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Pilot-scale sulfur autotrophic denitrification biofilter in denitrification of pig farm wastewater biological tail water

Qing Zhang

TAWA Technology Co., Ltd., Beijing 100176, China, email: hfutzhangqing@hotmail.com

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ABSTRACT

Pig wastewater is a type of effluent referred to as biochemical tailwater, characterized by its high concentrations of total nitrogen and nitrate. The reintroduction of manure into agricultural fields, namely through the use of pig wastes biochemical tailwater, can potentially lead to challenges associated with nitrate contamination in groundwater. The objective of this study was to investigate the efficacy of the sulphur limestone autotrophic denitrification system in removing nitrate nitrogen from wastewater. This system utilizes sulphur monomers as electron donors and carbonate as a source of inorganic carbon. The most cost-effective method for mitigating nitrate nitrogen in pig effluent is the utilization of a sulphur limestone autotrophic denitrification system. The aforementioned methodology is very suitable for the removal of impurities in agricultural produce due to its ability to achieve a complete reaction without the need for the addition of an organic carbon source. The concentration of nitrate–nitrogen in the biochemical tail water of pig wastewater has been seen to decrease significantly, from an initial level of 42 mg/L to a final level of 8 mg/L, as a result of the denitrification process carried out in the sulphur limestone autoclave reactor. When the hydraulic retention time is set to 3 h, the reactor's volumetric loading can reach a value of 0.45 kg·NO_3–N/(m^3·d). The nitrate nitrogen present in pig effluent can be extracted through the utilization of a sulphur limestone autoclave reactor following the process of denitrification. The utilization of this reactor has the potential to efficiently reduce the content of nitrate–nitrogen in the biochemical tail water of pig effluent, so mitigating the issue of groundwater pollution when the treated manure water is reintroduced into the field.

Keywords: Pig farm biochemical effluent; Nitrate nitrogen pollution; Sulfur-limestone autotrophic denitrification bioreactor

1. Introduction

The rapid expansion of the swine industry in China necessitates the effective management of pollution arising from numerous pig farms. The wastewater generated from pig farming exhibits a higher content of organic matter, elevated concentrations of contaminants such as chemical oxygen demand (COD), and significant levels of ammonia nitrogen. There are primarily two strategies utilized for the treatment of pig wastewater. There are two primary approaches for treating pig dung water: the usual discharge mode, which involves treating it as industrial wastewater, and a combined approach that integrates planting and breeding with the purpose of utilizing manure and water resources. Irrespective of the specific discharge manner or the utilization of planting and breeding techniques, the treated pig dung is reintroduced to the agricultural field for the purpose of irrigation. Following the remediation of groundwater pollution, a concern arises over the elevated levels of nitrate nitrogen found in the manure-contaminated water.

The predominant approach for managing the discharge of pig farm manure is through biochemical treatment, as depicted in Fig. 1.

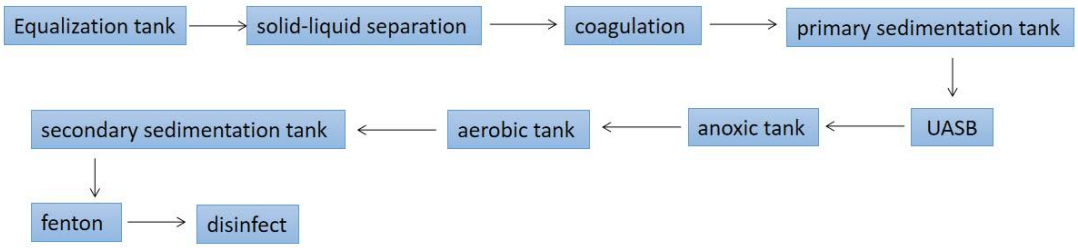

Fig. 1. Pig farm wastewater treatment process flow chart.

Once the treated manure permeates the surface layer, it will give rise to nitrate pollution within the shallow groundwater. The groundwater contamination by nitrates, as reported by local inhabitants, may result in several issues, including the occurrence of "blue baby disease". Hence, investigating the technological domains of lowering nitrate concentration in tailwater through enhanced treatment of pig farm manure and facilitating subsequent denitrification reactions hold significant study value.

Sulfur autotroph denitrification represents a novel approach to nitrate removal in water, constituting a distinct form of biological denitrification technology. In the absence of oxygen, sulfur bacteria utilize various inorganic carbon compounds such as CO_2, HCO_3^-, and CO_3^{2-} as their carbon source for growth. Additionally, they employ electron donors such as elemental sulfur, sulfide, sulfite, tetra thiosulfate, or thiosulfate. The process of nitrate reduction involves the occurrence of sulfur autotroph denitrification.

Desulfocapsa denitrification is a type of bacteria that exhibits facultative autotrophy and is characterized by a gram-negative staining pattern. The bacteria is capable of reducing nitrate to nitrogen, while simultaneously oxidizing elemental sulfur or its compounds to sulfate. The denitrification bacteria that are capable of tolerating the necessary environmental conditions for denitrification are often anaerobic or anoxic, with a preferred pH range of 6.5–7.5, and require an inorganic carbon source. The occurrence of denitrification reactions in sulfur autotrophs can be represented as follows:

$$6NO_3^- + 5S + 2H_2O \rightarrow 3N_2 + 5SO_4^{2-} + 4H^+ \quad (1)$$

A portion of nitrate undergoes synthesis to become organic nitrogen molecules, which subsequently serve as the constituents of bacteria. This process can be illustrated as follows:

$$NO_3^- \rightarrow NO_2^- \rightarrow NH_2OH \rightarrow \text{organic nitrogen} \quad (2)$$

The general equation for sulfur autotrophic denitrification, as presented by Zhang et al. [1] can be expressed as:

$$55S_0 + 50NO_3^- + 20CO_2 + 4NH_4^+ + 38H_2O \rightarrow 25N_2 + 55SO_4^{2-} + 64H^+ + 4C_5H_7O_2N \quad (3)$$

$$2CaCO_3 + 2H^+ \rightarrow Ca(HCO_3)_2 + Ca^{2+} \quad (4)$$

$$Ca(HCO_3)_2 + 2H^+ \rightarrow 2H_2CO_3 + Ca^{2+} \quad (5)$$

Hydrogen ions (H^+) are generated as a result of sulfur autotrophic denitrification, resulting in a reduction in pH levels. The ideal pH range for the autotrophic denitrification process carried out by *Desulfocapsa denitrificans* is approximately 6.8–7. Consequently, the bacteria require a particular level of alkalinity in order to thrive, as the produced H^+ ions interact with alkalinity to uphold a state of neutrality within the environment. Furthermore, the autotrophic denitrification process necessitates the presence of inorganic carbon for the purpose of synthesizing bacterial cells. Therefore, the introduction of limestone serves the function of supplying inorganic carbon to facilitate bacterial development and sustain a pH-neutral milieu. The utilization of the sulfur autotrophic denitrification biofilter reactor is a cost-effective approach for denitrification, which is commonly employed in the advanced treatment of municipal wastewater. Nevertheless, there is currently no documented application case for the denitrification of pig farm waste. The tailwater resulting from the disposal of pig farm manure is a form of wastewater characterized by a relatively low carbon-to-nitrogen (C/N) ratio. The denitrification treatment commonly employs the conventional denitrification procedure, which necessitates the addition of carbon sources to enhance the denitrification efficacy. Nevertheless, the introduction of carbon sources has the potential to augment the future treatment burden and elevate the COD value. Moreover, the constant administration of carbon sources will result in an escalation of the expenses associated with the denitrification process. The utilization of a sulfur autotrophic denitrification biofilter is a viable technological solution for the denitrification of sewage characterized by a low carbon-to-nitrogen (C/N) ratio. This approach obviates the necessity for the addition of an external carbon source during subsequent processes. Hence, the denitrification cost as a whole is quite cheap, rendering it very compatible with the denitrification needs of pig effluent tailwater. This study centers on the mitigation of nitrate nitrogen in the tailwater of swine wastewater by the utilization of sulfur autotrophic denitrification biofilter technology. The primary objective is to address the issue of nitrate pollution in groundwater by using this approach. The scientific investigation of nitrate removal in pig farm wastewater remains an area that has not yet been extensively explored.

The study examines the utilization of sulfur and limestone in autotrophic denitrification for the purpose of denitrifying municipal wastewater [2]. The nitrate denitrification reaction conducted in membrane bioreactor tests resulted in the production of a sulfur-based substance [3]. Zhang et al. [4] employed a membrane bioreactor to

investigate the denitrification of groundwater and the bacterial composition using sulfur-based material. In their study, Wang et al. [5] provide a comprehensive overview of the chemical reactions involved in sulfur autotrophic denitrification, as well as an analysis of the impact that various factors have on this process. The investigation of bacterial communities in the context of sulfur autotrophic denitrification is conducted, wherein a comparison of bacterial composition is made among different sulfur autotrophic denitrification reactors [6]. Wan et al. [7] investigated the integration of bioelectrochemical processes with sulfur autotrophic denitrification for the purpose of groundwater remediation. Zhou et al. [8] aim to mitigate nitrogen pollution in wastewater with low carbon-to-nitrogen (C/N) ratios. The coupling impact of electron-driven and sulfur-based autotrophic denitrification is also investigated by Chen et al. [9]. The process of removing nitrate and nitrite is carried out using sulfur-limestone autotrophic denitrification [10]. Yang et al. [11] have expressed their interest in investigating the emission of N_2O resulting from sulfide-driven autotrophic denitrification. The evaluation of kinetic factors pertaining to sulfur-limestone autotrophic denitrification in the biofilm process has been conducted [12]. Li et al. [13] have presented a pilot-scale use of a sulfur-limestone autotrophic denitrification biofilter for the treatment of municipal wastewater. The study conducted by Sahinkaya et al. [14] investigates the application of simultaneous sulfur-limestone autotrophic denitrification and heterotrophic denitrification in the treatment of drinking water with the aim of reducing sulfate production. Wang et al. [15] conducted a comparative analysis of sulfur autotrophic denitrification and heterotrophic denitrification, focusing on their hydrodynamic features and operational costs. Ucar et al. [16] conducted a study that employed a sulfur-based membrane reactor to examine and compare the efficacy of biogenic and sulfur as electron donors in autotrophic denitrification. Oh et al. [17] employed various carbon sources, including leachate and methanol, for the purpose of mixotrophic denitrification. Liu et al. [18] investigate the influence of iron on the augmentation of nitrate removal in mixotrophic denitrification.

2. Experimental materials and methods

2.1. Experimental set-up

Fig. 2 illustrates the pilot test reactor employed for the sulfur autotrophic denitrification biofilter. The dimensions of the pilot test apparatus are as follows: the length measures 5 m, the width measures 3 m, and the filling height measures 2 m. The height of the liquid level is 1 m. The reactor is partitioned into five filter units, with each unit being 1 m in length. Water holes serve as a means of connecting the various units. The sulfur autotrophic denitrification biofilter is composed of sulfur particles measuring 2 cm and calcium carbonate particles measuring 2 cm. These particles are meticulously combined and agitated in the reactor, adhering to a volume ratio of 3:1. Zhang et al. [1] examined the optimal impact of the sulfur and limestone ratio on autotrophic denitrification. Their findings indicate that a sulfur and limestone ratio of 3:1 yields the most advantageous results. A cover plate is installed on the upper section of the reactor in order to enhance the isolation of air's impact. The experimental configuration is designed to allow water to pass through it solely by the force of gravity. The operational parameters for the entire experiment are presented in Table 1.

2.2. Inoculation sludge and influent water quality

The experimental apparatus is linked to the terminus of the swine husbandry wastewater treatment plant. The waste product resulting from the biochemical reaction is directed into the reactor to undergo denitrification. In the initial stage of reactor operation, a specific quantity of lagoon sludge is introduced into the reactor. A circulating pump is employed to foster the growth of microorganisms within a sulfur autotrophic denitrification biofilter, which operates in a unidirectional flow for the initial 3 d. After a span of 3 d, the discharge from the sewage treatment system is introduced into the reactor. Table 2 displays the effluent water quality of the tail wastewater from the sewage treatment system of a pig farm.

Table 1
Operational conditions of the pilot scale reactor

Periods	1	2	3
Days (d)	0–63	63–121	121–181
NO_3–N (mg/L)	42	42	42
HRT (h)	3	2	1
Flow rate (m^3/h)	5	7.5	15
Nitrate loading rate NO_3–N·mg/(L·h)	14	21	42
Temperature (°C)	19	19	19

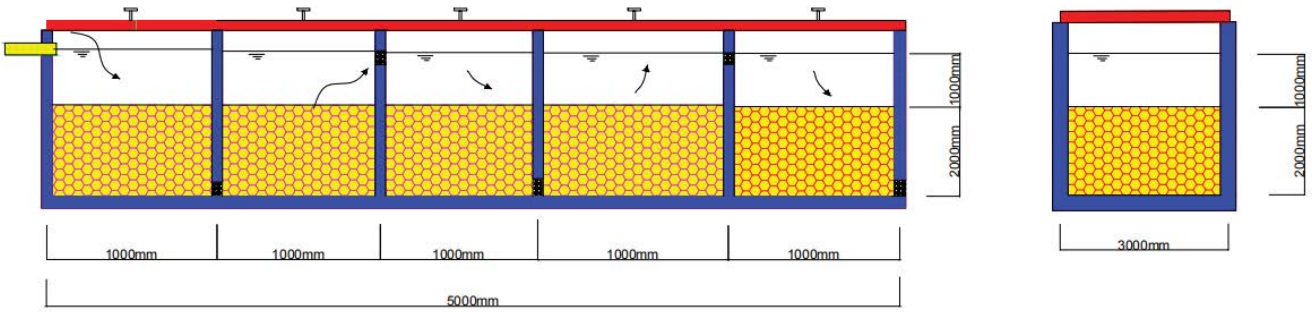

Fig. 2. Schematic diagram of sulfur autotrophic denitrification filter.

Table 2
Tail water quality of pig farm sewage treatment system

COD (mg/L)	120
TN (mg/L)	175
NH_3–N (mg/L)	12
TP (mg/L)	2
NO_3–N (mg/L)	42
SS (mg/L)	80

2.3. Analysis items and methods

The pH is quantified using the Leici PXB-286 portable tester, while the concentrations of Ca^{2+} and SO_4^{2-} are assessed by ion chromatography. The measurement of alkalinity is often conducted using acid titration. The determination of total phosphorus is conducted using the ammonium molybdate spectrophotometry technique. The concentration of NO_3–N is quantified using UV spectrophotometry.

2.4. High-throughput sequencing

Samples of biofilm were collected on days 1, 35, 85, and 135. The biofilm samples present on the surface of the elemental sulfur autotrophic denitrification biofilter were subjected to high-throughput sequencing analysis. The 16S rRNA fragment inside the V4-V5 region of the biofilm sample was specifically targeted for analysis. The polymerase chain reaction (PCR) amplification primers used for this purpose were 515F and 907R. In conclusion, the raw data of the acquired samples was compared and analyzed using the NCBI SRA database on the cloud platform. This process facilitated the extraction of relevant biological information and the generation of an analysis chart depicting the data on bacterial species.

3. Results and analysis

3.1. Start-up of elemental sulfur autotrophic denitrification filter

The commencement of operations for the pilot reactor was initiated in January and continued for a duration of 40 d. The hydraulic retention time (HRT) of the reactor was established as 3 h. During the initial phase of reactor start-up, the flow rate of the pump is regulated to 5 m^3/h in order to facilitate the controlled transfer of the inoculated bacteria solution into the filter tank at a reduced flow rate. The inoculum of heterotrophic bacteria is introduced into the reactor, and the presence of the organic carbon source within the reactor is confirmed. The process of domestication and assimilation of heterotrophic bacteria into the autotrophic bacteria family occurred gradually in an environment rich in sulfur and calcium carbonate as an inorganic carbon source. During the growing process of the strain in the reactor, the flow is conducted in a unidirectional circulation mode for the initial 3 d. The gradual drop in nitrate–nitrogen concentration in the water occurs as a result of the absence of continuous water inflow during the continuous circulation of the inflow water in the anoxic tank. Following the initial 3-d period, the subsequent inflow of tail wastewater into the pig farm wastewater facility was consistently facilitated by pumping mechanisms.

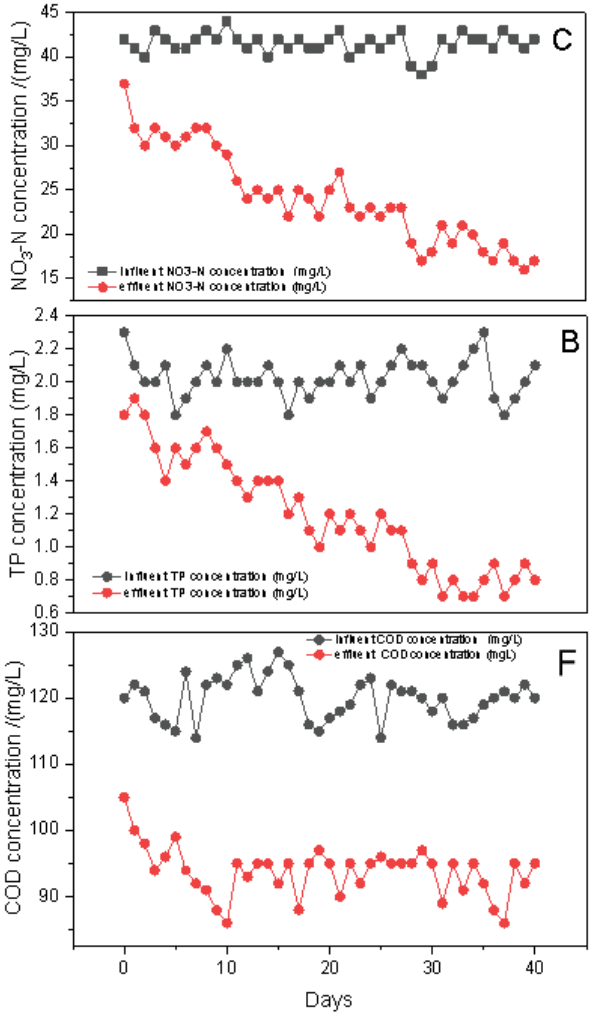

Fig. 3. Variations of COD TP NO_3–N throughout the microbial acclimatization.

According to the data presented in Fig. 3, during the initial 40-d period of domestication of sulfur autotrophic denitrifying bacteria, the influent COD was successfully stabilized at a concentration of 120 mg/L. It was observed that the COD in the effluent water exhibited a more pronounced drop within the first 10 d, ultimately reaching a minimum value of 80 mg/L. The concentration of COD in the effluent water remained constant at 90 mg/L for a period of 30 d. The monitoring of the water's total phosphorus concentration was conducted throughout the process of cultivating autotrophic bacteria. Starting on the second day, there was a noticeable decline in the concentration of total phosphorus in the water, accompanied by an increase in the volume load of the reactor for the purpose of total phosphorus removal. During the period spanning from the fourth to the tenth day, there was a gradual decline observed in the overall phosphorus concentration within the water, which was accompanied by a drop in the alkalinity that was dissolved in the water. The concentration

of total phosphorus in the water exhibited a significant decline, reaching a shallow level. Notably, the decrease in total phosphorus concentration was seen to occur rapidly between the 10th and 40th day. Empirical evidence has demonstrated that the dissolution rate of alkalinity in water is accelerated. After a period of 40 d following the initiation of acclimation of autotrophic bacteria, there was a significant decrease in the overall content of phosphorus in the water, reaching a relatively low level. During the initial 40-d period of domestication of sulfur autotrophic denitrifying bacteria, the concentration of nitrate in the influent water was maintained at a stable level of 42 mg/L. Furthermore, the nitrate content in the effluent water exhibited a declining pattern. The concentration of nitrate in the effluent water exhibited a decrease from an initial value of 38 mg/L on the first day to a final value of 16 mg/L on the 40th day.

According to the data presented in Fig. 4, after the initial 40-d period of domesticating sulfur autotrophic denitrifying bacteria, the influent water consistently maintained a sulfate content of 60 mg/L. Conversely, the effluent water exhibited a noticeable upward trend in sulfate concentration. The concentration of sulfate in the effluent water exhibited an increase from an initial value of 100 mg/L on the first day to 130 mg/L on the 40th day. According to the data presented in Fig. 4, there was a significant increase in the denitrification rate of the reactor for nitrate–nitrogen removal starting from the fourth day. The denitrification rate is consistently maintained at a value of 0.08 kg·NO_3–N/(m^3·d). The concentration of nitrate nitrogen exhibited a rapid decline between the 10th and 25th days, but the denitrification rate remained constant at 0.15 kg·NO_3–N/(m^3·d). Between the 20th and 40th day following the acclimation period of autotrophic bacteria, the denitrification rate was observed to attain a value of 0.2 kg·NO_3–N/(m^3·d). The concentration of nitrate nitrogen in the effluent decreases to a low level. Microscopic observation reveals the presence of sulfur denitrification bacteria within the biofilm adhered to the filter material.

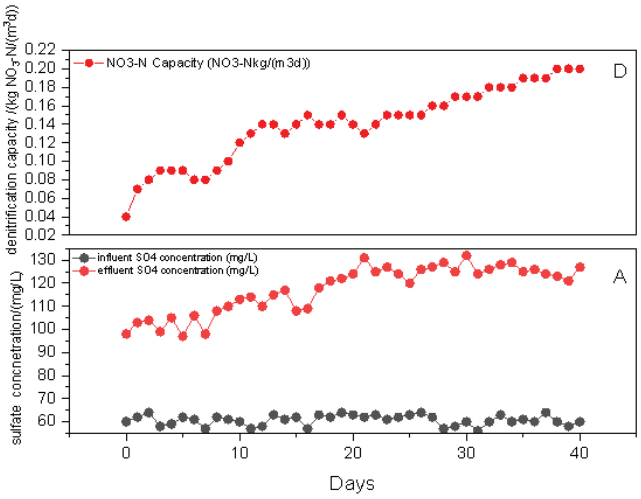

Fig. 4. Variations of denitrification rate and sulfate concentration throughout the microbial acclimatization.

3.2. Long-term operation effect of elemental sulfur autotrophic denitrification pilot test

Following the completion of microbial acclimatization, a constant influx and efflux of water is initiated in the experimental setup. The pump flow rate was adjusted to 5 m^3/h and the HRT was set to 3 h. Subsequently, the tailwater was continuously pumped into the sulfur autotrophic denitrification biofilter, and the concentration of NO_3–N in the filter water was measured every 24 h. During the first stage of the reactor operation period (0–60 d), the HRT of the reactor remained at 3 h. In the second stage (61–121 d), the HRT was reduced to 2 h by increasing the pump flow rate to 7.5 m^3/h. Finally, in the third stage (122–181 d), the HRT was further reduced to 1 h by increasing the pump flow rate to 15 m^3/h. The aforementioned data were obtained through continuous monitoring of the reactor's operation.

In Fig. 5 it can be observed that as the HRT of the reactor decreases, there is a continuous increase in the concentration of total phosphorus in the effluent. Conversely, a longer hydraulic retention time in the reactor leads to a higher concentration of Ca^{2+}. This increase in Ca^{2+} concentration results in the formation of more $Ca_3(PO_4)_2$, which in turn leads to

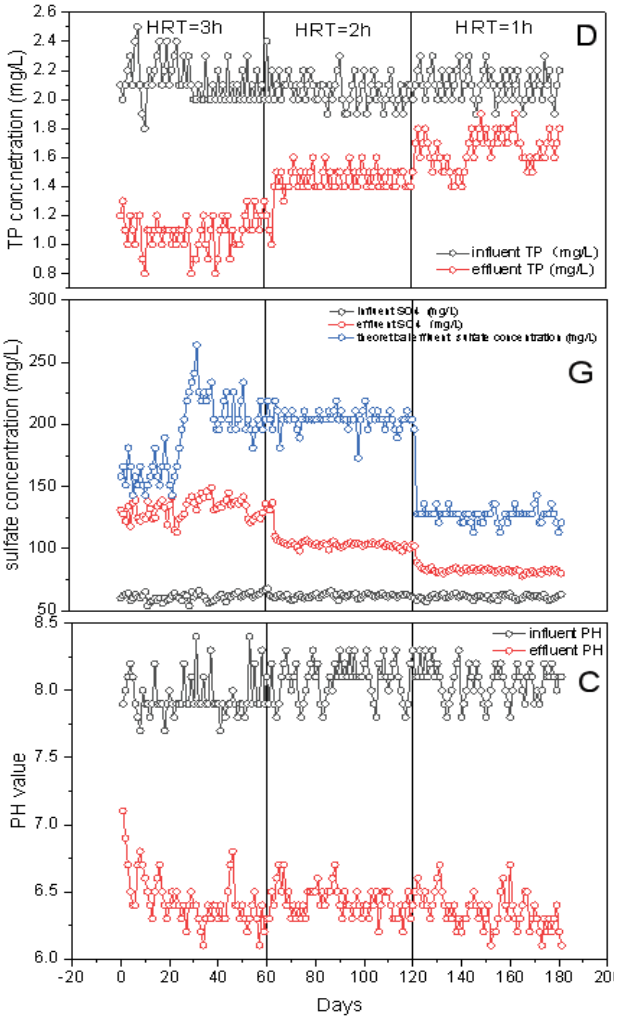

Fig. 5. Variation of TP sulfate concentration and pH value with hydraulic retention time.

a lower concentration of total phosphorus in the reactor. Throughout the three stages of reactor operation, there is no significant change in the pH value of the effluent. The influent water of the reactor has a pH value of approximately 7.8, while the effluent water maintains a stable pH value of 6.3. As the sulfur autotrophic denitrification reaction progresses, there is a downward trend in the pH within the reactor.

Fig. 6 illustrates the decrease in SO_4^{2-} concentration during sulfur autotrophic denitrification as the hydraulic retention time increases. The effluent exhibits higher levels of SO_4^{2-} compared to the influent. It is observed that the decline in SO_4^{2-} concentration in the sulfur autotrophic denitrification reaction is more pronounced with longer hydraulic retention times in the entire reactor. The stoichiometric ratio of sulfate production to nitrate removal is determined to be 7.54 mg/L sulfate per 1 mg/L nitrate. By considering the actual nitrate removal in the reaction, the theoretical sulfate production is calculated. However, the measured concentration of sulfate produced in the actual test is found to be lower than the concentration predicted by the theoretical calculations.

In Fig. 7, due to alkalinity consumed in the sulfur autotrophic denitrification reaction, the Ca^{2+} concentration in the effluent rises, compared to the Ca^{2+} concentration in the influent. As the hydraulic retention time of the whole reactor runs, the sulfur autotrophic denitrification reaction needs more alkalinity consumed. Therefore, more Ca^{2+} is released. A decrease in nitrate concentration accompanies the increase in Ca^{2+} concentration. The sulfur autotrophic denitrification process requires alkalinity. The alkalinity of the effluent is lower than that of the influent. The longer the hydraulic retention time of the whole reactor, the more alkalinity the sulfur autotrophic denitrification reaction consumes. The nitrate concentration in the influent water in the sulfur autotrophic denitrification reaction is higher than the nitrate concentration in the effluent water. The nitrate concentration in the effluent gradually increased as the residence time decreased. With the hydraulic retention time of the reactor decreasing, the concentration of nitrate in the effluent of the reactor increases continuously. In the experiment, the nitrate concentration in the effluent is higher than in previous studies because of the high level of non-dissolved suspended solids in the biochemical tail water of swine manure water.

Fig. 8 shows that with the decrease in the hydraulic retention time of the reactor, the denitrification rate of the reactor shows an upward trend. With the hydraulic retention time of the reactor being set at three h, the denitrification rate remains at 0.2 kg·NO_3–N/(m^3·d). When the hydraulic retention time of the reactor is reduced to 2 h, the denitrification rate rises to 0.35 kg·NO_3–N/(m^3·d). When the hydraulic retention time of the reactor drops to 1h, the denitrification rate climbs up to 0.45 kg·NO_3–N/(m^3·d). Sahinkaya et al. [2] reached a denitrification rate of around 0.3 kg·NO_3–N/(m^3·d) under nitrate loading rate 12–13 mg·NO_3–N/(L·h) and S/L ratio 1/1 operation conditions in pilot scale experiment, using municipal wastewater. In the Sahinkaya et al. [14] lab scale experiment, a bioreactor receiving simulated

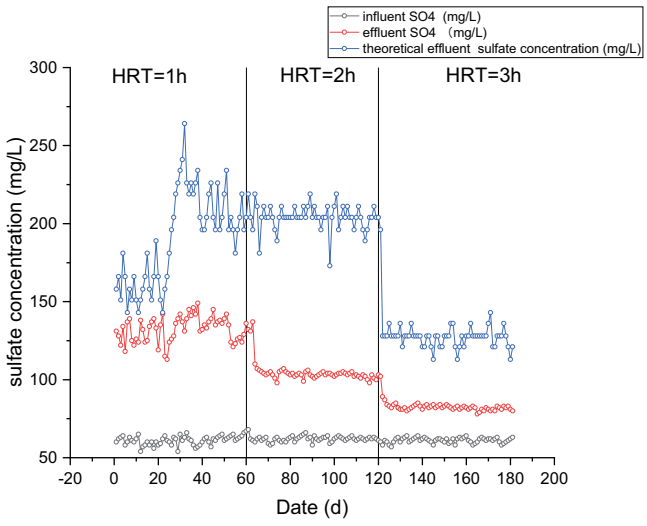

Fig. 6. Theoretical effluent sulfate concentration and measured sulfate concentration with hydraulic retention time.

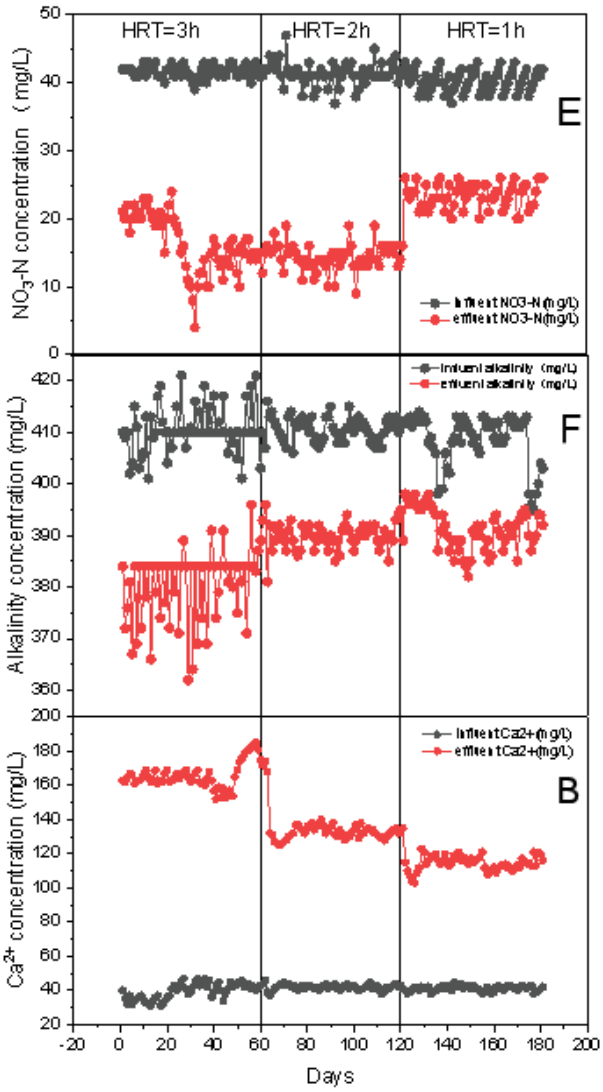

Fig. 7. Variation of various ions with hydraulic retention time.

groundwater obtained a 0.2 kg·NO_3–N/(m^3·d) denitrification rate. Sierra-Alvarez et al. [19] achieved a maximum denitrification rate of around 0.3 kg·NO_3–N/(m^3·d) in a lab-scale packed bioreactor with a S/L ratio of 1/1. The experiment's denitrification rate is slightly lower than that of Sahinkaya et al. [2]'s experiment with municipal wastewater. The denitrification rate in this experiment matches the interval values of the denitrification rate studied by others.

A modest decline in the concentration of COD suggests that a portion of the organic matter may have been consumed through a heterotrophic process. It is likely that heterotrophic bacteria in the upper region of the reactor are responsible for the consumption of this organic matter. The slight decrease in COD can primarily be attributed to the presence of COD within insoluble substances, which are predominantly composed of inert products that undergo slow and challenging biodegradation. The process of heterotrophic denitrification relies on a biodegradable fraction of the organic matter found in the wastewater. The remaining COD present in suspended materials is inert and cannot undergo further degradation.

Figs. 9 and 10 depict the collection of samples from all five units of the reactor, with water samples being obtained at progressively greater distances along the reactor's travel direction. The observed trends indicate a decrease in NO_3–N, TP, alkalinity, and pH values as the reactor distance increases. Conversely, the concentration of calcium ions and sulfate exhibits an increase along the reactor distance.

Figs. 11 and 12 depict the alteration pattern of the microbial population during the operation of an elemental sulfur

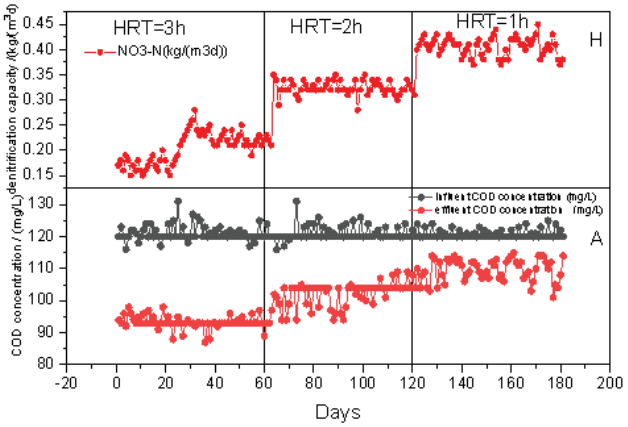

Fig. 8. Denitrification capacity and COD concentration change graph with different HRT.

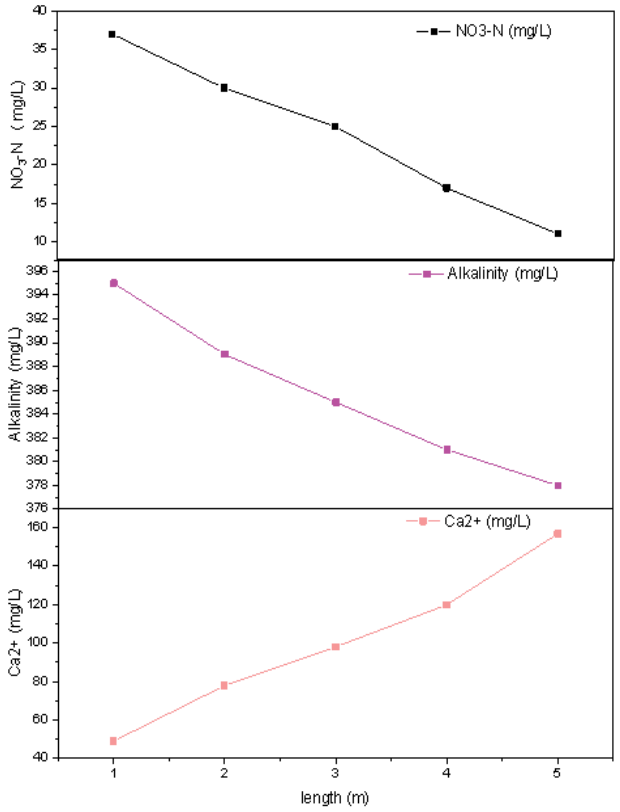

Fig. 9. NO_3–N alkalinity Ca^{2+} concentration change along with length distance of the reactor.

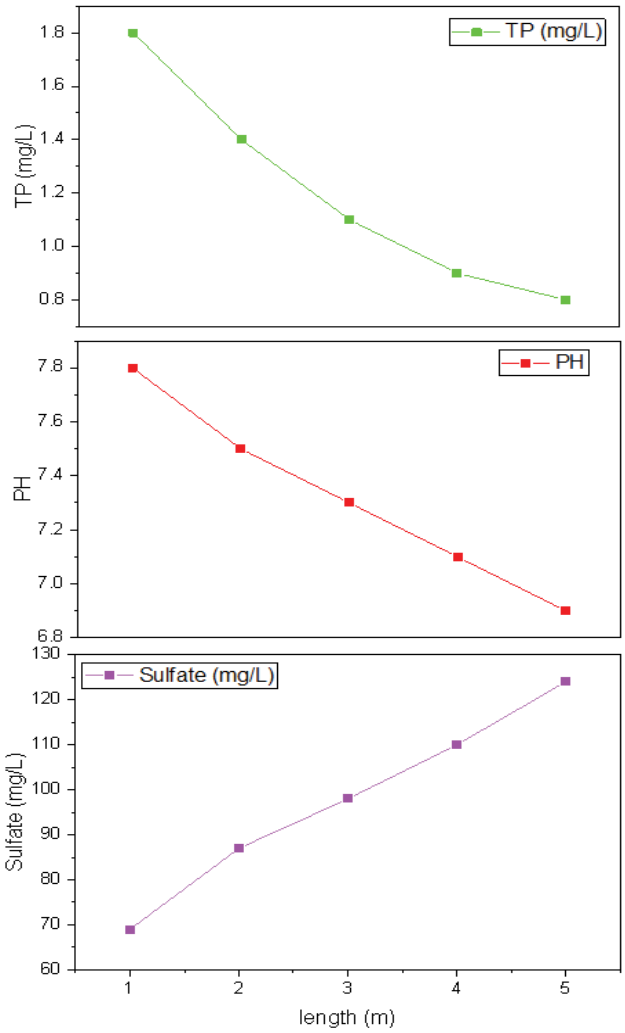

Fig. 10. TP pH sulfate concentration change along with length distance of the reactor.

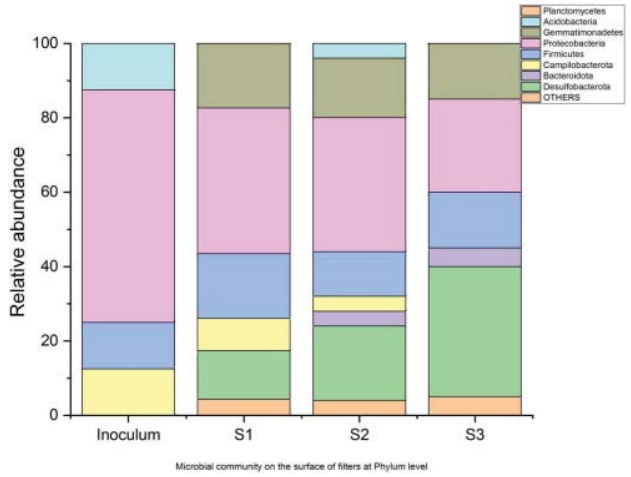

Fig. 11. Levels of microbial phylum on the filter surface.

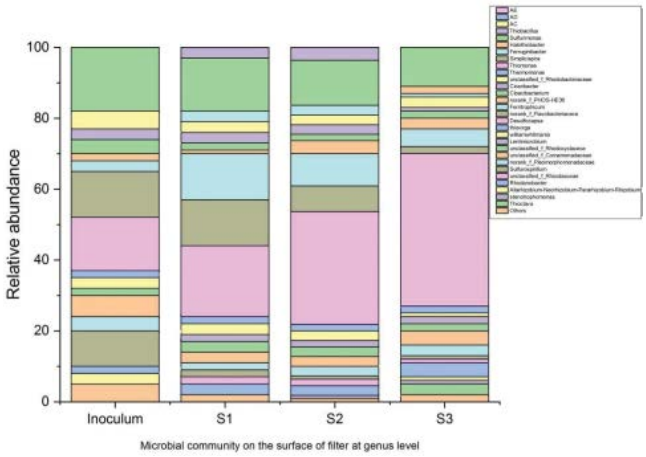

Fig. 12. Level of microbial genus on the filter surface.

autotrophic denitrification biofilter. This analysis is conducted at two taxonomic levels, namely phylum and genus. At the phylum level, the most prevalent microbial group in the biofilm samples is Proteobacteria, which is commonly observed in autotrophic denitrification systems, as evidenced by Fig. 11. This finding aligns with the research conducted by Chen et al. [20], who also identified Proteobacteria as the dominant denitrification phylum in denitrifying bioreactors. The observed changes in the microbial community structure provide compelling evidence for the functional transformation of the reactor. To further investigate the variations in biodiversity among the samples, we analyze the 20 most abundant genera across the four samples. The term "Inoculum" refers to the sample collected on the first day of period 1, while "S1" represents the sample obtained 35 d into period 2.

In a similar vein, the designation "S2" is used to refer to the sample collected from the surface of the biofilm in the filler on the 85th day of period 3, while "S3" represents the sample obtained on the 135th day of period 4. The Proteobacteria phylum exhibits the highest abundance in the initially inoculated sludge, but its prevalence decreases as the reactor operates for a longer duration. This trend was also observed by Li et al. [13] in their study involving a sulfur limestone autotrophic denitrification bioreactor utilizing municipal wastewater.

The continuous increase in the abundance of Desulfobacterota is observed with the initiation of domestication. Over time, the Desulfobacterota bacterium demonstrates an increased presence within the microbial community. It is worth noting that previous research failed to identify this phenomenon, potentially due to the utilization of biochemical tail water sourced from pig farm wastewater. Additionally, Acidobacteria and Firmicutes have been identified as the key contributors to nitrogen removal in the sulfur-limestone autotrophic denitrification bioreactor [13].

The experiment conducted by Li et al. [13] identified *Thiobacillus* and *Ferritrophicum* as the predominant genera. Specifically, *Thiobacillus* was found to be a dominant species in sulfur-based denitrification [21]. *Sulfurimonas*, which is typically found in sulfidic habitats and denitrifying reactors [22], was also detected in this study. Notably, *Desulfocapsa* and *Sulfurimonas* were identified as significant contributors to denitrification, exhibiting a high relative abundance within the genus. This observation represents a novel finding. Furthermore, the microbial populations observed in this experiment largely align with those previously studied by other researchers, particularly in relation to sulfur autotrophic denitrifying populations.

The abundance of the genus *Desulfocapsa* increases with increasing reactor operating time. *Desulfocapsa* belongs to the Proteobacteria phylum, one of the most common genera in the autotrophic denitrification reaction system, with elemental sulfur as the electron donor. *Desulfocapsa* is a typical genus of sulfur denitrification bacteria, proving that NO_3–N is removed in the main direction of autotrophic denitrification of elemental sulfur. Desulfobacillus, which consists of a series of denitrifying strains, is a typical sulfur autotrophic denitrifying bacteria widely distributed. The elemental sulfur autotrophic denitrifying bacteria domesticated from different raw sludge responded differently to the external environment. After the start-up of the biofilter is completed, the internal microorganisms are mainly elemental sulfur autotrophic denitrification functional bacteria, indicating that the elemental sulfur autotrophic denitrification reaction is the main metabolic path of the pilot plant. From the perspective of microbial populations, NO_3–N is removed by autotrophic denitrification of elemental sulfur.

4. Conclusion

The elemental sulfur autotrophic denitrification biological filter exhibited a start-up period of 40 d. Subsequently, it was able to maintain a NO_3–N removal load of 0.45 kg·NO_3–N/(m^3·d). Throughout the biofilter's long-term operation, the primary by-products generated in the reaction were SO_4^{2-} and Ca^{2+}. The optimal hydraulic retention time for the biofilter, as determined under experimental conditions, was 3 h. Following the successful start-up of the pilot scale biofilter, the predominant microorganisms present were elemental sulfur autotrophic denitrification functional bacteria. Consequently, the elemental sulfur autotrophic denitrification reaction became the principal metabolic pathway

within the biofilter. Proteobacteria, a bacterium of significant importance in the autotrophic denitrification reaction of sulfur in the biochemical tailwater of manure and water from swine farms, can be cultivated in raw water sourced from swine farm manure. As can be seen from the pilot experiment, the sulfur autotrophic denitrification reaction has a significant effect on the further removal of nitrate in the biochemical tail water of swine farm wastewater. At the same time, it has a role in removing pollutants such as COD and total phosphorus in biochemical tailwater. The reaction is also helpful for removing COD, total phosphorus and other pollutants in the biochemical tailwater. The pilot experiment shows that the sulfur autotrophic denitrification filter reactor is a kind of deep removal of nitrate for the biochemical tailwater of swine farm wastewater. It is a highly efficient reactor for the deep removal of nitrate from the biochemical tailwater of swine farm wastewater. This experiment is a sulfur autotrophic denitrification filter in the biochemical tailwater of swine farm wastewater. This experiment lays a theoretical foundation for applying sulfur autotrophic denitrification filters in the biochemical tailwater of swine farm wastewater. It provides a theoretical foundation for applying sulfur autotrophic denitrification filters in the biochemical tailwater of swine farms. Sulfur autotrophic denitrification filters show the direction of reduction of groundwater nitrate pollution.

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