Effect of nitrogen loading rate and alkalinity on partial nitrification in a continuous stirred tank reactor

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ABSTRACT

In order to achieve an appropriate effluent ratio of ammonium and nitrite as an influent of the anaerobic ammonium oxidation process, the optimum conditions for the partial nitrification in a continuous stirred tank reactor (CSTR) were investigated using real reject water. Hydraulic retention time (HRT) and influent alkalinity ($\text{HCO}_3^-/\text{NH}_4^+\text{-N}$ ratio) were major factors that greatly influenced the partial nitrification. As a result of continuous operation, ammonium conversion efficiency (ACE) and effluent nitrite to ammonium ratio (NAR) could be achieved at the HRT of 19 h corresponding to 0.71 kg/m³/d of nitrogen loading rate (NLR). Thereafter, the influent $\text{HCO}_3^-/\text{NH}_4^+\text{-N}$ ratio was adjusted from 0.5 to 2, and as a result, the optimum partial nitrification efficiency was maintained when the influent $\text{HCO}_3^-/\text{NH}_4^+\text{-N}$ ratio was one. The suitability of the determined operating conditions was verified in a CSTR over 30 d of operation time.

Keywords: AOB, $\text{HCO}_3^-/\text{NH}_4^+\text{-N}$ ratio, NLR, NOB, Partial nitrification

1. Introduction

Conventional ammonia nitrogen removal is based on autotrophic nitrification and heterotrophic denitrification [1-5]. In aerobic nitrification, ammonia is oxidized to nitrate by two different groups of bacteria. The first group, ammonia-oxidizing bacteria (AOB), converts ammonia to nitrite. In this reaction, each one mole of ammonium oxidation consumes 2 moles alkalinity theoretically, according to the nitrification chemical equation [6]. Then, the second group, nitrite-oxidizing bacteria (NOB), further oxidizes the intermediate product to nitrate [7, 8]. The relevant reactions are presented in Eq. (1) and Eq. (2). In the anoxic denitrification (Eq. (3)), nitrate and nitrite are converted to gaseous nitrogen by denitrifiers that is accomplished with a variety of external carbon source such as methanol, acetate, ethanol, and lactate [9]. Recently, the limitations of high oxygen requirement for nitrification and an external carbon source for denitrification is being the driving forces for developing new low-cost biological treatment processes for complete nitrogen removal [10, 11].

Novel nitrogen removal process via a newly discovered ANAMMOX (ANAerobic AMMonium OXidation) process has been recognized as a promising cost-effective and low energy alternative to conventional processes due to a significant reduction of aeration and alkalinity for nitrification and organic carbon for denitrification [7, 12-15]. In the ANAMMOX process, the *planctomycete*-like bacteria oxidize ammonium and nitrite simultaneously to nitrogen gas under the anoxic condition as shown in Eq. (4).

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To achieve an appropriate ratio to ammonium and nitrite for ANAMMOX process, PN (Partial Nitrification) is an essential process as shown in Eq. (5). In theory, the substrate ratio of $\text{NO}_2^-/\text{NH}_4^+$ for ANAMMOX bacteria is 1.32:1 [16]. However, considering the high toxicity to ANAMMOX bacteria of NO_2^- over NH_4^+ , the ratio of $\text{NO}_2^-/\text{NH}_4^+$ ratio was recommended as 1:1 [17]. This ratio can maintain the highest specific ANAMMOX activity (SAA) which is $0.32\text{gN}_2/\text{gVSS/d}$ during continuous operation [18].

To achieve successful PN, the following conditions must be considered. Firstly, NOB should be suppressed [19]. The following factors are considered as operating parameters for this: dissolved oxygen (DO), temperature, pH, free ammonia (FA), and free nitrous acid (FNA) [20-22]. Secondly, the appropriate ammonium oxidation efficiency for the subsequent ANAMMOX process should be controlled, resulting in $\text{NO}_2^-/\text{NH}_4^+$ ratio = 1. AOB requires approximately two moles of HCO_3^- per mole of NH_4^+ while NOB requires a lower amount of alkalinity [23]. Therefore, moles of alkalinity per mole of NH_4^+ consumed in nitrification or nitrification would be closely related to ammonium oxidation efficiency.

In this study, a whole cell entrapment technique was applied to inhibit the selective activity of NOB, which was demonstrated in previous studies [24]. On the other hand, the operating parameters for controlling ammonium oxidation efficiency have not been investigated, considering the properties of bacteria immobilized in PVA. Therefore, in order to control the ammonium oxidation efficiency in the nitrification reactor, hydraulic retention time (HRT) and influent alkalinity concentration were considered as the main variables, and the operation efficiency for each variable was evaluated.

2. Materials and Methods

2.1. Whole Cell Entrapment Procedure

The solution containing 15.0% (w/v) of polyvinyl alcohol (PVA) and 2.0% (w/v) of sodium alginate (SA) was autoclaved at 121°C for 20 min. After cooling to room temperature, the concentrated activated sludge was mixed with PVA/alginate solution. This solution mixture was dropped into a solution of saturated H_3BO_3 and CaCl_2 to form spherical carriers. The carriers were kept in the H_3BO_3 and CaCl_2 solution for 2 h, then immersed in a 0.5 M orthophosphate solution (KH_2PO_4) for 1 h, and finally washed with distilled water.

2.2. Experiment Set-Up

As shown in Table 1, the PN was carried out in an aerated and continuous stirred tank reactor (CSTR) that had a working volume of 1.74 L. A raw reject water, which consisting of NH_4^+-N ($680 \pm 120\text{ mg/L}$) as the main substrate was taken from the wastewater treatment plant, was used as the feeding for the reactor. Other influent characteristics were as follows; pH 8.1 ± 0.2 , alkalinity $2,430 \pm 415\text{ mg CaCO}_3/\text{L}$, BOD $125 \pm 23\text{ mg/L}$, NO_2^--N $0.1 \pm 0.1\text{ mg/L}$ and NO_3^--N $0.7 \pm 0.6\text{ mg/L}$. Because of the low growth rate of AOB, this study used PVA/alginate beads with a 300 mL

Table 1. Operation Conditions

Conditions	
NH_4^+-N (mg/L)	680 ± 120
BOD/ NH_4^+-N ratio	0.2 ± 0.05
Alkalinity (mgCaCO ₃ /L)	$2,430 \pm 415$
Temperature ($^\circ\text{C}$)	30 ± 2
Air flow rate (L/min)	3
Working volume (L)	1.74
PVA/alginate beads size (mm)	3.34 ± 0.3
HRT (h)	12, 15, 19

volume (packing ratio of 17.2%). In the reactor, aeration was conducted by an air diffuser stone, and DO concentration was controlled around 3.5-4 mg/L during the experiment. The temperature was maintained at $30 \pm 2^\circ\text{C}$ in the temperature room. The pH was not controlled.

2.3. Operation Conditions

The experiments in this study were split into 4 period: In period 1, the CSTR reactor was started by gradually increasing NH_4^+-N concentration from 200 mgN/L to 400 mgN/L (tap water was used for dilution) at HRT 12 h for 40 d. In period 2, Raw reject water was fed, at different HRT 12, 15 and then 19 h for 25, 24, and 29 d, respectively. In period 3, the influence of alkalinity on PN was evaluated by changing the $\text{HCO}_3^-/\text{NH}_4^+$ ratios from 0.5:1, 1:1, 1.5:1, and 2:1 for 15 d at HRT 19 h. In period 4, verification of optimum conditions for the stable PN by maintaining the $\text{HCO}_3^-/\text{NH}_4^+$ ratios at 1:1 and HRT 19 h for 30 d.

2.4. Analytical Methods

To monitor the PN performance in the reactor, alkalinity and the concentration of NH_4^+-N , NO_2^--N , and NO_3^--N in influent and effluent were routinely measured according to Standard methods [25]. The analytical methods are summarized in Table 2.

Table 2. Analytical Methods of Operation Factors

Factors	Analytical methods
NH_4^+-N	Kjeldahl nitrogen analysis (Kjeltec 2200 Auto Distillation Unit, FOSS)
NO_2^--N	Colorimetric method
NO_3^--N	Ion Chromatography (Dionex DX 500/High pressure liquid chromatography)
Alkalinity	Titration method
DO	Periodic measurement (D-55, Horiba Ltd, Kyoto, Japan)
pH, temperature	Periodic measurement (F-51, Horiba Ltd, Kyoto, Japan)

3. Results and Discussion

3.1. Adaptation of Bacteria to New Operation Conditions

During the adaptable period, the CSTR was operated at constant HRT 12 h when NLR was increased from 0.42 to 0.84 kgN/m³/d by increasing the influent NH₄⁺-N concentration gradually from 200 to 400 mg/L. Fig. 1 represented the evaluation of the PN during this period. Despite the high influent ammonium concentration, a stable ammonium conversion efficiency (ACE) was reached about 50.2 ± 0.5% and the detective nitrate concentration was lower than 3 mg/L. In the PN process with the wastewater containing a high concentration of nitrogen, a strategy for controlling DO concentration is most commonly used as a method of selectively inhibiting NOB. Its concentration is reported to be less than 0.5 mg/L [26]. Considering this, whole cell entrapment is a very effective technique. Even if DO concentration in the bulk phase is maintained high, the DO concentration diffused into the carrier is very rapidly limited. This is a mechanism for selective inhibition of NOB [27]. This has already been verified in many studies [28, 29].

Nitrite production rate of 0.21 ± 0.02 kgN/m³/d was achieved at 0.42 kgN/m³/d of NLR and reached 0.43 ± 0.02 kgN/m³/d at 0.84 kgN/m³/d of NLR. It meant that half of influent ammonium was converted to nitrite nitrogen. And an effluent nitrite to ammonium ratio (NAR) of one was achieved during the majority of period. In the beginning, bacteria quickly adapted to new operative conditions for 40 d by maintaining HRT at 12 h.

Fig. 1. The nitrogen loading rate (NLR), nitrite produced rate (NPR) and nitrite-to-ammonium nitrogen ratio (NAR) in the CSTR during adaptable section.

3.2. Effect of NLR on Partial Nitrification

After the previous adaptation, the CSTR was operated for 68 d using reject water (without dilution), which was studied at different HRT of 12, 15, and 19 h. The influent ammonium concentration was around 680 ± 120 mg/L. The NLR gradually increased with decreasing the HRT and the effect of NLR on PN was presented in Fig. 2. The results showed that the ACE and the effluent NAR were well correlated to the HRT. At the HRT 12 h, 45.1 ± 0.42% of influent NH₄⁺-N was oxidized to NO₂⁻-N, resulting in NAR of 0.8 ± 0.05. At the HRT 15 h, ACE showed 49.1 ± 0.11% and NAR was 0.93 ± 0.03. ACE presented 51.9% and NAR was 1.03 ± 0.03 at 19 h of HRT. Moreover, effluent nitrogen speciation

Fig. 2. Effect of nitrogen loading rate (NLR) on partial nitrification at different hydraulic retention time (HRT).

was mainly composed of ammonium and nitrite in an equimolar ratio, resulting in nitrate concentration lower than 4 mgNO₃⁻-N/L. Consequently, suitable HRT for the PN was 19 hours, corresponding to 0.71 kgN/m³/d of the NLR. It could be concluded that the HRT posed a significant effect on the PN in the CSTR.

3.3. Effect of influent alkalinity on partial nitrification

For stable nitrogen removal in the ANAMMOX process suppression of complete nitrification in the PN is essential [30, 31]. In addition, an appropriate is required [32, 33]. In this study, not only the accumulation of nitrate in the PN reactor was effectively suppressed by utilizing the characteristics of PVA/alginate bead, but also the NAR control factor according to the influent nitrogen load fluctuation was investigated. Among them, the effect of HRT in the PN reactor with PVA/alginate beads was verified in this study.

As shown in Fig. 3, the influence of alkalinity on PN was evaluated by changing the HCO₃⁻/NH₄⁺ ratios from 0.5 to 1, 1.5, and 2. The influent pH was maintained at 7.7 ± 0.6. At HCO₃⁻/NH₄⁺-N ratio of 1, the ACE showed 51 ± 0.52% as well as nitrite accumulation efficiency (NAE) represented 50.3 ± 0.53%. By adding sodium bicarbonate to increase HCO₃⁻/NH₄⁺ ratio to 1.5, the ACE and NAE increased to 60 ± 1.56% and 59.4 ± 1.5%, respectively. In the case of the influent HCO₃⁻/NH₄⁺ ratio of 2, the ACE of 72 ± 1.19% and the NAE of 71.6 ± 1.1% were achieved. However, at a condition

Fig. 3. Change of ammonium conversion efficiency (ACE) and nitrite accumulation efficiency (NAE) at different alkalinity conditions.

where influent $\text{HCO}_3^-/\text{NH}_4^+$ ratio was 0.5, not only ACE but also NAE dropped to $33 \pm 5.0\%$ and $33.1 \pm 4.89\%$, respectively, although the effluent pH remained relatively constant at 6.8 ± 0.2 .

Alkalinity plays two functions for AOB which are the carbon source for AOB and the acidification buffer for the hydrogen ion produced by ammonia oxidation [34, 35]. Only approximately 10% of inorganic carbon is assimilated into bacteria, and the majority of the HCO_3^- is utilized to neutralize hydrogen ion [23]. Although the mechanisms are not clear yet, many researchers emphasized the role of HCO_3^- to effectively achieve PN, and influent $\text{HCO}_3^-/\text{NH}_4^+-\text{N}$ was considered as a representative operating factor [22, 36, 37]. In this study, influent wastewater characteristic with the $\text{HCO}_3^-/\text{NH}_4^+$ ratio of 1 was suitable for PN-ANAMMOX system without external alkalinity.

3.4. Implications of Operating Parameters for Partial Nitritation

In this study, the PN was achieved under the conditions where the HRT was 19 h corresponding with $0.71 \text{ kgN/m}^3/\text{d}$ of NLR. In addition, when the influent $\text{HCO}_3^-/\text{NH}_4^+-\text{N}$ ratio was 1:1, the most effective partial nitritation efficiency was shown. In general, the raw reject water has a property of $\text{HCO}_3^-/\text{NH}_4^+-\text{N}$ ratio of 1:1, which leads to the advantage of biological treatment by the ANAMMOX/PN process. However, when CO_2 stripping that consumes alkalinity in the reactor [38] and denitrification reaction that generates alkalinity [39] react at the same time, separate operating factors should be studied to maintain about 50% of ACE.

4. Conclusions

In summary, the PN was carried out in the CSTR treating raw reject wastewater. PVA/alginate beads were used to inhibit NOB activity selectively, and NLR and influent $\text{HCO}_3^-/\text{NH}_4^+-\text{N}$ ratio were considered as the major operating parameters for PN.

PN reactor was operated in a CSTR at HRT of 19 h corresponding with $0.71 \text{ kgN/m}^3/\text{d}$ of NLR. Under these conditions $51.9 \pm 0.14\%$ of ACE and 1.03 ± 0.03 of NAR could be achieved. At $\text{HCO}_3^-/\text{NH}_4^+-\text{N}$ ratio of 1:1, ACE showed $51 \pm 0.52\%$ as well as NAR was 1.01 ± 0.01 , which was appropriate to the requirements of subsequent ANAMMOX process. The determined operating parameters were verified through continuous operation over 30 d.

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Author Contribution

D.C. (Ph.D.) designed all experiments and wrote the manuscript. T.T. (Undergraduate student) and W.Y. (Ph.D. student) conducted all the experiments. D.J. (Ph.D.) and K.K. (Professor) supported writing manuscript. J.J. (Professor) revised and reviewed the manuscript.

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