

EFFECT OF SALT CONCENTRATION ON SOY SAUCE WASTEWATER TREATMENT USING THE SNAP MODEL

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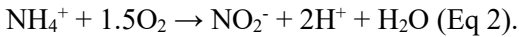
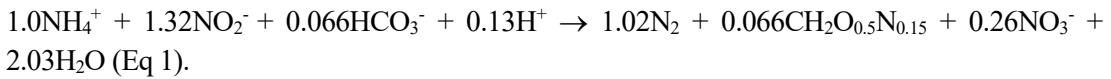
ABSTRACT

This study applies the SNAP model (Single-stage Nitrogen removal using Anammox and Partial nitrification) to remove organic compounds, especially N-NH₄, from wastewater. Experiments were conducted using real wastewater taken from the anaerobic treatment tank of the Mekong Sauce Processing Company's wastewater treatment system. The study was carried out across four phases with corresponding salt concentrations of 0.29%, 0.59%, 0.89%, and 1.18%. The results showed that at pH 7.24 ± 0.08 and a hydraulic retention time of 24 hours, the N-NH₄ removal efficiency peaked at 92.8% when the NaCl concentration was 0.59%. At the same salt concentration, the COD and P-PO₄ removal efficiencies were 42.4% and 56.5%, respectively. Additionally, the microbial density reached 1.5×10^8 CFU/mL at the end of phase 2.

Keywords: SNAP, wastewater treatment, ammonium nitrogen (N-NH₄), Sodium chloride (NaCl).

1. INTRODUCTION

Advancements in nitrogen removal from wastewater have gained increasing attention due to the significant environmental risks associated with nitrogenous compounds, particularly their detrimental impact on aquatic ecosystems. Excessive nitrogen discharge can lead to eutrophication, oxygen depletion, and severe disruptions in water quality. From an economic and operational standpoint, anaerobic ammonium oxidation (anammox) has emerged as a more efficient and cost-effective alternative to conventional nitrification-denitrification processes. Unlike traditional methods, anammox directly converts ammonium and nitrite into dinitrogen gas under anoxic conditions, minimizing energy consumption and the need for external carbon sources, as illustrated in Equation (Eq 1) [1]. However, since wastewater often lacks sufficient nitrite, an initial partial nitrification step is necessary to oxidize ammonium to nitrite at a controlled ratio of 1:1.32, as described in Equation (Eq 2) [2], ensuring optimal conditions for anammox activity. To enhance nitrogen removal efficiency, a novel integrated approach, termed the Shortcut Nitrification-Anammox Process (SNAP), has been successfully implemented in a Sequencing Batch Biofilm Granular Reactor (SBBGR), demonstrating robust performance in wastewater treatment applications [3]. The overall nitrogen removal efficiency of SNAP systems can be mathematically characterized by Equation (Eq 3) [4, 5].



Compared to conventional nitrification-denitrification, this way saves 100% of the external organic carbon source for de-nitrification and more than 50% of the oxygen supply for nitrification in the operational costs and a decrease in the energy demand. The principal steps of traditional and novel nitrogen removal processes are partially depicted in Fig. 1 [6].

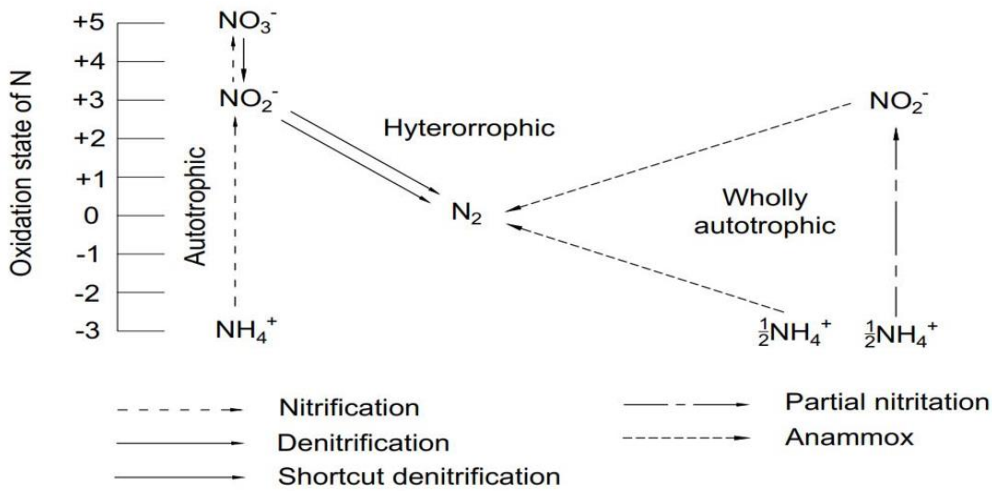
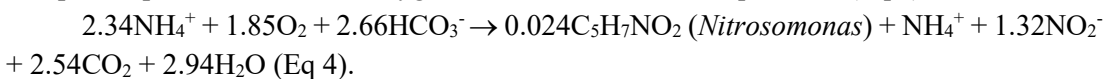
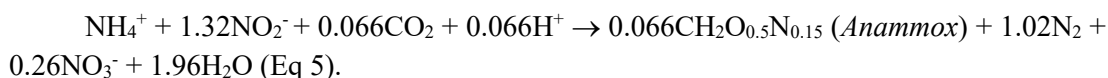


Figure 1. Principal steps of N-removal processes [6]

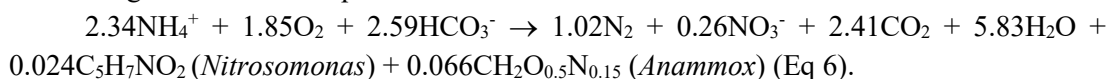
Many industries, such as seafood processing, petroleum production, and landfill leachate treatment, generate wastewater with high salinity (>1%) and hypersalinity (>3%), which adversely affects biological nitrogen removal processes [7]. Elevated salinity can disrupt functional microbial cells, leading to a significant decline or complete inhibition of nitrogen removal efficiency [8]. While physicochemical pretreatment methods can remove salts, their high operational costs make them less economically viable. Enhancing the performance of nitrogen removal processes under high-salinity conditions could reduce treatment costs and improve overall efficiency.

Anammox-based processes have attracted increasing attention due to their potential for efficiently treating ammonium-rich wastewater. Specifically, the SNAP model refers to a Single-stage Nitrogen removal using Anammox and Partial nitritation process. It is a method used in wastewater treatment to remove nitrogen compounds, particularly ammonium (NH_4^+), through the combined activity of two groups of bacteria: *Nitrosomonas* and *Anammox* bacteria. This process is carried out under controlled oxygen conditions to optimize the removal efficiency of nitrogenous compounds. The process begins with the autotrophic aerobic phase, in which ammonium-oxidizing bacteria (AOB) catalyze the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) with oxygen (O_2) acting as the electron acceptor, as described in reaction equation 4 (Eq 4). Following this, the process transitions to the autotrophic anaerobic phase, where *Anammox* bacteria perform the reaction, with nitrite (NO_2^-) serving as the electron acceptor in place of molecular oxygen, as shown in reaction equation 5 (Eq 5) [9, 10].





Combining the above two equations is as follows:



The SNAP model offers several advantages over the nitrification-denitrification process in wastewater treatment, including reduced operational and investment costs, high treatment efficiency, and lower energy consumption due to its lower oxygen demand. Additionally, the SNAP model is stable and capable of self-regulating under harsh conditions, while also generating less sludge, which reduces sludge treatment costs and improves overall system performance [11]. However, the application of the SNAP model combined with salt-tolerant bacteria in wastewater treatment remains underexplored. Therefore, this study serves as a critical foundation for future research and holds promising potential for practical implementation in the future.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Activated sludge

1 liter of *Anammox* sludge with a volatile suspended solids (VSS) concentration of 5120 mg/L and 10 g of a culture (a mixture of salt-tolerant *Nitrosomonas* sp. and *Nitrobacter* sp. with a density of 10^9 CFU/g) from the microbial culture collection of the Microbiology Department, Institute of Life Sciences, was added to the SNAP model.

2.1.2. Wastewater

The wastewater used in the experiment was collected from the effluent after the anaerobic tank of the 90 m³/day treatment system at Mekong Fish Sauce Processing Co., Ltd. (Duc Hoa Ha Commune, Duc Hoa District, Long An Province), with the parameters as shown in Table 1.

Table 1. Composition and characteristics of the influent wastewater

No.	Parameters	Units of calculation	Average value
1	pH	-	7.05 ± 0.08
2	COD	mgO ₂ /L	313.3 ± 5.7
3	N-NH ₄	mg/L	351 ± 2.4
4	N-NO ₃	mg/L	1.4 ± 0.1
5	P-PO ₄	mg/L	19.1 ± 0.3
6	Salinity	%	0.59 ± 0.02

2.1.3. Experiment model

The experimental model was designed and constructed based on the published data of authors Tran Trung Kien and Tran Quang Vinh (2011); Le Cong Nhat Phuong et al. (2012) [9, 12]. The reaction vessel is rectangular in shape, open at the top, with dimensions of 270 × 125 × 450 mm (total volume = 15.2 liters; useful volume = 12 liters). The vessel is divided into two sections: a 10-liter reaction chamber with polyacrylic sheet-based carrier material and a 2-liter settling chamber, which are connected at the bottom. The design ensures that the influent wastewater enters from the top of the reaction chamber, flows through the entire reaction chamber, then passes through the settling chamber from the bottom, rises to the top

of the settling chamber, and exits through the outlet pipe. This design extends the flow path of the wastewater, preventing flow short-circuiting and enhancing the treatment efficiency. The system is operated at the Applied Microbiology Laboratory, Tropical Biology Institute. The setup includes a 12-liter influent wastewater storage tank (1), a dosing pump (2) to introduce wastewater into the SNAP reactor at a flow rate of 10 liters/day, an air pump (3) supplying air to the reaction chamber (4), and a 12-liter effluent wastewater storage tank (6) (Fig. 2).

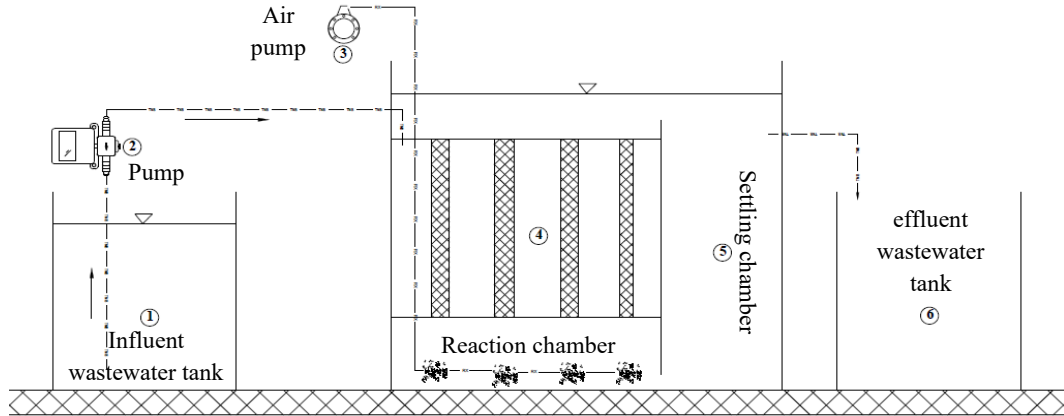


Figure 2. Experimental model of shortcut nitrification-anammox process system

2.1.4. Experimental methods

The experimental model was operated continuously for 120 days, starting with the startup phase (salt concentration 0.29%, pH 7.05; COD 138 mgO₂/L; N-NH₄ 176 mg/L; N-NO₃ 0.7 mg/L; P-PO₄ 9.6 mg/L). Once the performance reached over 50% for the N-NH₄ parameter (after 14 days), the model transitioned to Phase 1, as detailed below:

Phase 1 (P 1): Salt concentration 0.29%; pH 7.05; COD 138 mgO₂/L; N-NH₄ 176 mg/L; N-NO₃ 0.7 mg/L; P-PO₄ 9.6 mg/L, operated for 30 days.

Phase 2 (P 2): Salt concentration 0.59%; pH 7.05; COD 313 mgO₂/L; N-NH₄ 351 mg/L; N-NO₃ 1.4 mg/L; P-PO₄ 19.1 mg/L, operated for 30 days.

Phase 3 (P 3): Salt concentration 0.89%; pH 7.05; COD 313 mgO₂/L; N-NH₄ 351 mg/L; N-NO₃ 1.4 mg/L; P-PO₄ 19.1 mg/L, operated for 30 days.

Phase 4 (P 4): Salt concentration 1.18%; pH 7.05; COD 313 mgO₂/L; N-NH₄ 351 mg/L; N-NO₃ 1.4 mg/L; P-PO₄ 19.1 mg/L, operated for 30 days.

Throughout the operation, influent and effluent samples were collected every 2 days over the 120-day period and analyzed in triplicate for pH, N-NH₄, N-NO₃, P-PO₄, and COD, with immediate analysis conducted after sampling.

2.2. Methods of data analysis and processing

The pH values were measured using a HI2210 pH Meter (HANNA Instruments). Dissolved oxygen (DO) concentration was determined using a DO-802 meter (APEL Instruments). Chemical oxygen demand (COD) was measured after sample digestion using an ECO25 Thermoreactor (VELP Scientifica, Italy) and analyzed with a Primelab 2.0 meter (Germany). The concentrations of N-NH₄, N-NO₂, N-NO₃, and P-PO₄ were analyzed according to the "Standard Methods for the Examination of Water and Wastewater" and measured using a Jasco V-730 Spectrophotometer [13]. The data were processed using Microsoft Office Excel 2010 software, and the analytical results were statistically treated based on the method of three replicate analyses for each parameter.

The arithmetic mean (\bar{x}) was calculated as:
$$\bar{x} = \frac{\sum_{i=1}^n x_i}{n}$$

The standard deviation (S) was calculated using the formula:
$$S = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}}$$

3. RESULTS AND DISCUSSION

3.1. pH variation during the experimental operation

Figure 3 showed the pH change during the experiment's operation. A biological system can adapt to high or low pH levels over extended periods. However, rapid and unstable changes in pH can have an immediate impact on the microbial biomass in activated sludge.

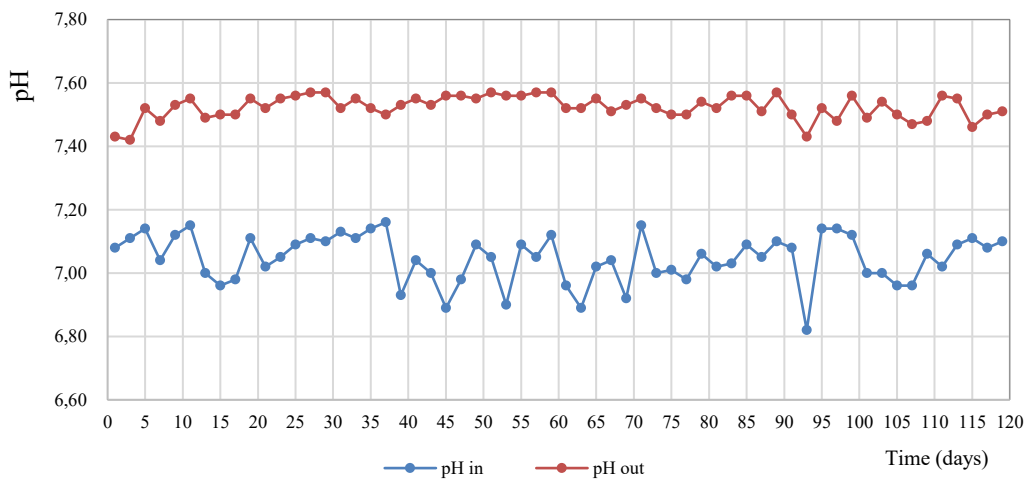


Figure 3. pH variation over experimental time.

The influent pH fluctuated between 6.82 and 7.16, while the output pH ranged from 7.42 to 7.57. The results indicate that the pH in the SNAP model remained relatively stable. This can be attributed to the simultaneous presence and activity of two groups of bacteria, *Nitrosomonas* and *Anammox*, in the SNAP model. *Nitrosomonas* bacteria consume HCO_3^- ions (alkalinity), which lowers the pH of the environment (Eq 4), whereas *Anammox* bacteria consume H^+ ions, leading to an increase in pH (Eq 5). The interaction between these two bacterial groups helps maintain a relatively stable pH, as reflected in Equation 7 (Eq 7) [14, 15].
$$\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{HCO}_3^- + 0.13\text{H}^+ \rightarrow 0.066\text{CH}_2\text{O}_{0.5}\text{N}_{0.15} + 1.02\text{N}_2 + 0.26\text{NO}_3^- + 2.03\text{H}_2\text{O}$$
 (Eq 7).

3.2. N-NH₄ removal efficiency across the experimental phases

Despite variations in salt concentrations and loading rates across different experimental phases, Figure 4 showed that the N-NH₄ conversion efficiency remained relatively high and stable, around 90%. The highest removal efficiency was observed in Phase 2, reaching 92.8% on the 49th day of operation. The applied load in the SNAP system, utilizing nonwoven polyester fiber as the carrier for treating synthetic wastewater, resulted in a N-NH₄ removal efficiency of 60-80% at a loading rate of 0.48 kg N-NH₄/m³/day [14]. Additionally, the influent nitrite concentration was either negligible or very low, fluctuating between 0 and 0.1 mg/L. The effluent nitrite concentration showed a slight increase compared to the influent, ranging

from 0 to 0.3 mg/L across all four phases. The activity of *Nitrosomonas* bacteria plays a crucial role in the SNAP model, as they accumulate nitrite, which serves as the substrate for *Anammox* bacteria to complete the ammonium oxidation process into nitrogen gas.

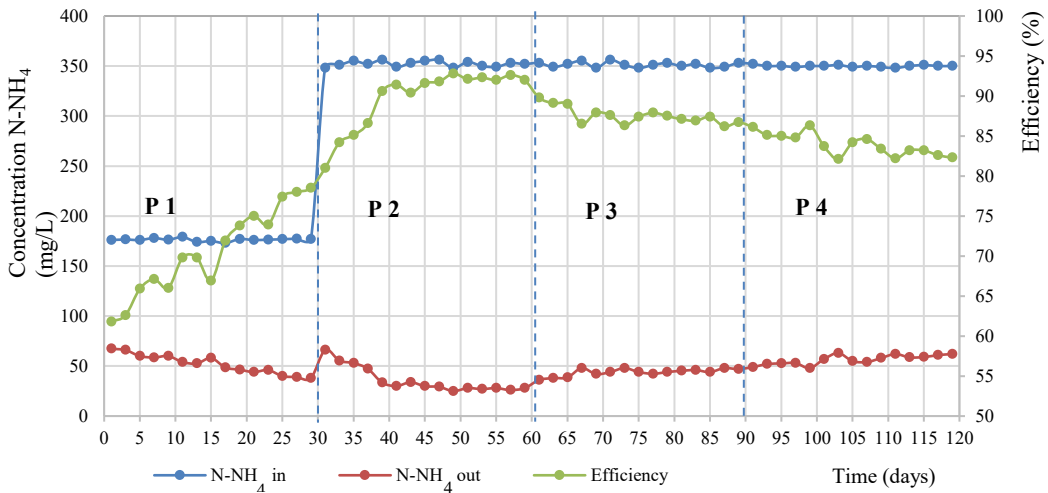


Figure 4. N-NH₄ removal efficiency through the experimental phases

Furthermore, the formation of nitrate, as shown in Figure 5, suggests that nitrite generated from ammonium oxidation continues to be oxidized into nitrate by other microbial groups (*Nitrobacter* sp) present in the reactor. Additionally, some nitrogen loss may occur in the form of microbial biomass or nitrogen gas due to the activity of *Anammox* bacteria. The aerobic AOB bacteria participate in the oxidation of ammonia to nitrite with oxygen as the terminal electron acceptor. When the oxygen concentration in the wastewater decreases, favorable conditions are created for *Anammox* bacteria to carry out the ammonium oxidation process into nitrogen gas, with nitrite serving as the electron acceptor in anaerobic conditions, replacing molecular oxygen [11, 16, 17].

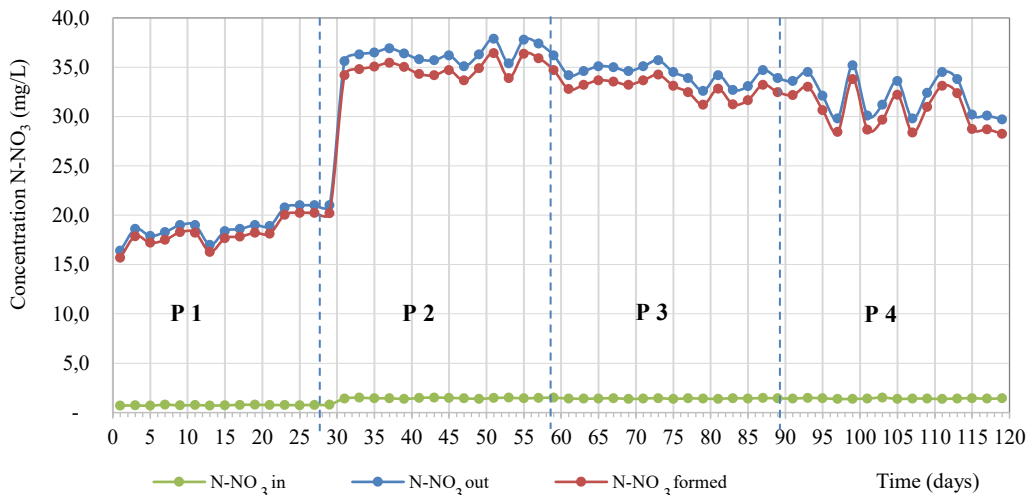


Figure 5. Variation of N-NO₃ concentration through experimental phases

3.3. P-PO₄ removal efficiency through experimental phases

The concentration of P-PO₄, as shown in Figure 6, significantly decreased in the SNAP model, with the highest removal efficiency of 56.5% observed on the 39th day of operation

(Phase 2). This could be attributed to the fact that under oxygen-rich conditions, microorganisms accumulated condensed phosphate within their biomass from the free phosphate present in the environment (Equation 8) [18].

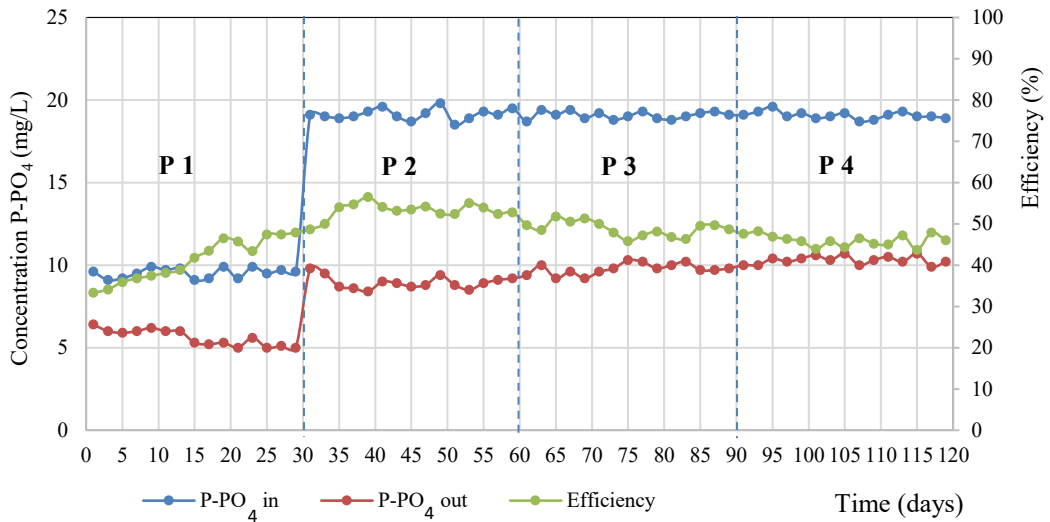
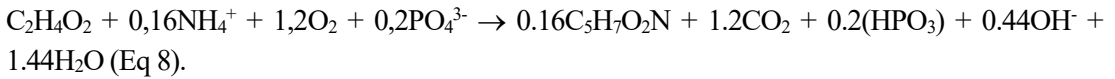


Figure 6. P-PO₄ removal efficiency throughout the experimental phases

3.4. COD removal efficiency through experimental phases

Overall, the COD removal efficiency in the experimental model was not high, remaining below 40% across all four phases (Figure 7). This can be attributed to the fact that the experimental model only supplemented with Nitrosomonas culture and Anammox sludge. These two bacterial groups did not utilize the available carbon sources in the environment. The reduction in carbon sources within the experimental medium could be due to the growth and utilization of carbon by other aerobic heterotrophic bacteria naturally present in the wastewater.

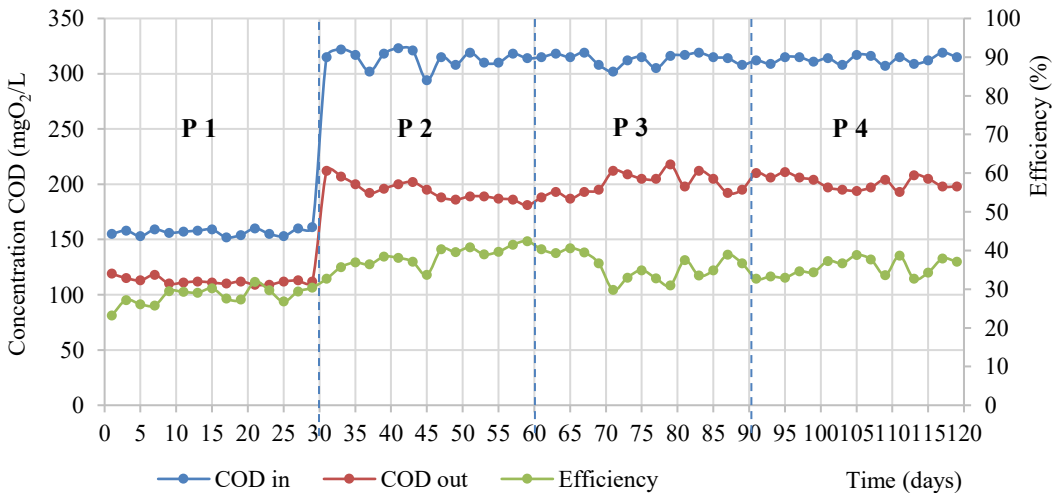


Figure 7. COD removal efficiency through experimental phases

3.5. The microbial density during the operation of the experimental model

The variation in microbial density in the SNAP model across different phases is presented in Table 2. The results of the investigation showed that the microbial density in the SNAP model is not only high but also stable and increases progressively through each phase. After 30 days of operation, the microbial density increased by 10.9 times compared to the initial value. During phases 1 and 2, the microbial density increased steadily and stabilized, reaching 1.2×10^8 CFU/mL and 1.5×10^8 CFU/mL, respectively.

Table 2. The microbial density during the operation of the experimental model

Operation phases	Initial density	Adaptation phase	End of Phase 1	End of Phase 2	End of Phase 3	End of Phase 4
Microbial Density (CFU/mL)	1.1×10^6	1.4×10^7	1.2×10^8	1.5×10^8	1.3×10^6	1.2×10^6

However, by the end of phase 3, the microbial density in the SNAP model decreased by 11.5 times compared to phase 2. This suggests that the wastewater treatment efficiency of the SNAP model is directly proportional to the microbial density in the activated sludge. A high and stable microbial density, consistently maintained throughout the operational process, maintained over time contributes to the effective operation of the system.

4. CONCLUSION

This study showed that the SNAP model operates under room temperature conditions with a pH of 7.52 ± 0.04 and a hydraulic retention time of 24 hours. The N-NH₄ removal efficiency achieved the highest value of 92.8% at a NaCl salt concentration of 0.59%. At this same salt concentration, the removal efficiencies for COD and P-PO₄ were 42.4% and 56.5%, respectively. The microbial density reached 1.5×10^8 CFU/mL at the end of Phase 2.

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TÓM TẮT

ẢNH HƯỞNG CỦA NỒNG ĐỘ MUỐI TRONG QUÁ TRÌNH XỬ LÝ NƯỚC THẢI TỪ HOẠT ĐỘNG SẢN XUẤT NƯỚC TƯƠNG BẰNG MÔ HÌNH SNAP

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Nghiên cứu này áp dụng mô hình SNAP (Single-stage Nitrogen removal using Anammox and Partial nitritation) để loại bỏ các hợp chất hữu cơ, đặc biệt là N-NH₄, từ nước thải. Các thí nghiệm được thực hiện bằng cách sử dụng nước thải thực tế lấy từ bể xử lý kỵ khí của hệ thống xử lý nước thải Công ty TNHH chế biến nước chấm Mekong. Nghiên cứu được thực hiện qua bốn giai đoạn với các nồng độ muối tương ứng là 0,29%, 0,59%, 0,89% và 1,18%. Kết quả cho thấy, tại pH 7,24 ± 0,08 và thời gian lưu nước là 24 giờ, hiệu quả loại bỏ N-NH₄ đạt cao nhất là 92,8% khi nồng độ NaCl là 0,59%. Tại nồng độ muối này, hiệu quả loại bỏ COD và P-PO₄ lần lượt là 42,4% và 56,5%. Ngoài ra, mật độ vi sinh vật đạt 1,5 × 10⁸ CFU/mL vào cuối giai đoạn 2.

Từ khóa: SNAP, xử lý nước thải, N-NH₄, NaCl.