Study on the Removal of High Nitrate-nitrogen Concentration from Pig Farm Wastewater by Heterotrophic and Sulphur Autotrophic Synergistic Denitrification Filter Process

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Abstract

Following the anaerobic fermentation process in a lagoon, the effluent from pig wastewater exhibited elevated levels of total nitrogen and nitrate nitrogen. The utilization of simple sulphur-limestone autotrophic denitrification resulted in a sluggish reaction rate and relatively moderate removal load. To enhance the efficacy of the sulphur autotrophic denitrification process, a strategy was implemented involving the introduction of a limited quantity of corncobs as a slow-release carbon source. This addition was made to a sulphur-limestone autotrophic denitrification reactor that was being operated in a continuous and stable manner. Furthermore, the volume ratio of the corncobs added was progressively increased. This study examines the alterations in nitrate nitrogen, Chemical Oxygen Demand (COD), sulphate, and alkali consumption within the sulphur-limestone autotrophic denitrification reactor, both prior to and after to the introduction of corncobs. This study examines the operational efficiency and reaction mechanism of a sulfur-limestone autotrophic denitrification reactor that is enhanced by the utilization of corncobs as a slow-release carbon source. The findings of the study indicate that the inclusion of maize cob carbon source in the sulphur limestone autotrophic denitrification process resulted in an improved effectiveness in removing nitrate and nitrogen. Additionally, when considering the three different ratios of corn cob carbon source, the process exhibited a Chemical Oxygen Demand (COD) removal rate exceeding 42%. The observed trend in the consumption of alkalinity in both the pure sulphur limestone autotrophic denitrification process and the sulphur limestone autotrophic denitrification process augmented by the three types of corncob slow-release carbon sources indicated an increase with time. The concentration of nitrate-nitrogen in the influent water exhibited a negative correlation with the length of the reactor flow-through. The addition of a slow-release carbon source derived from corncob greatly enhanced the autotrophic denitrification and denitrification impact in the three reactors. Moreover, there was an observed increase in the trend of heterotrophic denitrification as the amount of carbon source added increased. The experiments investigating mixotrophic denitrification reaction observed a positive correlation between the content of corn cob and the relative abundance of microbial flora. Specifically, an increase in corn cob content led to an increase in the relative abundance of Proteobacteria at the phylum level. Additionally, the relative abundance of various other flora at the genus level also increased proportionally. The efficacy of the mixotrophic denitrification process surpassed that of the sulphur-limestone autotrophic denitrification method in the treatment of nitrate-nitrogen wastewater characterized by a high concentration of nitrate-nitrogen. The former exhibited a higher denitrification rate and resulted in lower levels of sulphate ions and alkalinity production.

Keywords: Pig farm wastewater • High nitrate concentration • Corncob slow-release • Mixtrophic denitrification • carbon source • Microbial community composition

Introduction

The pig breeding business in China is seeing significant growth in scale, leading to increased attention towards the management of manure water created during the breeding process [1]. The current methods of pig manure treatment can be categorized into two primary modes: the utilization mode, where the manure water is returned to the field, and the discharge mode, where the manure water is released. In the northern region of China, there exists a substantial expanse of cultivated land, with a predominant practice of returning manure to the fields for consumption, as noted by Zhilhi N, et al. [1]. In the southern region of China, there is a relatively smaller area of cultivated land, and a majority of farmers employ a method of managing manure water that meets established standards. Lagoons are frequently employed in the northern region of China for the purpose of anaerobic fermentation of swine dung water, followed by its subsequent utilization in agricultural fields [2] (Figure 1). The manure water, which has undergone anaerobic fermentation, is reintroduced to the field usage model in its entirety by means of a combined water and fertilizer integration process. Swine dung is characterized by a significant concentration of nitrate nitrogen, which has the potential to infiltrate the soil and then enter the shallow groundwater as part of the manure return process. The presence of nitrate nitrogen in groundwater has the potential to induce

Figure 1. Illustrates the anaerobic treatment process employed for the treatment of pig wastewater in a lagoon.

adverse health effects when it is introduced into human drinking water sources [1]. The consumption of nitrate in water can have detrimental effects on human health [3]. Upon ingestion by individuals, nitrate undergoes conversion to nitrite, thereby exerting an influence on the blood's ability to transport oxygen and potentially resulting in the occurrence of methemoglobinemia or blue baby syndrome. The investigation of nitrate nitrogen removal from swine farm manure prior to its application in agricultural fields is a subject of considerable interest in current research. Notably, there exists a study gap pertaining to the removal process of nitrate nitrogen from manure that is reintroduced to the field under conditions of high load [3]. Monolithic sulfur autotrophic denitrification is mostly employed for the denitrification of wastewater containing low concentrations of nitrate nitrogen. Nevertheless, the rate of denitrification employed in the context of sulphur autotrophic denitrification is characterized by sluggishness, hence impeding the advancement of the sulphur autotrophic denitrification process [2]. Corn cob, an inexpensive agricultural residue, has the ability to sequester carbon sources when subjected to an alkaline solution, making it a commonly employed material in agricultural practices [4]. The primary objective of this investigation is to conduct an empirical examination of the denitrification process in swine manure water. Specifically, the study aims to evaluate the efficacy of utilizing maize cob as a slow-release carbon source inside a sulfur-limestone autotrophic denitrification system. The ultimate goal is to achieve synergistic denitrification through the collaboration of heterotrophic and autotrophic mechanisms, thereby effectively reducing the presence of high nitrate-nitrogen loads. The utilization of both heterotrophic denitrification and sulphur-autotrophic denitrification offers several advantages compared to the sole use of sulphur-autotrophic denitrification. Firstly, the alkali generated through heterotrophic denitrification and the acid produced through sulphur-autotrophic denitrification exhibit complementary properties. Secondly, the incorporation of heterotrophic denitrification can minimize sludge production during the denitrification process. Lastly, the inclusion of heterotrophic denitrification can effectively reduce the production of sulphate during autotrophic denitrification [5]. According to a study conducted by Liu D, et al. [6]. The alkalinity generated during heterotrophic denitrification was subsequently utilized by autotrophic denitrification. In their study Liang B, et al. [7] investigated the utilization of sulphur as an electron donor in autotrophic denitrification, as well as the use of corn cobs as a solid carbon source for heterotrophic denitrification, in the context of municipal wastewater treatment. The study conducted by Liu H, et al. [8] investigated the utilization of sulphur as an electron donor for autotrophic denitrification and methanol as a liquid carbon source for heterogeneous denitrification in the treatment of denitrification in municipal wastewater.

The investigation of nitrate nitrogen removal from swine farm manure prior to its application in the field is a compelling area of research. Notably, there exists a research gap concerning the process of nitrate nitrogen removal for high loads in the manure return mode, as highlighted by Zhilhi N, et al. [1]. Monotrophic sulphur autotrophic denitrification is primarily employed for the denitrification of wastewater with low concentrations of nitrate nitrogen. However, the denitrification rate associated with sulphur autotrophic denitrification is sluggish, thereby impeding the widespread adoption of this denitrification process [9]. Mixotrophic denitrification overcomes the limitations associated with heterotrophic denitrification, which requires substantial carbon inputs and a prolonged period for autotrophic denitrification to initiate. The study conducted by Li R, et al. [10] investigated the utilization of woodchips as a carbon source in the context of heterotrophic denitrification. The researchers developed a novel approach known as woodchips-sulfur-based mixotrophic denitrification. The Liu D, et al. [6] devised a mixotrophic denitrification process using corncob-sulfur-shell powder, where corncob was employed as a carbon source for heterotrophic denitrification. In their study, Li Y, et al. [5] employed sulphur as the principal substance in a fluid-bed membrane reactor to introduce a novel form of mixotrophic denitrification. In their study, Tang L, et al. [2] employed a mixotrophic denitrification mechanism to fabricate composite fillers using melted polycaprolactone and elemental sulphur as packing materials. In their study, Weng Z, et al. [4] conducted a comparative analysis of corncob and pyrite as carbon sources for heterotrophic denitrification within a mixotrophic denitrification process. In their study, Tong S, et al. [11] utilized oyster shells as a source of alkalinity and pyrite as the organic component in an up-flow bed-packed biofilter, employing mixotrophic denitrification. The individual in question is named Lee D, et al. [12] employed mixotrophic denitrification to treat high-concentration nitrate leachate wastewater Sahinkaya E, et al. [13] employed methanol as an organic carbon source in a mixotrophic denitrification process, in combination with sulphur-based autotrophic denitrification, with the aim of reducing nitrate levels in municipal wastewater. Sulfide serves as an electron donor in mixotrophic denitrification, whereas methanol is utilized as an organic carbon source [3] conducted a comparative analysis of two organic carbon sources, namely leachate and methanol, in conjunction with sulfur-based electron donors, within the context of mixotrophic denitrification. Liang Z, et al. [3] conducted a comparative analysis between sulfur-based autotrophic denitrification and biomass sulfur-based mixotrophic denitrification in terms of their effectiveness in reducing nitrate levels. Liu H, et al. [14] investigated the impact of iron (Fe) on the enhancement of nitrogen reduction by the utilization of sulphur-based autotrophic denitrification coupled with heterotrophic denitrification, also known as mixotrophic denitrification.

According to a study conducted by Liu D, et al. [6] corn cobs were utilized as a carbon source in the heterotrophic denitrification process, serving as a slow-release electron donor for the entirety of the reaction. Equation (1) demonstrates that the hydrolyzed glucose derived from the corncob served as the predominant carbon supply for the reactor, facilitating the denitrification reaction. The denitrifying bacteria could potentially utilize the aforementioned resource Sahinkaya E, et al [13].

$$C_6H_{12}O_6 + 2.8NO_3^- + 0.5NH_4^+ + 2.3H^+ \rightarrow 0.5C_5H_7NO_2 + 1.4N_2 + 3.5CO_2 + 6.4H_2O$$
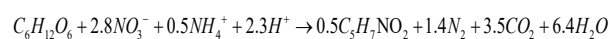
The use of a liquid carbon source in mixotrophic denitrification testing can have implications on the denitrification reaction and result in secondary pollution issues. Insufficient or excessive amounts of the liquid carbon source might restrict the denitrification process [15]. The utilization of a solid carbon source in the mixotrophic denitrification reaction offers the benefits of cost-effectiveness and rapid reaction kinetics, as evidenced by studies conducted by Liang Z, et al. [3] and Sahinkaya E, et al. [13]. Historically, the domain of agricultural pollution has predominantly relied on environmental engineering techniques to a lesser extent, resulting in inadequate attention towards the effective mitigation of deep nitrogen removal from swine dung and water. The primary objective of this study was to investigate the combined impact of sulphur-limestone autotrophic denitrification and heterotrophic denitrification on the efficient removal of nitrate nitrogen from pig farm wastewater with high concentrations [16-19].

Materials and Methods

Experimental configuration and effluent source

Residential Figure 2 illustrates the experimental configuration of the biofilter reactor. The dimensions of the entire reactor are as follows: the length measures 1 meter, the height measures 0.2 meters, and the width measures 0.2 meters. The reactor is partitioned into five distinct cells, each of which is compartmentalized. The length of each compartmentalized cell measures 0.2 meters. The interconnectedness of individual cells is facilitated by the presence of water channels. Experimental packing was introduced into the lowest section of each cell, and the liquid level within the reactor was maintained at a height of 0.1m above the packing layer. A sampling valve was installed beneath each reaction cell to ease the collection and analysis of water samples from various reaction cells. The total volume of the reactor was determined by measuring the distance between the liquid level and the packing surface. The experimental setup comprises four parallel reactors, with each reactor housing distinct packing materials. The inoculum and experimental water samples for reactor startup were obtained from the supernatant of fermented fecal water in the oxidation pond of pig farms located in northwest China. The water quality of the experimental water samples is presented in Table 1. The nitrate-nitrogen concentration in the supernatant of the oxidation pond was measured to be 180 mg/L, indicating that it falls under the category of high-concentration nitrate-nitrogen agricultural wastewater (Table 1) (Figures 2 and 3).

Empirical material

Startup of synergistic denitrification reactor (experimental phase I):

Table 1. Water quality analysis of supernatant derived from oxidation ponds in Chinese pig farms.

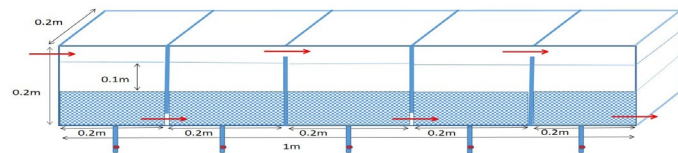| NO.	date (d)	HRT (h)	Packing Volumetric Ratio	COD (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	SO_4^{2-} (mg/L)	Alkalinity (mg/L)
F1	0-40	3	S:$CaCO_3$:corncob=3:1:0	678	180	1	10	120
F2	40-80	3	S:$CaCO_3$:corncob=3:1:1	678	180	1	10	120
F3	80-120	3	S:$CaCO_3$:corncob=3:1:2	678	180	1	10	120
F4	120-160	3	S:$CaCO_3$:corncob=3:1:3	678	180	1	10	120

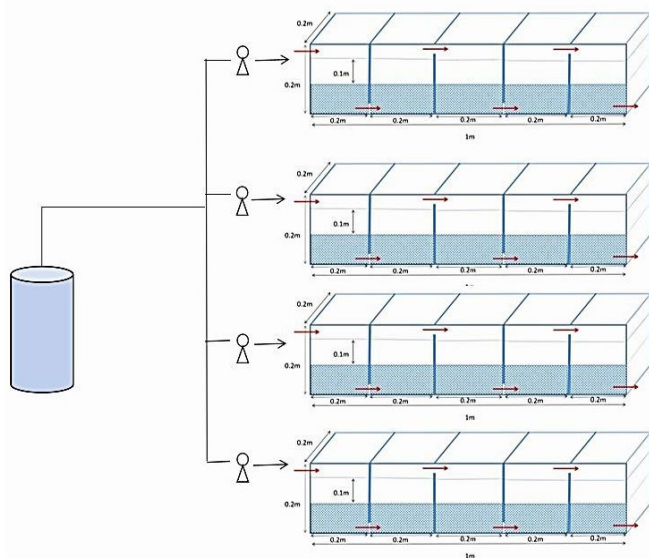**Figure 2.** Individual unit of the pilot reactor.

Figure 3. The system diagram depicting the experimental setup for small-scale trials.

Four identical small-scale experimental reactor devices were utilized in this study. These devices were filled with packing material according to certain ratios of sulphur monomers to limestone and corn cob volumes. The ratios used were 3:1:0, 3:1:1, 3:1:2, and 3:1:3, respectively. The devices were consecutively numbered as F1, F2, F3, and F4. The Hydraulic Retention Time (HRT) for the small-scale reactors was established as 3 hours. Experimental water samples were introduced into the reactors by a peristaltic pump, which maintained a consistent pumping speed. This process involved continuous pumping of the experimental feed water and continuous water flow. The concentration of nitrate nitrogen (NO_3-N) in the water was measured at 24-hour intervals. The pilot reactor was operated constantly over a period of several days until the volumetric loading of the reactor reached a stable state.

Implementation of the co-operative denitrification reactor (Experimental phase II): Following the completion of the startup process for the four parallel sets of pilot reactors, the F1 reactor underwent continuous operation for a period of 120 days. During this time, regular monitoring of the reactor effluent was conducted at 24-hour intervals to observe any variations in the concentrations of NO_3-N, NO_2-N, alkalinity, and sulphate. Table 2 displays the operational parameters of the F1 pilot reactor at every stage (Table 2).

The effluent of the F1 reactor was subjected to continuous testing for a period of 40 days. Parameters such as Chemical Oxygen Demand (COD), nitrate-nitrogen concentration, alkalinity, and sulphate content were monitored. The reactor operated at a Hydraulic Retention Time (HRT) of 3 hours, with an influent nitrate-nitrogen concentration of 180 mg/L. Subsequently, an assessment was conducted on the effluent water from the F2 reactor over a period of 40 days, with an operational Hydraulic Retention Time (HRT)

of 3 hours and an influent nitrate-nitrogen (NO_3-N) concentration of 180 mg/L. The parameters examined included Chemical Oxygen Demand (COD), nitrate-nitrogen concentration, alkalinity, and sulphate content. The continuous measurement of Chemical Oxygen Demand (COD), nitrate-nitrogen concentration, alkalinity, and sulphate content was conducted on the effluent from reactors F3 and F4 for a period of 40 days. These measurements were taken under specific operational circumstances, including a Hydraulic Retention Time (HRT) of 3 hours and an influent nitrate-nitrogen concentration of 180mg/L. Continuous analysis was conducted on the effluent discharged from the F3 and F4 reactors to determine its chemical Oxygen Demand (COD), concentration of nitrate-nitrogen, alkalinity, and sulphate content (Table 3).

Analytical approaches

The concentration of NO_3-N was assessed using UV spectrophotometry, whereas the concentration of NO_2-N was assessed using N-(1-naphthalene)-ethylenediamine photometry. The concentration of SO_4^{2-} was identified by barium chromate photometry, and alkalinity was established *via* acid-base indicator titration. The pH of the solution was determined using a portable pH meter. The quantification of total phosphorus was conducted using the method of ammonium molybdate spectrophotometry.

High-throughput sequencing

High-throughput sequencing analysis was conducted on the biofilm samples in the F1-F4 biofilters at intervals of 40, 80, 120, and 160 days, respectively. The V4-V5 region fragment within the 16S rRNA of the sludge sample was specifically targeted for analysis. The PCR amplification primers used for this purpose were 515F and 907R. The V4-V5 region segment within the 16S rRNA of the sludge sample was specifically chosen, and the primers used for PCR amplification were 515F and 907R. The experiment employed the NCBISRA database provided by the Meizhou Municipal Government. The experiment utilizes the NCBI SRA database on the Meiji cloud platform to conduct a comparative analysis of the raw data from the acquired samples. This analysis aims to derive relevant biological information and generate analytical charts for the sludge samples. Please provide the necessary information and generate an analysis chart based on the data pertaining to the bacterial species.

Results and Discussion

Initiation of synergistic denitrification reactors

The pilot reactor was initiated for a duration of 25 days, during which the nitrate-nitrogen concentration in both the influent and effluent was assessed at 24-hour intervals. The content of nitrate-nitrogen in the influent water of the reactor remained rather constant, with values ranging approximately from 180 mg/L. The rate of nitrate-nitrogen removal was seen to be slower in F1 (sulfur:$CaCO_3$:Corncob=3:1:0), while F2 (sulfur:$CaCO_3$:Corncob=3:1:1), F3 (sulfur:$CaCO_3$:Corncob=3:1:2), and F4 (sulfur:$CaCO_3$:Corncob=3:1:3) exhibited quicker rates of nitrate nitrogen removal. The study provided evidence that the combined use of heterotrophic and sulfur-limestone autotrophic denitrification

Table 2. Presents the operational conditions of the F1 reactor.

Periods	1	2	3
Days(d)	0-40	41-81	81-121
NO_3-N (mg/L)	180	180	180
HRT(h)	3	2	1
flow rate (L/h)	6.5	10	20

Table 3. The operational conditions of reactors F1-F4 are presented.

NO.	date (d)	HRT (h)	Packing Volumetric Ratio	NO_3-N (mg/L)	Flow Rate (L/h)
F1	0-40	3	S:$CaCO_3$:corncob=3:1:0	180	6.5
F2	40-80	3	S:$CaCO_3$:corncob=3:1:1	180	6.5
F3	80-120	3	S:$CaCO_3$:corncob=3:1:2	180	6.5
F4	120-160	3	S:$CaCO_3$:corncob=3:1:3	180	6.5

resulted in a higher rate of nitrate nitrogen removal compared to the exclusive utilization of sulfur-limestone autotrophic denitrification. During the initial 25-day period, there was a continuous decline in the concentration of nitrate-nitrogen in the effluent water of reactors F1-F4. Simultaneously, the nitrate nitrogen volumetric loading of these reactors exhibited a continuous increase. Specifically, after 25 days, the volumetric loading of F1 reached 0.5kg NO_3-N/(m^3/d), while F2, F3, and F4 experienced an increase in volumetric loading to 1.1kg NO_3-N/(m^3/d), 1.2kg NO_3-N/(m^3/d), and 1.3kg NO_3-N/(m^3/d), respectively. Upon the completion of the Startup process, it was seen that the volumetric loading of the F1-F4 reactors reached a stable state after a period of 25 days. This indicates that the four groups of small test reactors have successfully undergone the necessary procedures to initiate their operation (Figure 4).

The operational efficacy of sulphur autotrophic/hetero-trophic co-denitrification

In reactor F1, the Hydraulic Retention Time (HRT) was manipulated to values of 3, 2, and 1, in the absence of a solid carbon source. The content of NO_3-N in both the influent and effluent water was then determined. The autotrophic denitrification process was seen to occur consistently, resulting in the presence of residual nitrate-nitrogen when the Hydraulic Retention Time (HRT) ranged from 1 to 3 hours. According to Figure 5, it is evident that there is a positive correlation between the decrease in Hydraulic Retention Time (HRT) and the increase in nitrate-nitrogen concentration in the effluent of the F1 reactor during the autotrophic denitrification stage. In the context of Hydraulic Retention Time (HRT) of 3 hours, it is shown that the initial nitrate-nitrogen content of 180mg/L in the influent water may be effectively decreased to a concentration of 120mg/L. At a Hydraulic Retention Time (HRT) of 2 hours, the concentration of nitrate-nitrogen in the F1 reactor exhibited a drop to 130 mg/L by the process of sulphur autotrophic denitrification. When the Hydraulic Retention Time (HRT) was decreased to one hour, there was a corresponding reduction in the content of nitrate-nitrogen in the effluent water to 150 mg/L. The denitrification process involved a two-step reaction sequence: initially, the conversion of NO_3-N to NO_2-N, followed by the conversion of NO_2-N to N_2. Autotrophic denitrification involves the conversion of NO_3-N to NO_2-N. Furthermore, during the autotrophic denitrification process, NO_3-N is further transformed into N_2. Autotrophic denitrification exhibits a lower reaction rate for the conversion of NO_3-N to NO_2-N compared to the conversion of NO_2-N to N_2. Conversely, heterotrophic denitrification demonstrates the opposite trend, with a higher reaction rate for the conversion of NO_3-N to NO_2-N compared to the conversion of NO_2-N to N_2. Consequently, the introduction of a carbon source during the sulphur autotrophic denitrification process might augment the efficacy of heterotrophic denitrification, resulting in a substantial enhancement of the denitrification rate (Figure 5).

The operational efficacy of sulphur autotrophic/heterotrophic co-denitrification is investigated in Figure 6. In this study, four distinct reactor configurations, namely F1 (with a sulfur to calcium carbonate to corncob ratio of 3:1:0), F2 (with a sulfur to calcium carbonate to corncob ratio of 3:1:1), F3 (with a sulfur to calcium carbonate to corncob ratio of 3:1:2), and F4 (with a sulfur to calcium carbonate to corncob ratio of 3:1:3), were subjected to experimental testing to evaluate their performance in reducing NO_3-N contents in both influent and effluent water samples, specifically under co-denitrification circumstances. In this study, four different types of reactors were utilized, each containing varying proportions of solid slow-release carbon derived from maize cobs. The objective of the studies was to measure the concentration of NO_3-N in both the inlet and exit water, while operating under circumstances that promote synergistic denitrification. As illustrated in Figure 6, at a residence time of 3 hours the concentration of nitrate-N in the effluent of the F1 sulphur autotrophic denitrification reactor was measured to be 120 mg/L. At a residence time of 3 hours, the concentration of nitrate-N in the effluent of the F2-F4 mixotrophic reactors exhibited a drop to 60 mg/L, 50 mg/L, and 40 mg/L, respectively. The concentration of nitrate-N in the effluent of the reactors exhibited a consistent reduction as the content of corncobs within the reactors increased. The concentration in the effluent of the reactor experienced a reduction (Figure 6).

Figure 7 displays the comparative outcomes of alkali consumption across various dosages of solid slow-release carbon sources. The figure 7 based on
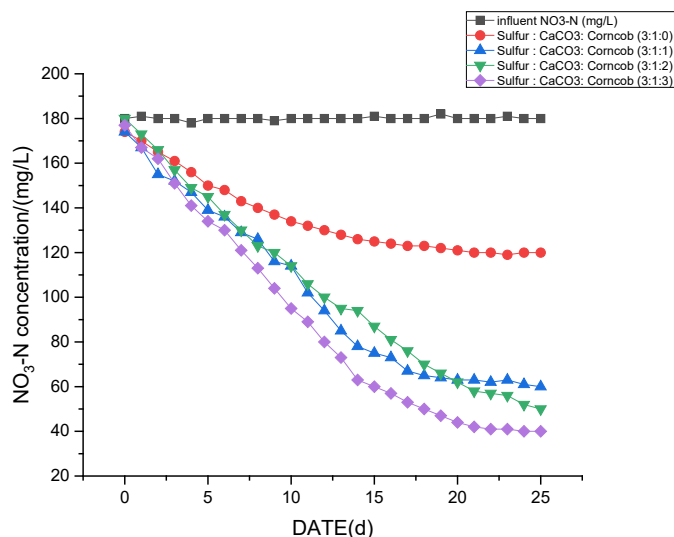

Figure 4. Presents the recorded concentrations of nitrate-nitrogen in both the influent and effluent water samples throughout the initial phase of the pilot reactor's operation.
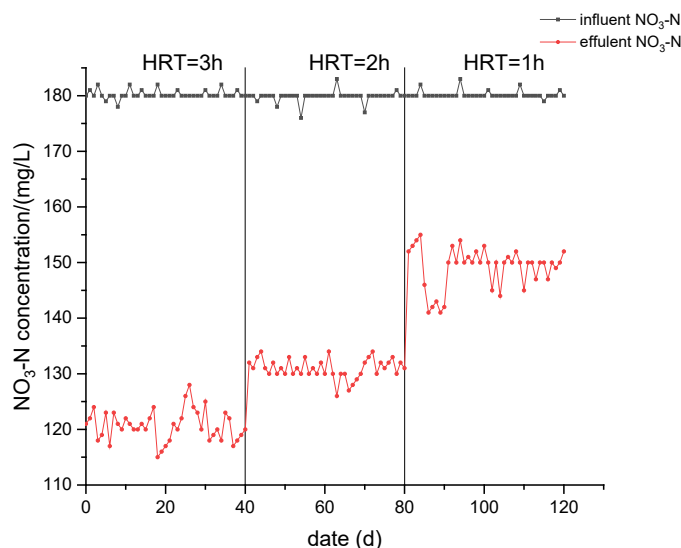

Figure 5. Illustrates the various effects of different HRT (Hydraulic Retention Time) on the operation of the F1 reactor.
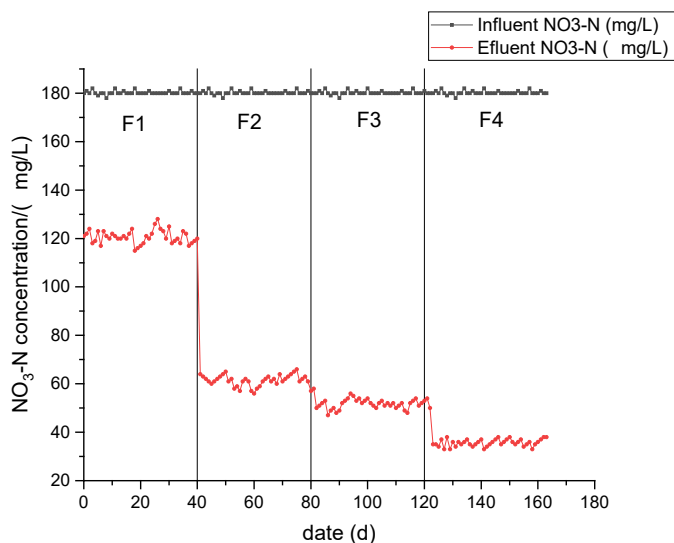

Figure 6. Examines the variation in effluent nitrate-nitrogen content across multiple reactors, each operating at three different hydraulic retention times.

the analysis of alkalinity levels and the removal of nitrate and nitrogen in the water at the intake and outflow during the operational phase, it is possible to determine the precise alkalinity consumption associated with co-denitrification and autotrophic denitrification, both of which achieve the same level of removal. The alkalinity consumption during the application of F2 at different carbon dosages exhibits minor variations when compared to the autotrophic denitrification phase. During the autotrophic denitrification phase, the observed alkali consumption was found to be 80 mg/L lower than the anticipated theoretical value. Similarly, in the F2 carbon dose, the alkali consumption was shown to be 200 mg/L lower than the theoretical value. The F1 carbon dosage experiment observed a behavior in which the carbon supply was completely consumed without any heterotrophic denitrification. This occurrence is likely attributed to the activity of parthenogenetic autotrophic/heterotrophic denitrifying bacteria. Significant variations in alkalinity consumption were seen under the F3 and F4 carbon dose circumstances, with reductions of 280 mg/L and 370 mg/L compared to the corresponding theoretical values. In a theoretical scenario, the removal of 1g of NO_3-N under autotrophic settings would result in the consumption of 4.57g of $CaCO_3$ alkalinity. Conversely, the removal of 1g of NO_3-N under heterotrophic conditions would lead to the production of 3.57g of $CaCO_3$ alkalinity. The disparity between theoretical and observed alkalinity consumption exhibited a positive correlation with the dosage of F3 and F4 carbon sources, suggesting the presence of a discernible heterotrophic denitrification phenomena within the reaction (Figure 7).

The NO_3-N concentration in the effluent was tested at various stages of the hydraulic retention period, while employing varying quantities of carbon sources. The initial phase of the autotrophic denitrification process exhibits a comparatively low denitrification rate. The predominant occurrence of the reaction is primarily observed in the central and posterior regions. The introduction of additional carbon source resulted in an enhanced denitrification rate during the initial phase of the process, and this reaction rate remained consistent throughout the entire duration of the F2 reactor. Similarly, in the F3 and F4 reactors, the degradation rate of NO_3N was improved during the initial phase of the carbon source period. These findings suggest that the inclusion of an appropriate quantity of carbon source in the sulphur autotrophic denitrification system can substantially enhance denitrification efficiency. Furthermore, it was observed that the denitrification rate progressively increased with higher amounts of carbon source supplementation (Figure 8).

Analysis of the mechanism of the synergistic denitrification reaction

This study aims to conduct a comparative examination of the Chemical Oxygen Demand (COD) levels in water, both in aquatic environments and outside of water, particularly under synergistic settings. The purpose of this study was to examine the correlation between Chemical Oxygen Demand (COD) consumption and nitrogen removal efficiency in the synergistic denitrification process, while considering various carbon dosage circumstances. The findings of this investigation are presented in Figure 9. The concentrations of Chemical Oxygen Demand (COD) in the influent and effluent water were investigated for the F1, F2, and F3 reactors, with varying carbon dosages. The concentration of Chemical Oxygen Demand (COD) was determined to be 270 mg/L, 310 mg/L, and 360 mg/L across the three experimental settings. The reduction in COD use suggests that the utilization of COD may be influenced by autoxidative denitrification or bacteria (Figure 9).

The impact of effluent and effluent sulfate in the presence of synergistic circumstances. To ascertain the precise quantity of nitrate and nitrogen eliminated from the autotrophic fraction in the presence of synergistic circumstances, the effluent sulphate concentration was assessed across several reaction conditions. The outcomes of this analysis are depicted in Figure 10. The levels of sulfate produced under the three different dosages of carbon sources were measured to be 780 mg/L, 820 mg/L, and 960 mg/L, respectively. Based on the measured nitrate and nitrogen levels in the effluent, the quantities of sulphate produced through complete autotrophic denitrification were determined to be 874 mg/L, 927 mg/L, and 1093 mg/L, respectively. In comparison to the theoretical values, the concentration of sulphate exhibited an increase of 72 mg/L, 44 mg/L, and 1093 mg/L under
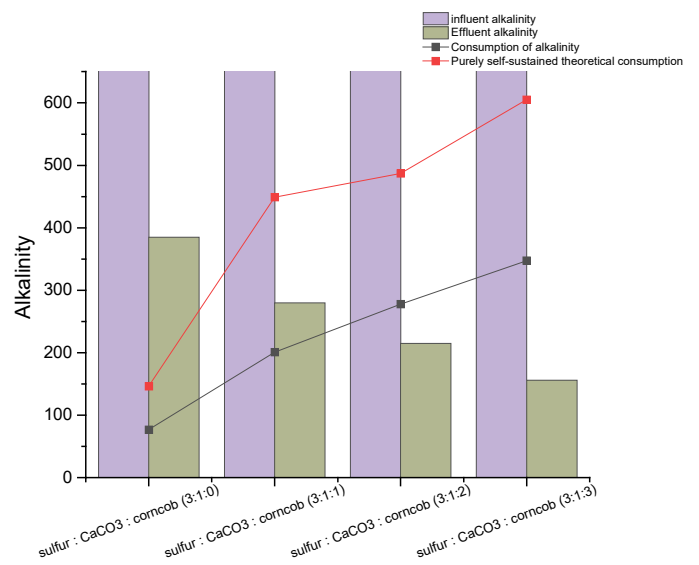

Figure 7. Examines the variability of alkalinity across four distinct types of reactors.
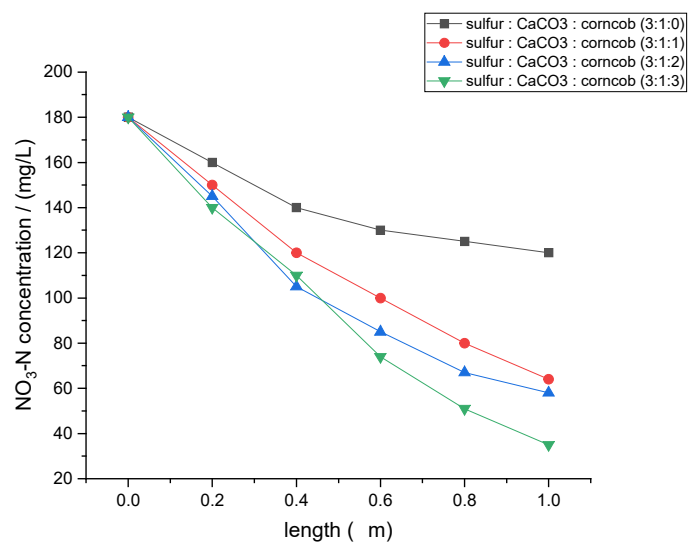

Figure 8. The concentration of nitrate-nitrogen exhibits variation over the course of four reactors.
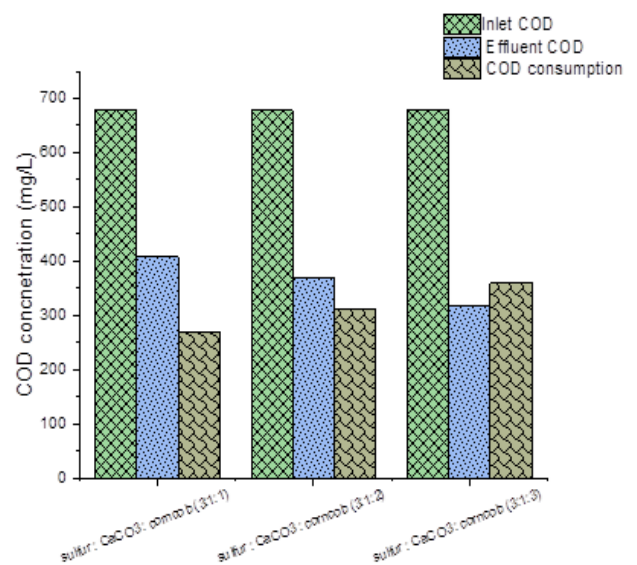

Figure 9. The alterations in the Chemical Oxygen Demand (COD) levels of the incoming and outgoing water are depicted.

the synergistic conditions, respectively. The observed sulphate production under the synergistic conditions exhibited an increase of 72 mg/L, 44 mg/L, and 18 mg/L, respectively, in comparison to the corresponding theoretical values. Furthermore, the theoretical sulphate production under the synergistic conditions, as calculated based on COD consumption, was found to be lower than the actual production. The measurement of COD consumption indicated the presence of heterotrophic denitrification, whereas the quantification of sulphate production provided insights into the extent of autotrophic denitrification. The occurrence of heterotrophic denitrification within the synergistic denitrification process leads to a partial reduction in sulphate generation. Empirical findings indicate a notable decrease in sulphate production as carbon consumption increases (Figure 10).

The impact of effluent and effluent sulfate in the presence of synergistic circumstances. To ascertain the precise quantity of nitrate and nitrogen eliminated from the autotrophic fraction in the presence of synergistic circumstances, the effluent sulphate concentration was assessed across several reaction conditions. The outcomes of this analysis are depicted in Figure 10. The levels of sulfate produced under the three different dosages of carbon sources were measured to be 780 mg/L, 820 mg/L, and 960 mg/L, respectively. Based on the measured nitrate and nitrogen levels in the effluent, the quantities of sulphate produced through complete autotrophic denitrification were determined to be 874 mg/L, 927 mg/L, and 1093 mg/L, respectively. In comparison to the theoretical values, the concentration of sulphate exhibited an increase of 72 mg/L, 44 mg/L, and 1093 mg/L under the synergistic conditions, respectively. The observed sulphate production under the synergistic conditions exhibited an increase of 72 mg/L, 44 mg/L, and 18 mg/L, respectively, in comparison to the corresponding theoretical values. Furthermore, the theoretical sulphate production under the synergistic conditions, as calculated based on COD consumption, was found to be lower than the actual production. The measurement of COD consumption indicated the presence of heterotrophic denitrification, whereas the quantification of sulphate production provided insights into the extent of autotrophic denitrification. The occurrence of heterotrophic denitrification within the synergistic denitrification process leads to a partial reduction in sulphate generation. Empirical findings indicate a notable decrease in sulphate production as carbon consumption increases (Figure 10).

The analysis focused on the calculation of nitrate-nitrogen removal derived from sulphate generation. Figure 11 illustrates the ratios of autotrophic and heterotrophic denitrification under synergistic conditions, as determined by the effluent SO_4^{2-} concentration and the autotrophic denitrification ratio calculated from COD consumption. The calculation of the proportion of heterotrophic denitrification (r_h) and the fraction of autotrophic denitrification (r_a) was performed according to equations (2).

$$r_h = 1 - \frac{S}{N \cdot S_n}$$
$$r_a = 1 - \frac{R}{N \cdot R_n}$$

(2)

In this context, the variables are defined as follows: S represents the measured concentration of sulphate generation in milligrams per liter (mg/L); N represents the measured concentration of nitrate nitrogen removal in mg/L; S_N represents the concentration of sulphate generation required to remove one mg/L of nitrate nitrogen, also measured in mg/L; R represents the measured Chemical Oxygen Demand (COD) consumption in mg/L; and R_N represents the concentration of COD generation required to remove one mg/L of nitrate nitrogen, also measured in mg/L. A strong negative connection was seen between the proportions of autotrophic and heterotrophic denitrification. The percentage of heterotrophic denitrification, when three different carbon sources were injected, were found to be 10.82%, 11.58%, and 12.19%, respectively. The relative proportions were 10.82%, 11.58%, and 12.19%. The utilization of carbon source for heterotrophic denitrification exhibited an upward trend as the dosage of carbon source increased. Additionally, the combined ratios of autotrophic and heterotrophic denitrification under the three conditions were found to be 100.1, 101.2, and 102.1, respectively. These results suggest that there is a synergistic effect between autotrophic and heterotrophic removal processes as the dosage of carbon source increases (Figure 11).
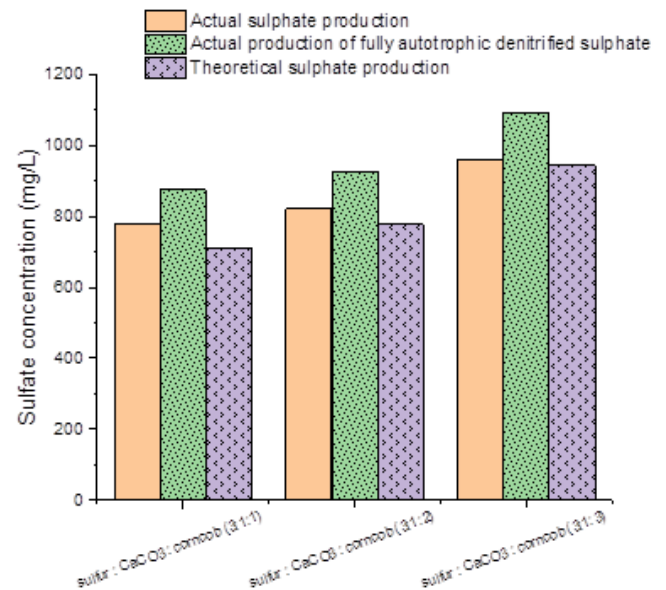

Figure 10. Illustrates a comparison between the theoretical and actual values of sulphate present in effluent water.
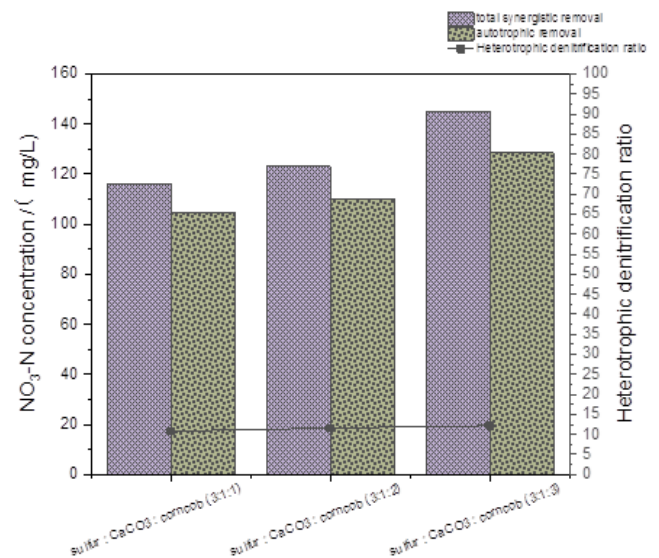

Figure 11. Examines the proportion of heterotrophic contribution.

Analysis of alkalinity in the presence of synergistic circumstances. The present study aimed to investigate the correlation between the removal of nitrate-nitrogen and the consumption of alkalinity, with the objective of gaining a deeper understanding of the involvement of heterotrophic denitrification in synergistic denitrification processes. The analysis focused on the alkalinity consumption during synergistic denitrification conditions. The alkalinity consumption of autotrophic organisms was excluded from the analysis to eliminate any deviation from the theoretical value. It was observed that the addition of a carbon source resulted in a reduction in alkalinity consumption, as depicted in Figure 12. The value of 11 represents the measured alkali consumption, which signifies the alkalinity required for the simultaneous removal of nitrate and nitrogen under fully autotrophic conditions. On the other hand, the theoretical alkali consumption, expressed in terms of Chemical Oxygen Demand (COD), represents the alkalinity generated through heterotrophic denitrification resulting from COD consumption, as well as the alkalinity consumed by the synergistic denitrification process combined with autotrophic denitrification. Additionally, the theoretical alkalinity consumption, measured in terms of sulfate (SO_4^{2-}) generation, indicates the alkalinity that would be consumed in the theoretical production of sulfate. The recorded alkali consumption values were 530, 562, and 662 mg/L for the three different

doses of carbon source. The amount of alkali used as a result of Chemical Oxygen demand (COD) consumption was determined to be 493 mg/L. The alkali consumption resulting from the formation of sulphate was found to be 472 mg/L for the F2 carbon source dose. This value was observed to be lower than the measured alkali consumption. The findings indicated that while COD was employed during the reaction procedure, there was no noteworthy production of alkalinity. Hence, the amount of alkali consumed exceeded the anticipated theoretical value. The observed disparity between the measured and predicted levels of alkali consumption, specifically in relation to the presence of SO_4^{2-} ions, suggests that sulphate was not generated. Simultaneously, the consumption of alkalinity was observed, suggesting that the presence of parthenogenetic denitrifying bacteria led to the depletion of alkalinity during the utilization of the organic carbon source. When the F3 carbon dosage was applied, the alkalinity consumption, as determined by COD consumption, was found to be 524 mg/L. This value was observed to be lower than the actual alkalinity consumption. Similarly, the alkalinity consumption based on sulphate generation was determined to be 497 mg/L, which was also lower than the actual alkalinity consumption. The Chemical Oxygen Demand (COD) was employed and simultaneously depleted a portion of the alkalinity. Simultaneous occurrences of autotrophic and heterotrophic processes were observed. In conjunction with the production of sulphate, the incorporation of a carbon source demonstrated a notable augmentation in the efficacy of autotrophic denitrification. This behavior was also observed when a carbon source was included. The alkalinity consumption, as determined by the consumption of Chemical Oxygen Demand (COD), was measured to be 605 mg/L. Additionally, the alkalinity consumption, as determined by the formation of sulphate, was found to be 581 mg/L. Theoretical alkalinity consumption, in relation to Chemical Oxygen Demand (COD), as well as actual consumption, were shown to be lower than the observed consumption. Theoretical alkali consumption (measured in terms of Chemical Oxygen Demand or COD) and actual alkali consumption both exhibited a decrease, suggesting that the heterotrophic effect had an inclination to intensify as the quantity of carbon source introduced increased (Figure 12).

Microbial community analysis

The microbiological phase of the biofilm on the packing material surface in the four groups of reactors was assessed on days 40, 80, 120, and 160 of the experiment (Figure 13).

The sulphur-limestone autotrophic denitrification reactor of F1 was primarily dominated by Proteobacteria, with a composition ratio of sulphur: $CaCO_3$:Corncob being 3:1:0. Similarly, in the heterotrophic, autotrophic co-denitrification reactors of F2, F3, and F4, where the composition ratios were sulphur: $CaCO_3$:Corncob of 3:1:1, 3:1:2, and 3:1:3 respectively, Proteobacteria also exhibited dominance. Furthermore, Proteobacteria were found to dominate in the heterotrophic-autotrophic synergistic denitrification reactors. The F2-F4 reactors exhibited a reduced relative abundance of Proteobacteria compared to the F1 sulfur-limestone autotrophic denitrification reactor. The F2-F4 reactor exhibited a comparatively reduced relative abundance of Proteobacteria compared to the F1 sulphur-limestone autotrophic denitrification reactor supplemented with corn cobs. The prevalence of Proteobacteria exhibited an upward trend as the corn kernel concentration grew in the F2-F4 reactors. Additionally, the relative abundance of Bacteroidetes surpassed that of the F1 sulfur-limestone autotrophic denitrification reactor in the F2-F4 reactors as the corn kernel content increased. The prevalence of Bacteroidetes exhibited a positive correlation with the augmentation of corn kernel content in F2-F4 reactors, thereby establishing Bacteroidetes as the prevailing species in the heterotrophic reaction. The mixotrophic denitrification process was seen to result in an increase in the relative abundance of Chloroflexi bacteria. This increase exhibited a declining trend as the concentration of the slow-release carbon source derived from corn cobs rose. A comparable scenario was observed in the case of Acidebateria, wherein the prevalence of Acidebateria bacteria exhibited an upward trend in the mixotrophic reactor, while it showed a decline in response to escalating concentrations of the slow-release carbon source derived from corncob (Figure 14).

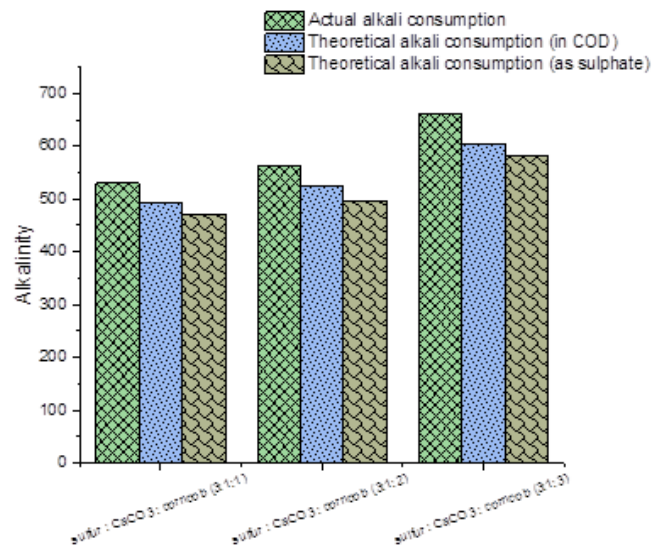In the sulfur-limestone autotrophic denitrification reactor of F1, a

Figure 12. The present study aims to compare the real alkali consumption with the theoretical alkali consumption.

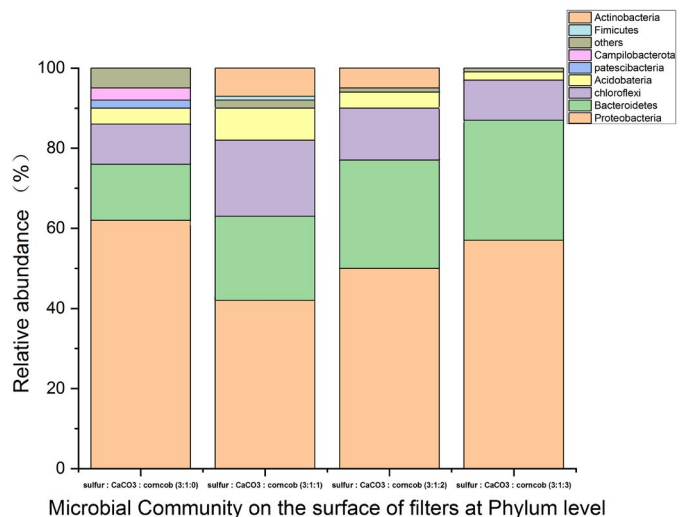Microbial Community on the surface of filters at Phylum level

Figure 13. The present study focuses on conducting a gate-level examination of microbial communities present on the surface of the reactor packing.

predominance of mixed bacteria was observed. Similarly, in the heterotrophic, autotrophic co-denitrification reactors of F2, F3, and F4, mixed bacteria also dominated. These reactors had different ratios of sulfur, $CaCO_3$, and corncob, with F2 having a ratio of 3:1:1, F3 having a ratio of 3:1:2, and F4 having a ratio of 3:1:3. Additionally, the heterotrophic-autotrophic synergistic denitrification reactors also exhibited a predominance of mixed bacteria. The prevalence of Thiobacillus in F2-F4 reactors was found to be comparatively lower than its prevalence in F1 sulfur-limestone autotrophic denitrification reactors. The prevalence of Thiobacillus in F2-F4 reactors exhibited a negative correlation with the proportion of corn cobs present. The F2-F4 reactors exhibited a greater relative abundance of Thiobacillus compared to the F1 sulfur-limestone autotrophic denitrification reactor. The prevalence of Themomonas in the F2-F4 reactors shown a positive correlation with the augmentation of maize kernel content. The Monomonas bacteria play a significant role as indicator species in the heterotrophic denitrification process.

Conclusion

The utilization of the sulphur autotrophic/heterotrophic synergistic denitrification system proves to be a viable option for effectively reducing nitrate levels in the secondary treatment effluent of pig wastewater, particularly
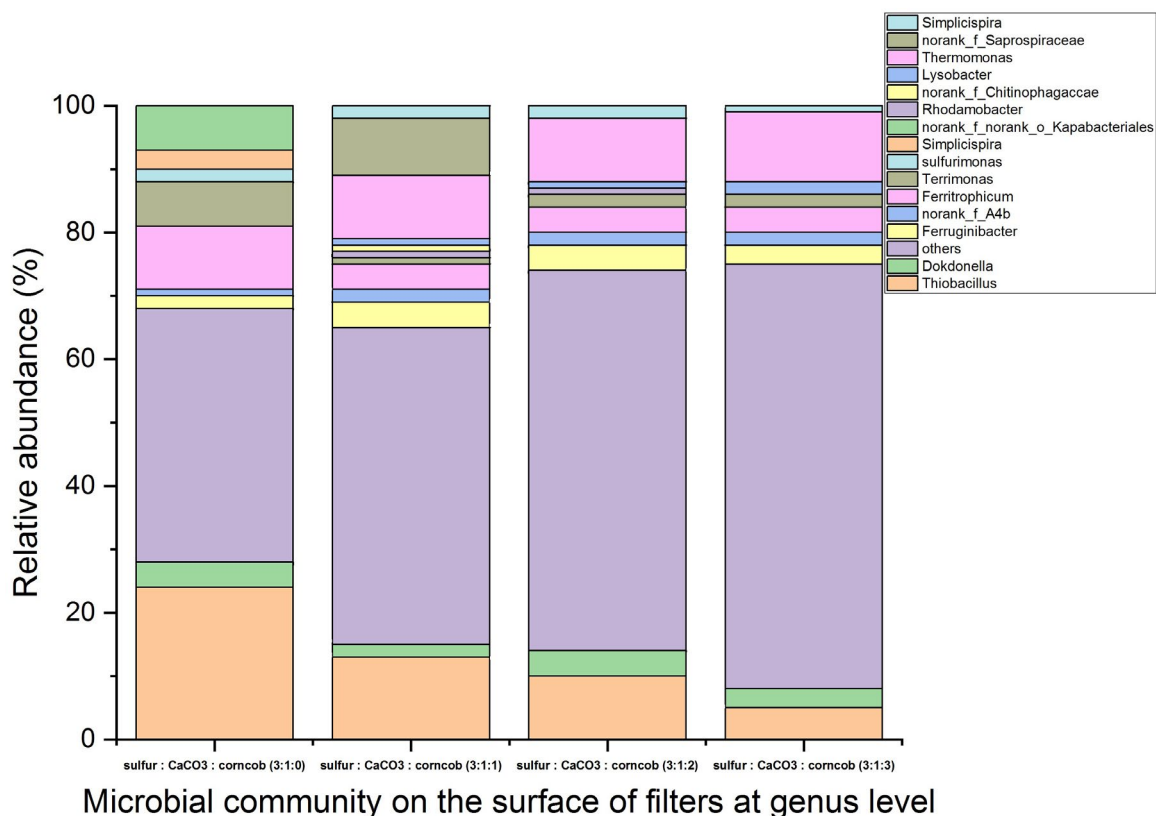
Figure 14. Focuses on conducting a genus-level investigation of the microbial community residing on the surface of the reactor packing.

in cases when deep denitrification is required. The treatment efficacy of this method is notable, demonstrating robust resistance to shock loads in wastewater with elevated concentrations of NO_3-N (NO_3-N>75 mg/L). The ultimate elimination of NO_3-N reached a level of 1.3 kg/(m^3/d), indicating a high rate of denitrification. The introduction of corn cob slow-release carbon into the autotrophic denitrification system resulted in an augmented removal of nitrate and nitrogen during the synergistic denitrification process, as compared to the autotrophic process. This observation suggests that the incorporation of a minimal quantity of carbon had a substantial positive impact on the denitrification process. In the context of the synergistic denitrification process, the consumption of carbon sources resulted in the utilization of Chemical Oxygen Demand (COD) across all three dosage conditions of the carbon sources. In situations when there is a reduced availability of carbon sources, it has been seen that heterotrophic denitrification does take place. However, it is important to note that the enhancement of nitrogen removal mostly stems from the heightened efficiency of autotrophic denitrification. The phylum Proteobacteria exhibited a prominent presence in all four groups of reactors, establishing its dominance at the phylum level. The genus Thiobacillus had the highest prevalence of a singular bacterial species among the four groups of reactors.

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How to cite this article: Zhang, Qing. "Study on the Removal of High Nitrate-nitrogen Concentration from Pig Farm Wastewater by Heterotrophic and Sulphur Autotrophic Synergistic Denitrification Filter Process." *J Civil Environ Eng* 13 (2023): 519.