Journal of Environment, Climate, and Ecology (JECE)
ISSN: 3079-255X (Online)
Volume 2 Issue 1, (2025)
https://doi.org/10.69739/jece.v2i1.625
https://journals.stecab.com/jece

Published by
Stecab Publishing

Research Article

Research on Ammonium Treatment from Cassava Starch Factory Wastewater Using the SNAP Model

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About Article

Article History
Submission: April 21, 2025
Acceptance : May 25, 2025
Publication : June 05, 2025

Keywords
Ammonium, Anammox, Cassava Wastewater Treatment, Nitrosomonas, SNAP

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ABSTRACT

This study applies the Single-stage Nitrogen removal using *Anammox* and Partial nitritation - SNAP model to remove organic compounds, especially NH_4^+, from wastewater. The experiment was carried out using real wastewater taken from the anaerobic tank of the wastewater treatment system of Nam Bao Tin Import Export Co., Ltd. The study was conducted in three phases with NH_4^+ concentrations of 58 mg/L, 90 mg/L, and 120 mg/L, respectively. The NH_4^+ removal efficiency reached 91.8% on the 51st day of operation (phase 2) and formed small amounts of nitrate. Also at this stage, the PO_4^{3-} and COD removal efficiency were 61.9% and 40.7%, respectively. Microbial density reached 1.5×10^7 CFU/mL by the end of phase 2.

Citation Style:
Kien, T. T., Loan, L. Q., Ngoc, T. T. M., Nhung, V. T. T., Dao, D. T. H., Vinh, T. Q., Giap, D. D., Dung, N. H., & Quan, L. K. M. (2025). Research on Ammonium Treatment from Cassava Starch Factory Wastewater Using the SNAP Model. *Journal of Environment, Climate, and Ecology, 2*(1), 61-67. https://doi.org/10.69739/jece.v2i1.625

1. INTRODUCTION

Ammonium (NH_4^+) is a common form of nitrogen compound in municipal, industrial, and livestock wastewater, usually existing as a dissolved ion with varying concentrations depending on the source of its generation. If left untreated, NH_4^+ can cause eutrophication of water sources, toxicity to aquatic organisms, and affect human health through the food chain (Zhou *et al.*, 2023). Therefore, the removal of NH_4^+ from wastewater is a goal of biological treatment systems. Among the NH_4^+ treatment methods, the biological nitrification and denitrification process is widely applied due to its high efficiency and environmental friendliness. However, traditional systems such as SBR (Sequencing Batch Reactor) or MBR (Membrane Bioreactor) often require long retention times, large investment costs, and tight control of operating conditions (Zhang *et al.*, 2018; Jiang *et al.*, 2021).

The Single-stage Nitrogen removal using *Anammox* and Partial nitritation (SNAP) model is the result of a study on the simultaneous operation of two processes of partial nitrification and *anammox* in a device that reacts with the combination of two groups of bacteria *Nitrosomonas* and *Anammox*, under controlled conditions of oxygen supply. This is a method used in wastewater treatment to remove nitrogen, especially ammonium. This process creates a very efficient wastewater treatment technology with high concentrations of NH4+. The combination of two processes of partial nitriting and *anammox* forms a two-step process carried out by two different groups of bacteria. The first step is the aerobic autotrophic process by AOB (ammonium oxidizing bacteria) catalyzing the oxidation of ammonium to nitrite with oxygen as the electron acceptor according to equation 1 (1). The second step is the anaerobic autotrophic process carried out by *Anammox* bacteria in which nitrite is an alternative electron acceptor to molecular oxygen, equation 2 (2) (Le *et al*, 2023; Antwi *et al.*, 2019; Yokota *et al.*, 2021).

$$2.34NH_4^+ + 1.85O_2 + 2.66HCO_3^- \longrightarrow 0.024C_5H_7NO_2\ (\textit{Nitrosomonas}) + NH_4^+ + 1.32NO_2^- + 2.54CO_2 + 2.94H_2O \quad (1)$$

$$NH_4^+ + 1.32NO_2^- + 0.066CO_2 + 0.066H^+ \longrightarrow 0.066CH_2O_{0,5}N_{0,15}\ (\textit{Anammox}) + 1.02N_2 + 0.26NO_3^- + 1.96H_2O \quad (2)$$

2. LITERATURE REVIEW

The treatment process because of combining partial nitriting and *anammox* has many advantages over nitrification-denitrification treatment, which greatly reduces the treatment cost. Since partial nitrification and *anammox* are biological metabolites, carried out by groups of autotrophic bacteria with different growth characteristics, the key issue is to study the reaction technique to achieve the best control of both metabolisms (Pham & Furukawa, 2008). While the SNAP process has been increasingly studied, most previous research has utilized synthetic wastewater. In contrast, this study is among the first in Vietnam to investigate the application of the SNAP model on real cassava starch factory wastewater, a type of high-strength industrial effluent characterized by fluctuating ammonium, phosphate, and COD levels. The use of actual wastewater enhances the practical value of the findings and contributes significantly to the localization of advanced nitrogen removal technologies. Cassava starch production is one of the key agro-industrial activities in Vietnam, especially in tropical and subtropical regions where cassava cultivation is widespread due to favorable climate and soil conditions. However, the wastewater generated from cassava processing contains high concentrations of ammonium, organic compounds, and phosphate, posing serious environmental risks if not adequately treated. Traditional biological treatment systems often face challenges in these settings due to the variable and high-strength nature of the influent, the need for chemical inputs, and limited infrastructure in rural or decentralized areas. While the SNAP model has shown promise in controlled settings, its effectiveness in real-world tropical wastewater contexts has not been well-documented. This study provides critical data and practical insights into adapting the SNAP model to Vietnam's agro-industrial sector, supporting the development of cost-effective, environmentally friendly treatment solutions suitable for local conditions.

3. METHODOLOGY

3.1. Materials

3.1.1. Activated sludge

1 liter of *Anammox* sludge with a volatile suspended solids (VSS) concentration of 5020 mg/L and 10 g of a culture (a mixture of *Nitrosomonas* sp. and Nitrobacter sp. with a density of 109 CFU/g) from the microbial collection of the Department of Microbiology, Institute of Life Sciences - Vietnam Academy of Science and Technology, Vietnam, was added to the SNAP model.

3.1.2. Wastewater

The wastewater used in the experiment was collected from the effluent after the anaerobic tank of the 60 m3/day treatment system at Nam Bao Tin Import-Export Co., Ltd. (Linh Dong Ward, Thu Duc City, Ho Chi Minh City, Vietnam), with the parameters as shown in Table 1.

Table 1. Composition and characteristics of the influent wastewater

Parameters	Units of calculation	Average value
pH	-	6.91 ± 0.14
COD	mgO_2/L	320 ± 5.7
NH_4^+	mg/L	58 ± 2,4
NO_3^-	mg/L	1.4 ± 0.1
PO_4^{3-}	mg/L	14.1 ± 0.3

Source: Institute of Life Sciences

3.1.3. Experimental model

The experimental model was designed and built based on published data by Le *et al.* (2012). The reactor is in the form of a rectangular box at the top to be opened, the dimensions of length - width - height are 270 × 125 × 450 (mm) respectively (volume = 15.2 liters; useful volume = 12 liters). The device is divided into 2 compartments: a 10-liter reaction compartment with a substrate of polyacrylic fibers, in the form of a sheet and a 2-liter settling compartment, these 2 compartments are connected at the bottom. The design is so that the inlet wastewater will go from the end of the reaction compartment at the top, move to the end of the reaction compartment and flow through the settling compartment from the bottom, continue to go up to the top of the settling compartment and flow through the pipe mouth out of the equipment. The model is designed to extend the path of wastewater, avoid flow shortage, help the treatment process achieve high efficiency, The model is operated at the Department of Microbiology, Institute of Life Sciences - Vietnam Academy of Science and Technology, Vietnam. The model consists of an ìnfluent wastewater container (1) with a capacity of 12 liters. Metering pump (2) for pumping wastewater from the container into the SNAP tank with a flow rate of 10 liters/day. Air pump (3) supply gas to the reaction chamber (4). Effluent wastewater container (5), volume 12 liters (Figure 1).

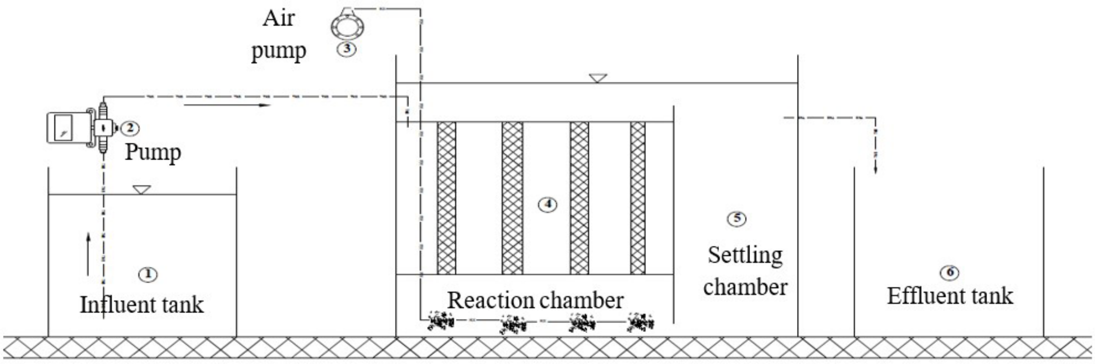

Figure 1. Experimental model

3.1.4 Experimental methods

The experimental model is continuously operated for 90 days (excluding the running phase to start the experimental model), influent and effluent samples are taken throughout the operation period, periodically every 2 days and analyzed 3 times with the following analysis parameters: pH, NH_4^+, NO_3^-, PO_4^{3-}, COD, analyzed immediately after sampling. Warm-up phase pH 6.91; DO 0.2-0.5 mgO_2/L; COD 160 mgO_2/L; NH_4^+ 29 mg/L; NO_3^- 0.7 mg/L; PO_4^{3-} 7.05 mg/L. When the performance reaches above 50% for the NH_4^+ parameter (actual follow-up in this period is 14 days), the model moves to phase 1, specifically as follows:

Phase 1: pH 6.91; DO 0.2-0.5 mgO_2/L; COD 320 mgO_2/L; NH_4^+ 58 mg/L; NO_3^- 1.4 mg / L; PO_4^{3-} 14.1 mg/L, operating the model for a period of 30 days. Phase 2: pH 6.91; DO 0.2-0.5 mgO_2/L; COD 320 mgO_2/L; NH_4^+ 90 mg/L; NO_3^- 1.4 mg/L; PO_4^{3-} 14.1 mg/L, operating the model for a period of 30 days. Phase 3: pH 6.91; DO 0.2-0.5 mgO_2/L; COD 320 mgO_2/L; NH_4^+ 120 mg/L; NO_3^- 1.4 mg / L; PO_{43-} 14.1 mg/L, operating the model for a period of 30 days.

3.2. Data analysis and processing methods

The pH value is measured using the HI2210 pH Meter (HANNA Instruments). DO is measured using the DO-802 dissolved oxygen concentration meter (APEL Instruments). The COD was sampled using the ECO25 Thermoreactor sampler (VELP Scientifica - Italy) and measured by the Primelab 2.0 meter (Germany). NH_4^+, NO_3^-, PO_4^{3-} were analyzed based on "Standard Methods for the examination of water and wastewater" and measured by Jasco V-730 Spectrophotometer. The data are processed based on Microsoft Office Excel 365 software; the results are processed according to the mathematical statistical method of 3 times of analysis of the same indicator.

The arithmetic mean (x) is calculated as follows:

$$\bar{x} = \sum_{i-1}^{n} \frac{x_i}{n}$$

The formula calculates the standard deviation (S):

$$S = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

4. RESULTS AND DISCUSSION

4.1. pH changes during experimental operation

Figure 2 shows the pH change during the operation of the experimental model. The input pH ranges from 6.82 to 7.08, while the output pH ranges from 6.88 to 7.16. The results indicate that the pH in the SNAP model remains relatively stable. This may be due to the simultaneous presence and activity of two groups of bacteria, *Nitrosomonas* and *Anammox*, in the SNAP model.

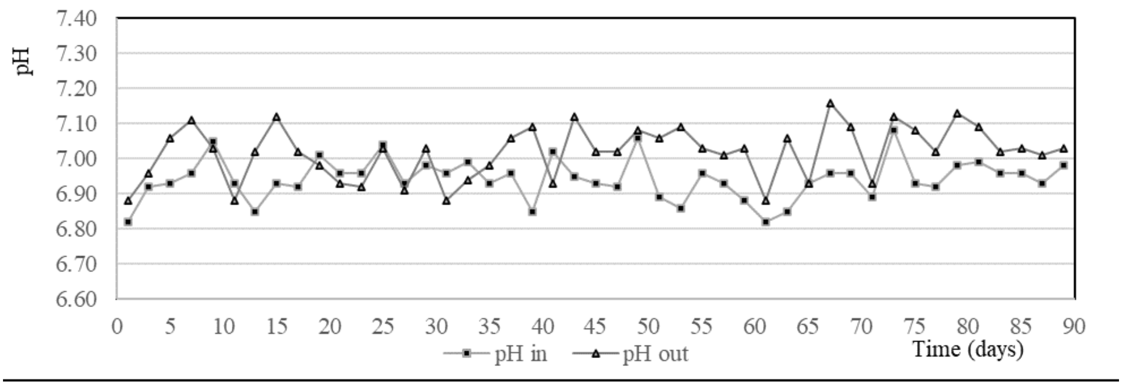

Figure 2. The change in pH over time experiments

Nitrosomonas bacteria consume HCO_3^- ions (alkalinity), which lowers the pH of the environment, while *Anammox* bacteria consume H^+ ions, increasing pH. The interaction between these two groups of bacteria helps maintain a relatively stable pH, as shown by equation 3 (3) (Yokota *et al.*, 2021; Furukawa *et al.*, 2006).

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H + \longrightarrow 0.066CH_2O_{0.5}N_{0.15} + 1.02N_2 + 0.26NO_3^- + 2.03H_2O \quad (3)$$

4.2. NH_4^+ processing performance through experimental phases

Figure 3 shows that the NH4+ processing performance is relatively high, ranging from 62.4% to 91.8%. The NH_4^+ removal efficiency in this study peaked at 91.8%, which is comparable to the performance reported by Furukawa *et al.* (2006), where SNAP systems achieved up to 90% ammonium removal under similar operating conditions. Moreover, this result is higher than the 85% removal observed in SNAP systems treating synthetic wastewater (Antwi *et al.*, 2019), indicating that real cassava wastewater did not compromise system efficiency when properly acclimated. The activity of *Nitrosomonas* bacteria plays a significant role in the SNAP model, they have a role in accumulating nitrite sources to provide substrates for *Anammox* bacteria to complete the oxidation of ammonium into nitrogen.

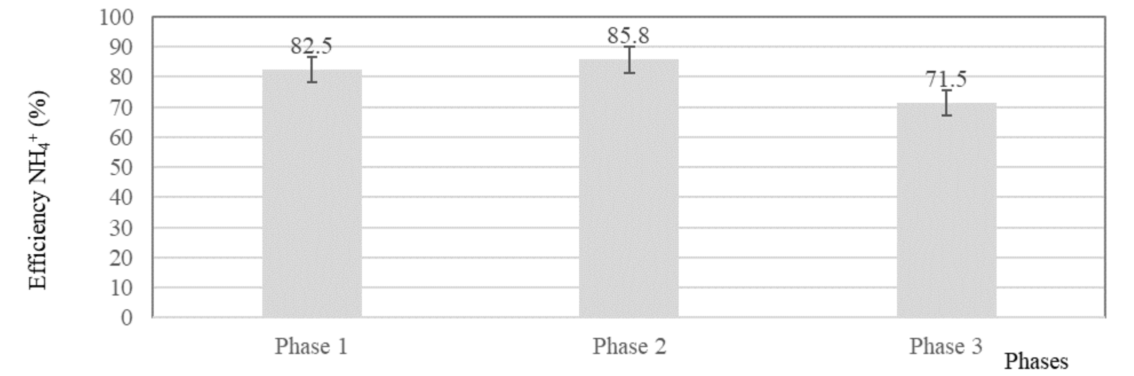

Figure 3. NH_4^+ treatment efficiency through experimental phases

In addition, there is nitrate formation, as shown in Figure 4, the increased amount of nitrate output may be due to the amount of nitrite formed from ammonium oxidation that continues to be oxidized to nitrate thanks to the microbial groups in the reaction compartment. In addition, some of the lost nitrogen can exist in the form of microbial biomass that forms and in the form of nitrogen gas made by *Anammox* bacteria. AOB aerobic bacteria participate in the oxidation of ammonium to nitrite with oxygen as the final electron acceptor and when the amount of oxygen present in the wastewater decreases, it facilitates *Anammox* bacteria to carry out the oxidation of ammonium to nitrogen gas with nitrite as an alternative electron acceptor for molecular oxygen sources under anaerobic conditions (Pham Khac Lieu & Kenji Furukawa, 2008; Le *et al.*, 2019; Zulkarnaini *et al.*, 2018).

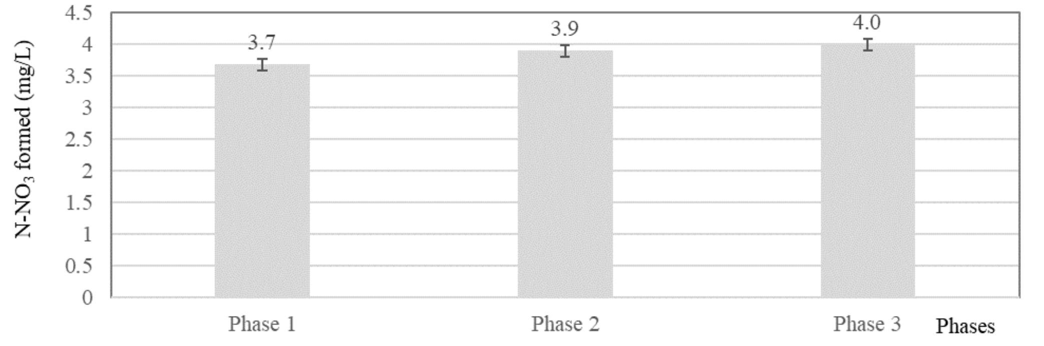

Figure 4. NO_3^- formed through experimental phases

4.3. PO_4^{3-} processing performance through experimental phases

Phosphorus removal reached up to 61.9%, close to values reported in systems with enhanced biological phosphorus removal (EBPR) under intermittent aeration, as described by Le (2007). This suggests that although the SNAP model was not designed for phosphorus removal, incidental uptake by polyphosphate-accumulating organisms (PAOs) may have occurred (4).

$$C_2H_4O_2 + 0.16NH_4^+ + 1.2O_2 + 0.2PO_4^{3-} \longrightarrow 0.16C_5H_7O_2N + 1.2CO_2 + 0.2(HPO_3) + 0.44OH^- + 1.44H_2O \quad (4)$$

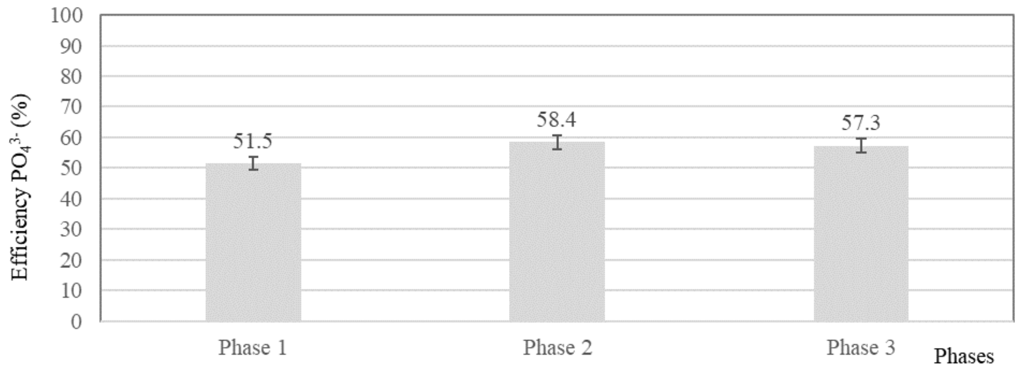

Figure 5. PO_4^{3-} treatment efficiency through experimental phases

4.4. COD Processing Performance Through Experimental Phases

The COD removal efficiency remained below 41% (Figure 6), which is lower than conventional systems involving heterotrophic bacteria (e.g., A2/O-MBR systems often report >80% COD removal, Zhang *et al.*, 2018). Due to the experimental model, only *Anammox* sludge and a mixture of preparations (*Nitrosomonas* sp. and Nitrobacter sp.) were added., these two groups of bacteria do not use the carbon available in the environment. The reduced carbon source in the laboratory environment may be due to other aerobic heterotrophic bacteria available in the wastewater that have grown and used.

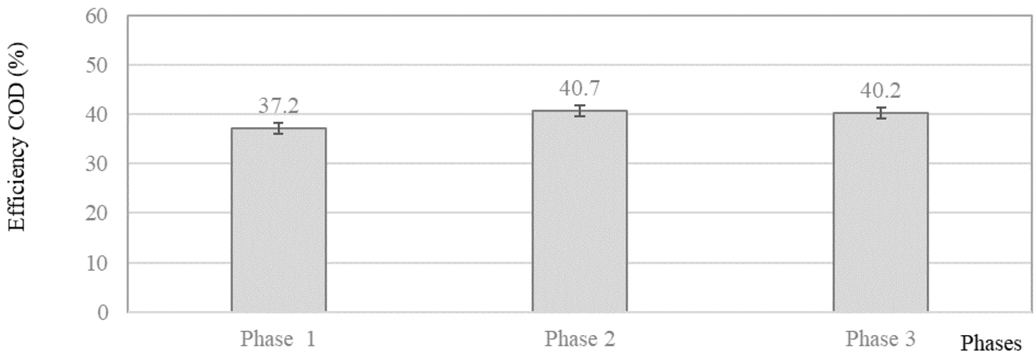

Figure 6. COD treatment efficiency through experimental phases

4.5. Microbial density during the operation of the experimental model

The change in microbial density during the experiment is shown in Table 2. The results show that the microbial density in the SNAP model is not only high, but also stable and gradually increases over each stage. After 30 days of operation, the microbial density increased by 10.8 times compared to the original value. In phases 1 and 2, microbial density increased steadily and steadily, reached 1.3 x 10^7 CFU/mL and 1.5 x 10^7 CFU/mL, respectively.

Table 2. Microbial density during the operation of the experimental model

Stages of operation	Initial density	End of Phase 1	End of Phase 2	End of Phase 3
Microbial density (CFU/mL)	1.2x10^6	1.3x10^7	1.5x10^7	1.2x10^6

However, by the end of phase 3, the microbial density in the SNAP model decreased by 12.5 times compared to phase 2. This shows that the wastewater treatment efficiency of the SNAP model is directly proportional to the microbial density in the activated sludge. High and stable microbial density, which is maintained throughout operation, is maintained over time, which contributes to the efficient operation of the system.

The performance of the SNAP model in ammonium removal was found to be high, reaching a peak efficiency of 91.8% on the 51st day of operation, particularly during phase 2 with an influent NH_4^+ concentration of 90 mg/L. This efficiency is comparable to or even higher than previously reported SNAP systems treating wastewater with similar ammonium loads (Furukawa *et al*., 2006; Antwi *et al*., 2019). The elevated removal efficiency at this stage suggests that the microbial consortium of *Nitrosomonas* and *Anammox* bacteria had reached a stable and active state under the controlled operational parameters.

One of the key factors contributing to stable system performance was the regulation of pH, which remained within the optimal range for both *Nitrosomonas* (6.8 - 7.3) and *Anammox* (6.7 - 7.2) activity. As previously reported (Yokota *et al*., 2021), the partial alkalinity consumption by *Nitrosomonas* and proton consumption by *Anammox* likely counterbalanced each other, maintaining a near-neutral pH environment without the need for chemical buffering.

Moreover, the drop in microbial density observed at the end of phase 3, despite higher influent NH_4^+ (120 mg/L), indicates a possible inhibition effect caused by ammonium overloading. High ammonium concentrations can lead to the accumulation of free ammonia (FA), which is known to inhibit the activity of both *Nitrosomonas* and *Anammox* bacteria (Zulkarnaini *et al*., 2018). This microbial decline suggests that although SNAP systems are resilient, there exists a threshold beyond which reactor performance deteriorates due to microbial stress or toxic inhibition.

Nitrate accumulation during all phases, as shown in Figure 4, also reflects the potential partial oxidation of nitrite by residual aerobic bacteria, such as Nitrobacter, that may have persisted in the reactor environment. This phenomenon may reduce the efficiency of total nitrogen removal if left unchecked and indicates the need for more precise dissolved oxygen (DO) control to suppress Nitrobacter growth and favor partial nitritation.

Phosphorus removal peaked at 61.9% in phase 2, likely due to enhanced polyphosphate-accumulating organisms (PAOs) activity under intermittent aeration. Although phosphorus removal is not the primary function of the SNAP process, its occurrence under optimized operational settings highlights the synergistic potential of nutrient removal when diverse microbial populations co-exist under controlled conditions.

On the other hand, COD removal remained relatively low (~ 40%), which aligns with expectations since the microbial inoculum lacked sufficient heterotrophic bacteria. The limited carbon consumption is consistent with the dominance of autotrophic *Nitrosomonas* and *Anammox* bacteria in the system. In future implementations, co-culturing with heterotrophs or optimizing retention time could potentially enhance organic matter removal while maintaining nitrogen efficiency.

In summary, the SNAP model demonstrated strong potential for treating ammonium-rich wastewater from cassava starch processing. However, operational limits such as ammonium overloading and nitrate accumulation require careful monitoring to sustain microbial activity and ensure long-term stability.

5. CONCLUSION

This study demonstrated the effectiveness of the SNAP model in treating ammonium-rich real wastewater from a cassava starch production facility. The system operated under room temperature, pH 6.91, and 24-hour water retention time. The maximum NH_4^+ removal efficiency reached 91.8% on the 51st day of operation (phase 2), with concurrent PO43- and COD removal efficiencies of 61.9% and 40.7%, respectively. Microbial density peaked at 1.5×10^7 CFU/mL, correlating with optimal treatment performance.

For future research, efforts could focus on refining dissolved oxygen control and integrating heterotrophic bacteria to enhance overall nutrient removal. When considering scale-up, challenges such as maintaining microbial balance, preventing inhibition under high ammonium concentrations, and adapting to fluctuations in wastewater composition will need to be carefully managed through pilot-scale.

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