. 90	ca. 30		
	180	360	ca. 20	ca. 5		
Expanded Granular Sludge Bed (EGSB)	166–666		40–58	64–71	SRB and denitrifying bacteria were mainly responsible for SO_4^{2-} and nitrogen removal.	[21]
	1000–2000	3600	40–70	66–82		
	>3000		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 $\text{NH}_4\text{-N}$ removal, even without $\text{NO}_2\text{-N}$ in the feed. Other autotrophic denitrifying bacteria, related to the species <i>Paracoccus Denitrificans</i> , were also present. These bacteria utilize S^0 as an electron donor, produce SO_4^{2-} and competitively use $\text{NO}_2\text{-N}$ with anammox.	[29]
Expanded Bed Reactor (EBR)	229	163	ca. 44	40	The reduction of SO_4^{2-} and $\text{NH}_4\text{-N}$ 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 $\text{NH}_4\text{-N}$ was considered as sulfammox only.	[31]
Non-Woven Rotating Biological Contactor (NWRBC)	ca. 198	ca. 528	ca. 100	ca. 70	The reduction of SO_4^{2-} and NH_4^+ 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^+ was considered as a sulfammox only.	[23]



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 $\text{NH}_4\text{-N}$. 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 $22 \text{ mg SO}_4^{2-}/\text{dm}^3$, there was a gradual increase in the AUR and SAA indicators from $1.2 \text{ mg N/g VSS}\cdot\text{h}$ to $4.4 \text{ mg N/g VSS}\cdot\text{h}$ and from $3.9 \text{ mg N/g VSS}\cdot\text{h}$ to $8.2 \text{ mg N/g VSS}\cdot\text{h}$, respectively. In R2 in phase I, over a 6 hour cycle, AUR and SAA increased from $1 \text{ mg N/g VSS}\cdot\text{h}$ to $2.1 \text{ mg N/g VSS}\cdot\text{h}$ and from $3.6 \text{ mg N/g VSS}\cdot\text{h}$ to $5.3 \text{ mg N/g VSS}\cdot\text{h}$; in phase II, over a 3 hour cycle, they increased to $9.7 \text{ mg N/g VSS}\cdot\text{h}$ and $22.5 \text{ mg N/g VSS}\cdot\text{h}$; and in phase III, over a 3 hour cycle, they dropped to $7.1 \text{ mg N/g VSS}\cdot\text{h}$ and $18.8 \text{ mg N/g VSS}\cdot\text{h}$, 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 $\text{NH}_4\text{-N}/\text{SO}_4^{2-}$ ratio on the process and identification of the bacteria responsible for sulfammox are suggested.

Author Contributions: Conceptualization, D.G. and J.M.; methodology, J.M.; software, J.M.; validation, J.M.; formal analysis, J.M.; investigation, D.G.; resources, J.M.; data curation, J.M.; writing—original draft preparation, D.G.; writing—review and editing, J.M.; visualization, D.G.; supervision, J.M.; project administration, D.G.; funding acquisition, J.M.

Funding: This research was funded by Narodowe Centrum Nauki grant number 2019/03/X/ST10/01127.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Duyar, A.; Ozdemir, S.; Akman, D.; Akgul, V.; Sahinkaya, E.; Cirik, K. Optimization of sulfide-based autotrophic denitrification process in an anaerobic baffled reactor. *J. Chem. Technol. Biotechnol.* **2018**, *93*, 754–760.
- Liu, C.; Zhao, D.; Yan, L.; Wang, A.; Gu, Y.; Lee, D. Elemental sulfur formation and nitrogen removal from wastewaters by autotrophic denitrifiers and anammox bacteria. *Bioresour. Technol.* **2015**, *191*, 332–336.
- Sarti, A.; Pozzi, E.; Chinalia, F.A.; Ono, A.; Foresti, E. Microbial processes and bacterial populations associated to anaerobic treatment of sulfate-rich wastewater. *Process Biochem.* **2010**, *45*, 164–170.
- Wei, C.; Wang, W.; Deng, Z.; Wu, C. Characteristics of high-sulfate wastewater treatment by two-phase anaerobic digestion process with Jet-loop anaerobic fluidized bed. *J. Environ. Sci.* **2007**, *19*, 264–270.
- Sinbuathong, N.; Khaodhiar, S.; Liengcharernsit, W.; Sirirote, P.; Watts, D. Effect of sulfate on the methanogenic activity of a bacterial culture from a brewery wastewater during glucose degradation. *J. Environ. Sci.* **2007**, *19*, 1025–1027.
- Zhang, D.; Cui, L.; Zhu, H.; Madani, R.M.A.; Liang, J. Treatment performance and microbial community under ammonium sulphate wastewater in a sulphate reducing ammonium oxidation process. *Environ. Technol.* **2020**, doi:10.1080/09593330.2020.1720305.
- Fdz-Polanco, F.; Fdz-Polanco, M.; Fernandez, N.; Uruña, M.A.; Garcia, P.A.; Villaverde, S. New process for simultaneous removal of nitrogen and sulphur under anaerobic conditions. *Water Res.* **2001**, *35*, 1111–1114.
- Kartal, B.; van Niftrik, L.; Keltjens, J.T.; Op den Camp, H.J.M.; Jetten, M.S.M. Anammox-Growth Physiology, Cell Biology, and Metabolism. *Adv. Microb. Physiol.* **2012**, *60*, 211–262.
- Liu, S.; Yang, F.; Gong, Z.; Meng, F.; Chen, H.; Xue, Y.; Furukawa, K. Application of anaerobic ammonium-oxidizing consortium to achieve completely autotrophic ammonium and sulfate removal. *Bioresour. Technol.* **2008**, *99*, 6817–6825.
- In 't Zandt, M.H.; de Jong, A.E.; Slomp, C.P.; Jetten, M.S. The hunt for the most-wanted chemolithoautotrophic spookmicrobes. *FEMS Microbiol. Ecol.* **2018**, *94*, fiy064.
- Bi, Z.; Wanyan, D.; Li, X.; Huang, Y. Biological conversion pathways of sulfate reduction ammonium oxidation in anammox consortia. *Front. Environ. Sci. Eng.* **2020**, *14*, 38.
- Rikmann, E.; Zekker, I.; Tomingas, M.; Tenno, T.; Loorits, L.; Vabamäe, P.; Mandel, A.; Raudkivi, M.; Daija, L.; Kroon, K.; et al. Sulfate-reducing anammox for sulfate and nitrogen containing wastewaters. *Desalin. Water Treat.* **2016**, *57*, 3132–3141.

13. Ali, M.; Okabe, S. Anammox-based technologies for nitrogen removal: Advances in process start-up and remaining issues. *Chemosphere* **2015**, *141*, 144–153.
14. Klein, K.; Kattel, E.; Goi, A.; Kivi, A.; Dulova, N.; Saluste, A.; Zekker, I.; Trapido, M.; Tenno, T. Combined treatment of pyrogenic wastewater from oil shale retorting. *Oil Shale* **2017**, *34*, 82–96.
15. Beller, H.R.; Chain, P.S.G.; Letain, T.E.; Chakicherla, A.; Larimer, F.W.; Richardson, P.M.; Coleman, M.A.; Wood, A.P.; Kelly, D.P. The genome sequence of the obligately chemolithoautotrophic, facultatively anaerobic bacterium *Thiobacillus denitrificans*. *J. Bacteriol.* **2006**, *188*, 1473–1488.
16. Yu, H.; Wang, A.; Chen, C. Structure and dynamics of microbial community in the denitrifying sulfide removal process. *Huanjing Kexue* **2013**, *34*, 1190–1195.
17. Wang, X.; Sun, G.; Zhu, Y. Thermodynamic energy of anaerobic microbial redox reactions couples elemental biogeochemical cycles. *J. Soils Sed.* **2017**, *17*, 2831–2846.
18. Di Capua, F.; Pirozzi, F.; Lens, P.N.L.; Esposito, G. Electron donors for autotrophic denitrification. *Chem. Eng. J.* **2019**, *362*, 922–937.
19. Kosugi, Y.; Matsuura, N.; Liang, Q.; Yamamoto-Ikemoto, R. Nitrogen flow and microbial community in the anoxic reactor of “Sulfate Reduction, Denitrification/Anammox and Partial Nitrification” process. *Biochem. Eng. J.* **2019**, *151*, 107304.
20. Zhang, D.; Cui, L.; Wang, H.; Liang, J. Study of sulfate-reducing ammonium oxidation process and its microbial community composition. *Water Sci. Technol.* **2019**, *79*, 137–144.
21. Wang, D.; Liu, B.; Ding, X.; Sun, X.; Liang, Z.; Sheng, S.; Du, L. Performance evaluation and microbial community analysis of the function and fate of ammonia in a sulfate-reducing EGSB reactor. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 7729–7739.
22. Zhang, L.; Zheng, P.; He, Y.; Jin, R. Performance of sulfate-dependent anaerobic ammonium oxidation. *Sci. China Ser. B* **2009**, *52*, 86–92.
23. Zhao, Q.I.; Li, W.; You, S.J. Simultaneous removal of ammonium-nitrogen and sulphate from wastewaters with an anaerobic attached-growth bioreactor. *Water Sci. Technol.* **2006**, *54*, 27–35.
24. Rikmann, E.; Zekker, I.; Tomingas, M.; Tenno, T.; Menert, A.; Looits, L.; Tenno, T. Sulfate-reducing anaerobic ammonium oxidation as a potential treatment method for high nitrogen-content wastewater. *Biodegradation* **2012**, *23*, 509–524.
25. Zhang, D.; Cui, L.; Madani, R.M.A.; Wang, H.; Zhu, H.; Liang, J. Effect of nitrite and nitrate on sulfate reducing ammonium oxidation. *Water Sci. Technol.* **2019**, *80*, 634–643.
26. Wu, L.; Yan, Z.; Li, J.; Huang, S.; Li, Z.; Shen, M.; Peng, Y. Low temperature advanced nitrogen and sulfate removal from landfill leachate by nitrite-anammox and sulfate-anammox. *Environ. Pollut.* **2020**, *259*, 113763.
27. Dapena-Mora, A.; Arrojo, B.; Campos, J.L.; Mosquera-Corral, A.; Méndez, R. Improvement of the settling properties of Anammox sludge in an SBR. *J. Chem. Technol. Biotechnol.* **2004**, *79*, 1417–1420.
28. Greenberg, A.E.; Clesceri, L.S.; Eaton, A.D. *APHA Standard methods for the examination of water and waste water, 21st edn.* American Public Health Association, American Water Works Association, Water Pollution Control Federation: Washington, DC, USA, 2005.
29. Prachakittikul, P.; Wantawin, C.; Noophan, P.; Boonapatcharoen, N. ANAMMOX-like performances for nitrogen removal from ammonium-sulfate-rich wastewater in an anaerobic sequencing batch reactor. *J. Environ. Sci. Health* **2016**, *51*, 220–228.
30. Cai, J.; Jiang, J.X.; Zheng, P. Isolation and identification of bacteria responsible for simultaneous anaerobic ammonium and sulfate removal. *Sci. China Chem.* **2010**, *53*, 645–650.

31. Yang, Z.; Zhou, S.; Sun, Y. Start-up of simultaneous removal of ammonium and sulfate from an anaerobic ammonium oxidation (anammox) process in an anaerobic up-flow bioreactor. *J. Hazard. Mater.* **2009**, *169*, 113–118.
32. Van De Graaf, A.A.; De Bruijn, P.; Robertson, L.A.; Jetten, M.S.M.; Kuenen, J.G. Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. *Microbiology* **1996**, *142*, 2187–2196.

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).