

Impact of Temperature and Alkalinity on Nitrogen Removal in the Start-up Period of Partial Nitrification in a Sequence Batch Reactor

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Abstract

The effect of temperature and influent alkalinity/ammonia (K/A) ratio on the start-up of the partial nitrification (PN) process for an activated sludge-based domestic wastewater treatment was studied. Two different sequence batch reactors (SBR) were operated at 26 °C and 32 °C. The relationship between temperature and the concentration of free ammonia (FA) and free acid nitrite (FNA) was investigated. A stable PN process was achieved in the 32 °C reactor when the influent ammonium concentration was lower than 150 mg-N/L. In contrast, the PN process in the 26 °C reactor had a higher nitrite accumulation rate (NAR) and ammonium removal efficiency (ARE) when the influent ammonia concentration was increased to more than 150 mg-N/L. Then three different ranges of the K/A ratio were applied to an SBR reactor. In the K/A range of 2.48~1.65, the SBR reactor achieved the highest NAR ratio (75.78%). This ratio helps to achieve the appropriate level of alkalinity to maintain a stable pH and provide a sufficient amount of inorganic carbon source for the activity of microorganisms. At the same time, FA and FNA values also reached the threshold to inhibit nitrite-oxidizing bacteria (NOB) without a significant effect on ammonia-oxidizing bacteria (AOB). Results showed that the control of temperature and K/A ratio during the start-up period may be important in establishing a stable and steady PN process for the treatment of domestic wastewater.

Keywords: Partial nitrification, Wastewater treatment, Temperature, Alkalinity, Start-up period, Nitrogen removal

1. Introduction

Ammonium is one of the most common pollutant parameters in many types of wastewater (industrial, agricultural, and domestic wastewater)[1,2]. In the natural environment, ammonium can be converted into nitrite (NO₂⁻) and nitrate (NO₃⁻), which are toxic to humans and other organisms and can pollute the natural environment. Therefore, the efficient removal of ammonium is a major concern for many treatment systems. Many methods of ammonium treatment in water and wastewater have been researched, tested, and applied in practice. Biological technologies are often widely applied, especially with wastewater with medium and low ammonium concentrations such as domestic wastewater, because of this technology's technical and economic benefits [3-5].

The most common biological treatment process used to treat nitrogen and ammonium in wastewater combines nitrification and denitrification. The combined application of these two treatment processes

also has certain disadvantages, including (1) some bacterial strains participating in the process have a slow growth rate[6], (2) high energy costs for aeration[7,8] (3) additional carbon sources may be required[6]. A partial nitrification process (PN) can eliminate these disadvantages. During this process, ammonium is mainly oxidized to nitrite and then reduced to N₂ gas via denitrification or Anammox. This technology is suitable for treating wastewater with high ammonium concentration and low carbon/nitrogen (C/N) ratio and can result in a 25% reduction in the energy required for aeration, a 30% reduction in sludge production, and a 20% reduction in CO₂ released into the atmosphere. This process also has a high and stable ammonium treatment efficiency. The most important thing to achieve the PN process is to prevent the oxidation of nitrite to nitrate, which also inhibits nitrite-oxidizing bacteria (NOB) while promoting the growth of ammonia-oxidizing bacteria (AOB). Therefore, it is necessary to control several key operating parameters, such as pH[9], temperature[10], dissolved oxygen concentration (DO)[11], alkalinity[12], free ammonia concentration (NH₃ or FA)[13], the concentration of free acid nitrite (HNO₂ or FNA)[13], hydraulic retention time (HRT)[14], toxic substance concentration.

AOB microorganisms can dominate over NOB microorganisms in environments with a high temperature above 24 °C[15,16], and conversely, NOB microorganisms have a fast growth rate at low-temperature conditions (< 15 °C). In another study, temperatures above 30 °C

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were optimal for the growth of AOB and suitable for operating the PN process[17]. Thus, different studies still have different temperature conditions ideal for partial nitrification. However, temperature also plays an important role in the PN process because it directly affects the existence of FA and FNA in water. FA and FNA have been shown to effect the competition between AOB and NOB[18]. Temperature can also affect the properties of the activated sludge and the operating costs of the treatment system.[16]. Therefore, it is necessary to investigate the temperature, especially during the start-up of PN process.

Meanwhile, alkalinity also plays an important role in the operation of the PN process. Alkalinity is directly related to the pH of the treated surface. pH will affect the concentration of FA and FNA. The concentration of FNA will increase when the pH drops too low due to a lack of alkalinity, thereby inhibiting NOB microorganisms. Conversely, when the alkalinity is too high, it prevents the pH from decreasing. As a result, the inhibition of FNA was significantly reduced[19,20]. Besides, about 10% of alkalinity in water serves as an inorganic carbon source for microorganisms AOB and NOB[12]. Therefore, maintaining the right alkalinity will help maintain a stable pH for the treatment reactor and thus create favorable conditions for microbial growth. However, not many studies investigate the influence of alkalinity and temperature on the start-up period of the PN process.

This study focuses on studying the effects of temperature and alkalinity on the start-up period of the PN process. The selection of suitable operating conditions for the start-up period will shorten the time and provide a good basis for the performance of the process.

2. Materials and methods

2.1. Configuration of the SBR reactor

The lab-scale treatment reactor was designed with a working capacity of 5L in mica. The reactor's length, width, and height are 150, 150, and 250 mm. The reactor was equipped with a 60 rpm agitator, a fine aeration disc at the bottom, and two peristaltic pumps, which pumps water in and out of the reactor. Besides, a thermostatic bath was used to control the temperature of the experiment, and an aerator has a capacity of 9W. The reactor was operated in batches with a 4-hour operating cycle, including the following main steps: filling (15 minutes), aeration (180 minutes), settling (30 minutes), and withdrawing (15 minutes). The water exchange rate of the treatment reactor was 50%. DO concentration was controlled at 0.5~2 mg/L for the duration of the experiment by a flowmeter. The general operation process was divided into 2 phases. Stage 1 was operated under a DO condition of > 2 mg/L, facilitating complete treatment of the remaining COD in the seed sludge. In stage 2, DO was reduced to 0.5~2 mg/L to promote the growth of AOB over the NOB.

2.2. Seed sludge and synthetic wastewater

The activated sludge used in the study was obtained from a laboratory-scale domestic wastewater treatment system. The sludge is light brown and has a good settling capacity with an SVI of 135. Firstly, the activated sludge is cleaned with distilled water to remove some

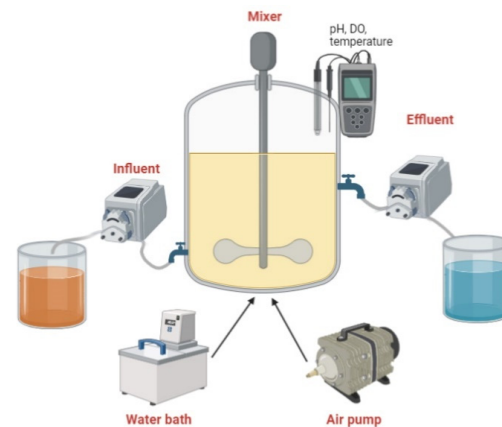


Figure 1. Schematic diagram of the SBR system.

Table 1. Characteristics of the Influent Wastewater in Different Phases

Phases	HRT (hour)	DO (mg/L)	NH ₄ ⁺ -N (In) (mg/L)	Alkalinity (mg CaCO ₃ /L)
1	8	> 2 mg/L	50	380
			70	525
			150	1125
			200	1500
2	8	0,5~2 mg/L	250	1875
			300	2250

major pollutants in the sludge to limit the influence on the analytical results in this study. The mixed liquor suspended solids (MLSS) concentration of the activated sludge in the treatment reactor was 4000 mg/L.

Synthetic wastewater has the following main components: 50~300 mg-N/L as NH₄⁺-N (NH₄Cl) and 380~2250 mg CaCO₃/L (NaHCO₃ as the alkalinity source). Besides, a mixture of nutrients and microelements necessary for the growth and development of microorganisms has also been prepared[21].

2.3. Experiment operation

Two identical treatment reactors were operated similarly, as described in Figure 1, to investigate the effect of temperature on the acclimatization phase of the PN process. One was operated at 32 °C, and the other was operated at ambient temperature (average temperature was 26 °C ± 1.5). Other main parameters was discussed in Table 1. Influent and effluents of the reactors were collected to analyze NH₄⁺, NO₂⁻, NO₃⁻, pH, and alkalinity. The experiment was carried out continuously for 40 days.

In the next experiment, the molar ratio of alkalinity/ammonium (K/A) in the input wastewater was adjusted from 4.44 to 0.72 to investigate the influence of alkalinity on the start-up period of the PN process. The ratio adjustment depended on the efficiency of the PN process through monitoring the ammonium removal efficiency (ARE) values and the nitrite accumulation rate (NAR) of the system. At the same time, the influent NH₄⁺ concentration was 50 mg-N/L. This ex-

periment was performed for 50 days.

2.4. Analytical methods and calculation

2.4.1. Analytical methods

The parameters of wastewater were analyzed according to the standard methods, including $\text{NH}_4^+\text{-N}$ (APHA 2005, 4500-NH₃, F), $\text{NO}_3^-\text{-N}$ (APHA 2005), $\text{NO}_2^-\text{-N}$ (APHA 2005, 4500), alkalinity (APHA 2005, 2320-Alkalinity, A)[30]. The pH and DO readings are measured using the HACH HQ-40b with separate probes. All analytical experiments were carried out in triplicate, and the final results used were the mean values.

2.4.2. Calculation of parameters related to partial nitrification

Nitrite accumulation ratio (NAR)[22]:

$$NAR(\%) = \frac{(\text{NO}_2^- - \text{N})_{out}}{(\text{NO}_2^- - \text{N})_{out} + (\text{NO}_3^- - \text{N})_{out}} \times 100 \quad (1)$$

Free ammonia (FA)[9]:

$$FA\left(\frac{\text{mg}}{\text{L}}\right) = \frac{17}{14} \times \frac{(\text{NH}_4^+ - \text{N}) \times 10^{pH}}{\exp[6334/(273 + T)] + 10^{pH}} \quad (2)$$

Free nitrous acid (FNA)[9]:

$$FNA\left(\frac{\text{mg}}{\text{L}}\right) = \frac{46}{14} \times \frac{\text{NO}_2^- - \text{N}}{\exp[-2300/(273 + T)] + 10^{pH}} \quad (3)$$

Ammonia removal efficiency (ARE)[23]:

$$ARE(\%) = \frac{(\text{NH}_4^+ - \text{N})_{in} - (\text{NH}_4^+ - \text{N})_{out}}{(\text{NH}_4^+ - \text{N})_{in}} \times 100$$

where T is the temperature of the reactor (°C), $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ are the concentrations in the treatment reactor; $\text{NO}_2^-\text{-N}_{out}$, and $\text{NO}_3^-\text{-N}_{out}$ is the concentration in the outlet of the reactor.

2.4.3. Statistical analysis

Statistical analyzes were performed using SPSS IBM 26 software. T-test was used to compare the difference between the results in the experiments at 2 different temperature conditions.

3. Results and discussion

3.1. Nitrogen removal performance of partial nitrification

The ammonium concentration in the influent wastewater was adjusted gradually from 50 ± 3.4 mg-N/L to 150 ± 8.6 mg-N/L in the first phase and increased to 300 ± 9.7 mg-N/L in the second phase (Figure 2). In the first phase, there was a significant difference in the ARE of the two reactors ($p < 0,05$). In the first 3 days, the 26 °C reactor achieved ARE of 99.9%. When the input ammonium concentration was increased to 75 mg-N/L, the ARE value decreased sharply to 3.6% and gradually increased again, reaching 99.9% after 3 days of adjustment. This phenomenon continued to repeat when the input $\text{NH}_4^+\text{-N}$ concentration was increased to 150 mg-N/L, and the efficiency rose rapidly to 99.9%.

Meanwhile, the nitrite concentration was almost not detected, and the effluent nitrate concentration gradually increased from 12.8 mg-N/L to 255 mg-N/L. This result shows that NOB microorganisms were not inhibited, and ammonium was completely oxidized to nitrate by the conventional nitrification process.

In 32 °C reactor, a decrease in ammonia removal efficiency was also observed with an increase in the influent ammonia concentration. However, the 32 °C reactor maintained a higher and more stable ammonium treatment efficiency of 99.9% than the other one. However, the main end product of oxidation was also nitrate and there was no significant difference between NAR of the reactors ($p > 0.05$)

In the second phase, the concentration of $\text{NH}_4^+\text{-N}$ was increased in the range of 200 ± 11 to 300 ± 13.1 mg-N/L. The removal efficiency of the two reactors was lower than that of the previous stage and there was a significant difference in the ARE of the two reactors ($p < 0,05$). The 26 °C and 32 °C reactors reached 95.67% and 95.13% of ARE, respectively. Thus, ARE of the reactors was not affected by temperature. However, the temperature of 32 °C makes the process more stable when there is a change in the input ammonium concentration.

In the 26 °C reactor, at day 30, the NAR started to appear at 56%, corresponding to an increase of the effluent concentration of $\text{NO}_2^-\text{-N}$ from 0 mg-N/L on day 29 to 165 mg-N/L. The concentration of $\text{NO}_3^-\text{-N}$ decreased by nearly 50% from 225.3 mg-N/L to 125.2

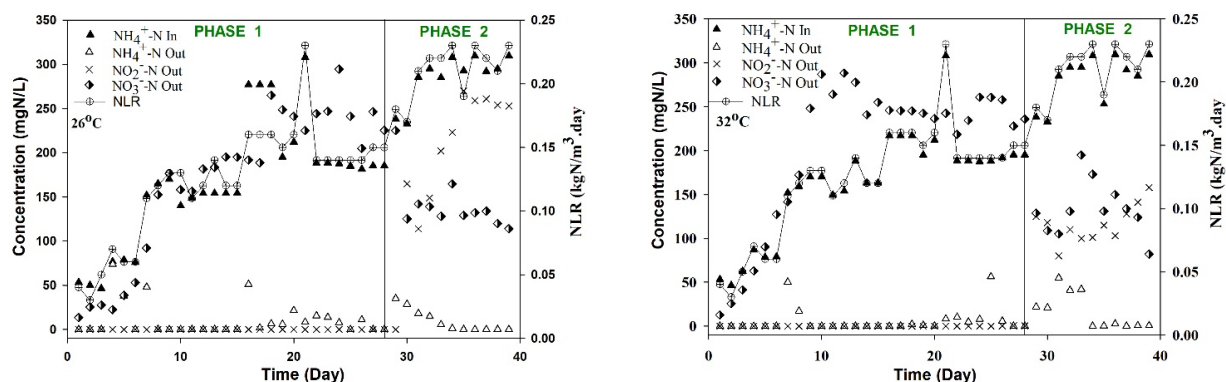


Figure 2. Changes of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$ concentrations in the 26 °C reactor and 32 °C reactor.

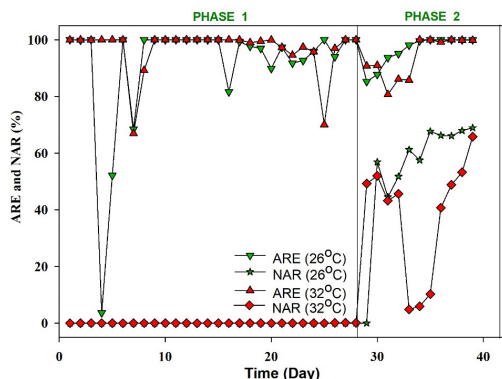


Figure 3. Ammonium removal efficiency (ARE) and nitrite accumulation rate (NAR) of the 26 °C reactor and 32 °C reactor.

mg-N/L. The NAR value continued to increase gradually and peaked at 68.9% on the last day of the experiment. It can be noted that NOB microorganisms were inhibited while AOB microorganisms were still growing steadily. The ARE value remained high throughout the experiment.

In the 32 °C reactor, the NAR ratio started to increase from the 29th day of the experiment with the corresponding value of 49.26% and decreased to the lowest level of 4% when the concentration of the influent $\text{NH}_4^+\text{-N}$ was increased to 300 mg-N/L (Figure 3). A significant difference between two reactors was also observed ($p < 0.05$). The adaptation of the microorganisms then caused the NAR rate to increase again gradually to 65.83% at the end of the experiment. It can be seen that although the ammonium concentration in the input water in the two treatment reactors is similar, there are significant differences between FA of 26 °C reactor and that of 32 °C reactor in both phases ($p < 0.05$). In the first stage, when the input $\text{NH}_4^+\text{-N}$ concentration ranged from 50~150 mg-N/L, the FA value in the first reactor ranged from 0.82~2.8 mg/L, and in the second reactor was 1.14~4.4 mg/L.

3.2. Effect of temperature on FA values of the reactors

AOB microorganisms are less sensitive to FA concentrations than NOB[18]. They were inhibited at FA concentrations from 0.1 to 1 mg/L, while this value for AOB microorganisms was 10~150 mg/L [9]. Some other similar value ranges have been published for different types of treatment reactors, such as in the study with batch treatment reactors, NOB microorganisms were inhibited at FA value of 0.1~4.0 mg/L. In an anaerobic-aerobic system, the inhibitory value is 1~5 mg/L. For systems with high influent ammonium concentrations, AOB microorganisms can be affected at FA concentrations above 7 mg/L and completely inhibited at 20 mg/L. In this study, the FA value in the first stage in the two treatment reactors was at a level that could inhibit NOB microorganisms and did not affect AOB microorganisms. High ARE during this period was observed. However, FA can only inhibit but not kill NOB microorganisms[24]. Therefore, during the ammonium treatment of AOB microorganisms, the FA value will gradually decrease and when it reaches a value of less influence, the NOB microorganisms can active again. This is also the reason that in phase

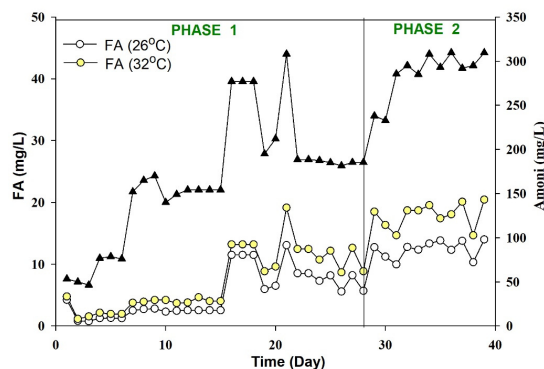


Figure 4. Changes of FA (mg/L) and NAR (%) of the 26 °C reactor and 32 °C reactor.

1, although FA concentrations of both reactors were > 5 mg-N/L, the NAR values were very low. At the later stage of the experiment, with $\text{NH}_4^+\text{-N}$ concentration from 200~300 mg-N/L, the difference in FA value was larger. The largest FA value at 26 °C reactor was only 13.9 mg/L, while it was 20.45 mg/L in the 32 °C reactor. There was also a significant difference between FA of the reactors ($p < 0.05$). High FA concentration at 32 °C tank affected AOB and caused ARE to drop sharply from 99% to 80%. The NAR ratio also increased but was not stable when AOB did not have favorable conditions to operate more strongly than NOB. Meanwhile, the lower FA concentration of the 26 °C reactor helped to stabilize the PN process (Figure 4). ARE recovered quickly after a change in input ammonium concentration and NAR steadily increased and reached 65.83%.

Thus, at an ammonium concentration of 50~150 mg-N/L, the temperature of 32 °C helps ammonium oxidizing microorganisms to grow more stable than in the lower temperature condition of 26 °C. At ammonium values above 200 mg-N/L, the higher the operating temperature, the higher the FA value will increase and the greater the effect on AOB microorganisms will be, limiting the PN process.

3.3. Effect of K/A ratio on nitrogen removal performance of partial nitrification

The results showed that the K/A ratio directly affected NAR and ARE (Figure 5). The experiment was started with the K/A ratio from 4.44 to 3.33, corresponding to the influent ammonium concentration of 55.5 ± 1.5 mg-N/L. The NAR of the reactor was from 21.8% to 39.15% and lowest in the entire experiment period. The ARE was similar when the highest treatment efficiency was only 72.52%, with the ammonium concentration in the output being 15.5 mg-N/L.

By the 8th day of the experiment, the NAR value began to increase rapidly from 38.15% to 69.57%. The K/A ratio was adjusted to 2.48 with an alkalinity of 500 mg $\text{CaCO}_3\text{/L}$. The ARE also rose sharply to 94.52%, and the effluent ammonium concentration was only 3.09 mg-N/L. Meanwhile, the nitrite concentration increased to 13.78 mg-N/L. At K/A from 2.48 to 1.65, the NAR reached 75.78% on the 32nd day of the experiment. The nitrite and nitrate concentrations in the effluent were 65.07 and 20.8 mg-N/L, respectively (Figure 7).

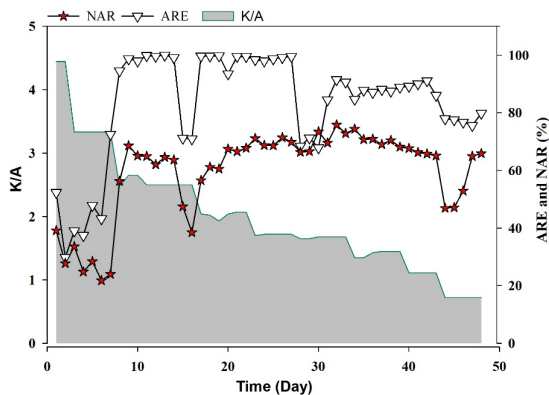


Figure 5. Changes of ARE and NAR at different K/A ratios.

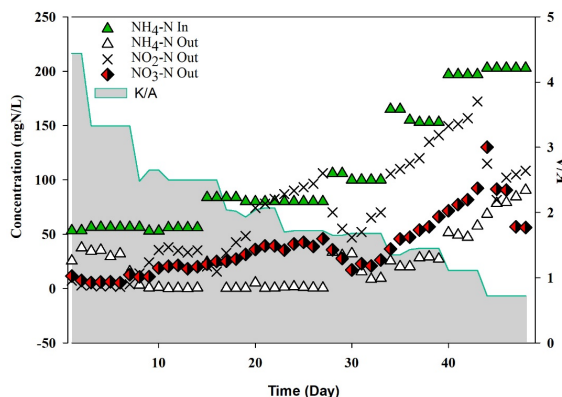


Figure 7. Change of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and $\text{NO}_2^-\text{-N}$ concentrations at different K/A ratio.

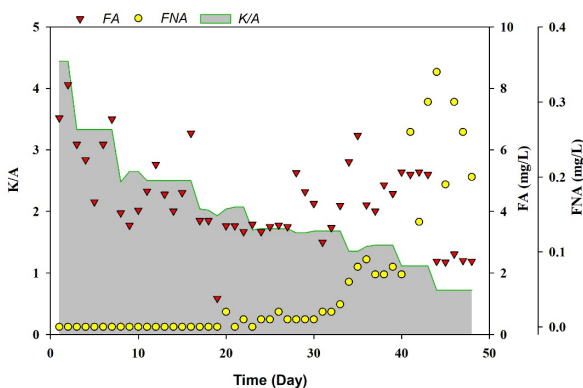


Figure 6. Change of FA and FNA concentrations at different K/A ratios.

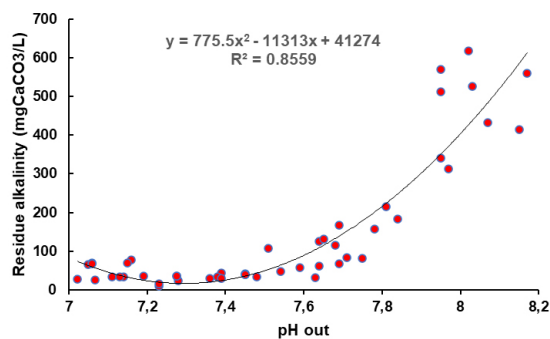


Figure 8. Relationship between pH and residual alkalinity of the effluent.

In contrast, the ARE decreased slightly on days 15 and 28, corresponding to the change in the influent ammonium concentration. Microorganisms need to adapt to changes in the environment. From days 34 to 48 of the experiment, the K/A ratio was reduced to a low range from 1.35 to 0.72, and ARE values dropped from 84.64% to 55.37% with a high concentration of ammonium in the effluent (90.06 mg-N/L). The NAR declined slightly to an average of $64.4\% \pm 8.4$ compared with $67.89\% \pm 4.8$ of the previous K/A ratio.

3.4. Selecting appropriate K/A ratio for partial nitrification in SBR

The three ranges of K/A ratios have different effects on the PN process, and in particular, the activity of the AOB and NOB microbial groups. At high values of 4.4 to 3.3, high alkalinity increased the pH of the wastewater and caused the FA concentration to remain between 7.0 and 8.1 mg/L. The pH of the wastewater was maintained at a level higher than the optimal pH of the nitrification process ($\text{pH} = 7.5$ [25, 26]), when the residual alkalinity in the wastewater was high (Figure 8). In addition, NOB activity can be significantly inhibited at FA levels below 9.0 mg-N/L, and AOB activity begins to decrease at FA levels between 10-300 mg-N/L[27, 28]. the AOB and NOB microbial activities were significantly affected, resulting in the lowest NAR and ARE ratios.

When K/A was adjusted to the lowest value ranged from 1.35 to 0.72, the ARE decreased the most. The low K/A ratio did not supply

enough inorganic carbon sources for the AOB microorganisms as each mg of ammonium oxidized would consume a minimum of 7.1 mg of alkalinity. The low K/A ratio also causes the pH of the treatment reactor to drop sharply during the treatment process[29]. This relationship was clearly shown when monitoring the residual alkalinity and pH of the effluent as shown in Figure 8. There is also not enough excess alkalinity to maintain the optimal pH[25], which increases the concentration of FNA in the reactor. Specifically, FNA in the previous stages ranged from 0 to 0.019 mg/L, while in this stage, it was 0.041 to 0.15 mg/L (Figure 6). The inhibition of AOB by FNA was further increased at low alkalinity conditions[30] and resulted in a sharp reduction of ARE to 55.37%. However, inhibition thresholds in 0.42~1.72 mg $\text{HNO}_2\text{-N/L}$, at which AOB activity was reduced by 50%, while for NOB, this value was lower (from 0.011 to 0.07 mg/L[30]). Therefore, the ability to accumulate nitrite was not greatly affected at the end of the experiment, even though the ammonium removal efficiency was strongly reduced.

Finally, maintaining K/A at 2.5 to 1.68 gave the best results with concurrently maintaining high NAR and ARE values. At this range, pH was maintained stable for the activity of microorganisms, and FA and FNA values also reached a threshold just enough to inhibit NOB without affecting AOB. The ratio from 2.5 to 1.68 selected in this study is also consistent with the results recorded in other studies on other wastewater and operating procedures[20,31,32]. However, according to

Marisol Belmonte[20], the inhibition caused by FA and FNA was only temporary due to fluctuations in pH and ammonium and nitrite concentrations during treatment. Therefore, maintaining DO below two mg/L was also used to control this study's K/A ratio of wastewater.

4. Conclusions

The study has determined the influence of temperature and the influence of the alkalinity/ammonium (K/A) ratio of influent wastewater on the adaptation and development of a partial nitrification process in an SBR reactor. Specifically, when the influent concentration of $\text{NH}_4^+\text{-N}$ was below 150 ± 8.6 mg-N/L, 32°C was an appropriate temperature condition, helping the treatment process to take place stably with high ARE and NAR values. At concentrations of 300 ± 9.7 mg/L or more, the reactor operated at 26°C showed better ARE and NAR. The highest ARE and NAR were 99% and 65.83%, respectively. The influence of temperature on the PN process was determined for each relationship between the temperature and the FA and FNA values of the reactor. At ammonia concentration above 300 mg-N/L, high temperature makes FA concentration reached 20.19 mg-N/L and 1.5 times higher than FA at 26°C , leading to AOB inhibition and reduced efficiency of PN. On the other hand, the K/A ratio 1.68–2.2 was considered the most suitable for the PN process because it helped maintain a stable pH value in the reactor by maintaining a reasonable amount of residual alkalinity. Maintaining a stable pH gave the FA and FNA reasonable levels to inhibit NOB while AOB remained active. As a result, the highest NAR ratio was achieved at 75.83%. This K/A ratio also ensured a suitable level of inorganic carbon source for ammonium oxidizing microorganisms to achieve high ammonium removal efficiency.

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