Abstract: In the treatment of industrial and municipal wastewaters, biological nitrification and denitrification processes have been widely applied methods. However, in recent years, experimental studies have been carried out on nitritation and denitritation processes in order to reduce the cost of wastewater treatment. In this study, the removal of synthetic wastewaters containing ammonium at various concentrations through nitritation and denitritation processes was investigated in a sequence batch reactor (SBR). The SBR was operated at a low dissolved oxygen concentration of 0.7±0.2 mg/L, while the initial pH level and temperature were kept constant at 7.5 and 25 °C, respectively, which were below the defined optimal level for nitritation. For the initial concentrations of 100, 150, and 200 mg NH$_4$-N/L, 91, 142, and 180 mg NO$_x$-N were produced, respectively with the specific nitritation rates of 0.69, 0.95, and 0.81 mg NH$_4$-N/mg MLVSS.hour. Considering the operating conditions of nitritation, approximately NO$_2$-N/NO$_x$-N ratio of 80% could be considered as successful with the combined effect of DO limitation and NH$_3$ inhibition on the nitrite oxidizing bacteria. During the first days of nitritation and denitritation processes, change of nitrogen concentrations was negligible because the adaptation of organisms to the oxic and anoxic conditions. After completing nitritation stage, denitritation was carried out by using methanol as an electron donor for the initial concentrations of 91, 142, and 180 mg/L of NO$_x$-N in 96, 120 and 264 hours of reaction times, respectively.

Keywords: nitritation, denitritation, SBR, NO$_2$-N/NO$_x$-N ratio
1. Introduction

Nitrogen removal performance should be improved in many wastewater treatment plants (WWTPs) due to the stringent directives for nutrient release into the aquatic environments. Simultaneous biological nitrogen removal (BNR) by nitrification-denitrification process, which is carried out under the oxic and anoxic conditions, respectively, is a widely applied in wastewater treatment. In the nitrification process, NH₄-N is oxidized into NO₂-N during the first step by ammonium oxidizing bacteria (AOB) and then NO₂-N is oxidized into NO₃-N during the second step by nitrite-oxidizing bacteria (NOB). Accumulated NO₂-N and NO₃-N in the nitrification process is converted to N₂ gas by the denitrification organisms which used organic carbon as an electron donor.

In the WWTPs, returning the wastewater (side stream) with high nitrogen content (1000-1400 g/m³) produced in the anaerobic digester to the biological reactor (main stream) negatively affect the treatment efficiency. However, the BNR process can be improved by a separate treatment of highly concentrated waters. While the biosludge decant flow might be relatively low, this stream contributes 15-20% of the total nitrogen load on the WWTPs.

Because of the high operational cost of classical BNR process, which are to a great extent related to the dissolved oxygen (DO) and organic matter requirements for nitrification and denitrification, respectively, new methods such as shortcut BNR (partial nitrification/nitritation and denitritation) are under investigation in last decades. Compared to the classical BNR processes, nitritation and denitritation processes provide 25% and 40% reduction of DO and organic carbon demand, respectively.

In order to effectively perform the nitritation process, the activity of nitrobacter spp. should be restricted without affecting nitrosomonas spp. Additionally, adaptation of the denitrifying microorganisms to NO₂-N must be provided, which might be toxic even at low concentrations.

By the limiting of NOB activity, NO₂-N accumulation could be achieved under the convenient operational conditions such as low DO concentrations (≥25°C) and pHs (≥ 8.0), and low sludge retention times (SRT). The mainstream/sidestream wastewaters contain high concentration of NH₄-N can be treated combined with nitritation and denitrification or anaerobic ammonium oxidation (anammox) processes in either a single or two-stage bioreactor system. Since the real wastewaters contain limited concentration of NO₂-N, partial nitrification is considered a suitable process before anammox. However, in the single-stage reactors, the most important conflict between anammox and NOB bacteria is the difference in SRT required for their growth, one of the key points to be resolved. The main restriction on the usage of process is sensitivity of anammox bacteria to the operational and environmental conditions. Additionally, the growth rate of anammox bacteria is quite slow, compared to the other bacteria. Considering the limitations of anammox process in application, combined nitritation and denitrification process in existing WWTPs, especially in the treatment of side stream wastewater, may be a more suitable option.

In the nutrient treatment, sequence batch reactor (SBR), which is a single sludge reactor, has been considered a viable option with their ease operation and low cost and comparatively shorter hydraulic retention time than an extended aeration process. The main purpose of this experimental study was to investigate the effect of initial NH₄-N concentrations on nitritation and denitritation processes in a laboratory-scale SBR. The SBR was operated at a low DO concentration. The temperature and pH of reactor were also kept constant at lower levels than defined as the optimal condition for nitritation.

2. Materials and Methods

2.1. Synthetic Wastewaters

Experiments were carried out with the synthetic wastewater, which was prepared by adding a nutrient mix and other chemicals to provide the trace metals and vitamins necessary to maintain the bacterial growth. The influent total nitrogen in synthetic wastewaters were 100, 150 and 200 mg/L in the nitritation step. Methanol as an organic carbon source was added at the end of nitritation process for the growth of anammox bacteria. All chemicals used in the experimental study were analytical grade.

2.2. Enrichment of Microorganisms

The SBR was inoculated with microorganisms taken from the nitrification-denitrification basins. Microorganisms were acclimatized to nitrogen with medium solution prepared daily.
in demineralized water. The inoculation lasted approximately one month for microbial growth with daily replenishment of NH$_4$-N and NO$_x$-N. The concentration of mixed liquor volatile suspended solids (MLVSS) was about 1.0 g/L after inoculation in the SBR. At the beginning of inoculation period, NH$_4$-N concentration was 50 mg/L and gradually increased to 100 mg/L during the nitritation studies. The SRT of SBR was adjusted to be 40 days by removing appropriate volume of mixed liquor. The reactor was filled with the synthetic wastewaters and was operated oxic and anoxic operations in sequence with aeration and mixing for several days to obtain a dense microbial culture.

2.3. SBR Set-up and Operation

The SBR was inoculated with the enriched nitrifying and denitrifying organisms, which were mentioned above. The completely stirred SBR with a volume of 4 L containing 3.5 L synthetic wastewater at the room temperature of 19±1 °C, was used in the experimental study (Figure 1). The reactor was operated by repeating a number of cycles of fill and draw throughout the experiments. The SBR was equipped with an aquarium type bubble air diffuser, peristaltic pump, magnetic stirrer, DO and pH probes. The liquor was mixed by a magnetic stirrer in both periods of oxic and anoxic. The DO concentration and initial pH value were kept at 0.7 ± 0.2 mg/L and 7.5 through the nitritation study, respectively. It was ensured that the pH of mixed liquor was kept about constant by dosing the NaOH solution with the peristaltic pump. Since it was reported to be the optimal value for NO$_2$-N accumulation$^{[5]}$, the SRT was kept constant at 40 days by monitoring the MLVSS concentration in the reactor.

The reactor was operated in the oxic-anoxic sequences, in which NO$_2$-N produced in the oxic condition and then anoxic stage was started to remove NO$_3$-N. The SBR was aerated continuously except the feeding-settling and denitrification periods. In order to evaluate the biological process, the mixed liquor in SBR was withdrawn and NH$_4$-N, NO$_2$-N, NO$_3$-N, COD and MLVSSs concentrations were determined daily depending on the process. The nitritation process was carried out to the concentration of NH$_4$-N was decreased to about ≤ 7 mg/L (oxidation of NH$_4$-N was over 92%) and then air pump was stopped and the DO concentration was adjusted to about zero by adding Na$_2$SO$_3$. The denitritation process was introduced by adding the nutrient solutions (Table 1), the pH of mixed liquor was adjusted to be 7.5 using 10 N NaOH solutions. Denitritation experiments were completed when the NO$_x$-N concentration was dropped to lower than 10 mg/L.

After settling of the biosludge, about 95% of liquid-phase was drawn by the peristaltic pump in 30 minutes at the end of each cycle. Prior to filling of the SBR with the synthetic wastewater, the biosludge in SBR was washed three times with demineralized water to remove remaining pollutants such as NH$_4$-N, NO$_2$-N, and COD. In each cycle, it was observed that the biosludge sedimentation was well and the supernatant was clear. Once the nitritation/denitrification stages were completed, another nitritation period was initiated.

2.4. Analytical methods

All analyses were performed on the grab samples taken from the SBR and completed in accordance with APHA.$^{[21]}$ Samples
were filtered using 0.45 mm, white, 47 mm radius filters. NH\textsubscript{4}-N concentration was determined by an ammonia-specific electrode, which was calibrated with known concentrations before each use. All samples were tested for NO\textsubscript{3}-N and NO\textsubscript{2}-N concentrations using a Hach DR 2800 spectrophotometer with Hach Chemicals Nitriver5 and Nitriver2, respectively and a Dionex DX-500 LC 20 Ion Chromatography with a CD 20 suppressed conductivity detector and a column model Dionex Ionpac AS9-SC 4×250 mm. The eluent used for this IC contained 1.7 mM NaHCO\textsubscript{3} and 1.8 mM Na\textsubscript{2}CO\textsubscript{3}. The pumping flow rate of eluent was 2 mL/min. The DO concentration was measured by DO meter (YSI 5100). Biomass concentrations as mixed liquor suspended solids (MLVSS) and the concentrations of COD at the beginning and end of reactions were determined according to the APHA.\textsuperscript{24}

\section*{2.5. Formulas}

The free ammonia (FA) and free nitric acid (FNA) (HNO\textsubscript{2}) concentrations were calculated using Eq. 1 and 2.\textsuperscript{25} The ratio of COD/NO\textsubscript{x}-N was determined with Eq 3. The removal efficiency (E) was calculated with Eq 4.

\begin{equation}
FA(\text{mg/L}) = \frac{17}{14} \frac{\sum NH_4 - N(\frac{mg}{L})}{e^{3934/[(725+7)]}+10^{394}}
\end{equation}

\begin{equation}
HNO_2(\text{mg/L}) = \frac{47}{14} \frac{\sum NO_2 - N(\frac{mg}{L})}{e^{X}d\text{exp}^{-3934/[725+7]}} \times 10^{394}
\end{equation}

\begin{equation}
\frac{COD}{NO_x - N} = \frac{COD, \text{mg} / \text{L}}{\text{influent}(NO_x - N)(\text{mg} / \text{L})}
\end{equation}

\begin{equation}
E(\%) = \frac{\text{(influent - effluent) concentration}}{\text{influent concentration}} \times 100
\end{equation}

\section*{3. Results and Discussion}

Results indicated that the nitritation process operated at the DO concentration of 0.7±0.2 mg/L, which was significantly lower than the classical BNR process followed by denitritation provided good nitrogen removal in the SBR. Significant nitrogen removal was not observed in the early days of each stage because environmental conditions change between the oxic and anoxic stages.

Experiments were carried out at least 3 times for each initial concentration of NH\textsubscript{4}-N and average values of data was presented in Figures. Due to the oxidation of NH\textsubscript{4}-N in nitritation process, which cause the release of H\textsuperscript{+}, the pH of mixed liquor solution was elevated to about 7.5 by pumping the NaOH solution when the level of pH dropped to about 7±0.1 in a day. The average MLVSS concentrations were between 0.7 and 0.85 g/L in the SBR.

Figure 2 presents the NH\textsubscript{4}-N removal and NO\textsubscript{x}-N accumulations during the nitritation and NO\textsubscript{x}-N removal in the denitritation stages. A decrease of NH\textsubscript{4}-N in the SBR was
noticeable at the beginning of nitritation stage. As it was expected, elevation of the influent NH$_4$-N concentrations in the reactor, increased the biological reaction times for NH$_4$-N oxidation to NO$_x$-N. In the nitritation stage, average 92\% of NH$_4$-N was oxidized at the total reaction times of 168, 240, and 312 hours for the concentrations of 100, 150 and 200 mg NH$_4$-N/L, respectively. For the inlet concentrations of 100, 150 and 200 mg NH$_4$-N /L, 73, 119, and 134 mg/L of NO$_2$-N were accumulated in the reactor, while NO$_3$-N concentrations were too low as 18, 23, and 38 mg/L, respectively. At the end of nitritation stage for the studied influent NH$_4$-N concentrations, it was determined that the ratio of NO$_2$-N/NO$_x$-N was 0.80. Results indicated that the nitritation was successfully carried out by the limitation of nitrobacter spp. activity and the most of NH$_4$-N was oxidized to NO$_2$-N, instead of NO$_3$-N. For the inlet concentration of 200 mg NH$_3$-N/L, Queiroz et al.\textsuperscript{1}
reported the ratio of NO$_2$-N/NO$_x$-N was higher than 95% in the SBR, which operated at the DO concentration and pH of about 1.0 mg/L and 8.3, respectively. Differences in SBR operating conditions are thought to result in the higher NO$_2$-N/NO$_x$-N ratio than that of our study.

The accumulation of NO$_x$-N may be provided by the restriction of nitrobacter spp. activity by adjusting the reactor operational and environmental conditions at a convenient SRT, DO concentration, pH, and temperature. Because nitrobacter spp. has longer growth time than nitrosomas spp., NO$_2$-N oxidation could be limited by operating at an appropriate SRT, which provides more growth of biomass capable of nitrification, in the suspended growth reactor. One of the important factors on the selection of SRT in nitritation process was NH$_4$-N oxidation, which decrease when the SRT was lower than the convenient level and accumulation of intermediate product like N$_2$O.\textsuperscript{26,27}

In our previous study, which were carried out under the same conditions without denitrification step, indicated that about 92% of inlet 200 mg NH$_4$-N/L was oxidized in the biological reaction time of 168 hours for the nitritation.\textsuperscript{15} However, the equal oxidation percent was achieved in 312 hours reaction time in this study. Simultaneous removal of NH$_4$-N and NO$_2$-N in the single sludge reactor caused the increase of reaction time. It could be drawn from the results that additional reaction time was necessary for the adaptation of microorganisms when the nitrogen removal was carried out in the single nitritation/denitrification unit. Reaction time can be minimized if the nitritation and denitrification stages are proceeded in different units.

It was reported that the activities of AOB and NOB were inhibited at the FA concentrations of 10-150 mg/L and 0.1-1.0 mg/L, respectively and the activity of all nitrifying bacteria were restricted when the FNA concentration was higher than 0.2 mg/L.\textsuperscript{30} The FA concentrations were determined as about 1.4, 2.1, and 2.8 mg/L, which were higher than the threshold concentration of the NOB inhibition, for the initial concentrations of 100, 150 and 200 mg NH$_4$-N, respectively (\textbf{Figure 3}). With the decrease of NH$_4$-N concentration to about 5 mg/L in the nitritation stage, the FA concentrations decreased to 0.08 mg/L, which was lower than the NOB inhibition level. There were studies that successfully removal of NH$_4$-N via nitrite even with the FA concentration was higher than 14.0 mg/L.\textsuperscript{4,7}

The DO is a co-substrate for the nitrification and its concentration affects the reaction in a dual manner. The NOB is more affected than that of AOB at low DO concentrations\textsuperscript{28}, due to the reported ranges of Monod constants of nitrosomonas and nitrobacter are 0.3-1.3 and 0.25-1.3 mg/L, respectively. The optimal DO concentration for NO$_2$-N accumulations depends on the reactor systems such as suspended and attached growth. Although the highest NO$_2$-N accumulation achieved when the DO concentration was over than 1.0 mg/L in the attached growth process\textsuperscript{29,30}, considerable accumulation was observed at low concentration of DO (1.0 mg/L) in the suspended growth process.\textsuperscript{15} Complete nitrification was occurred in the suspended growth reactors when the DO concentrations was higher than 1.0-1.5 mg/L.\textsuperscript{31} Experiments were carried out at low DO concentration about 0.7 ± 0.2 mg/L in this study and it was not observed the

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig3}
\caption{FA variation in the SBR.}
\end{figure}
limitation of NH$_4^+$-N oxidation as reported in our previous study.\textsuperscript{15}

When the DO limitation is the main factor responsible for NO$_2^-$-N accumulation, inhibition of NOB by FNA can also play a role in this phenomenon.\textsuperscript{32} Because the FNA concentration was below 0.2 mg/L throughout the experimental period for the studied NH$_4^+$-N concentrations, the time course of FNA levels in the study was negligible (Figure 4). It was reported that NO$_2^-$-N accumulation was not possible on a long term basis using pH as a key parameter.\textsuperscript{33,34}

The AOB grow faster than the NOB at high temperatures and the maximum specific growth rate of NOB is approximately half of that for the AOB at the temperature of 35 °C.\textsuperscript{35} The AOB can effectively out compete the NOB only at temperatures above 25 °C.\textsuperscript{36} The temperature in the laboratory through the experimental study was kept constant at 19±1 °C which was lower than the reported optimum value of 25-30 °C for nitrifiers.\textsuperscript{10,31} However, compared with the previous studies, which were carried out about optimal temperature for nitrifiers; considerable NO$_2^-$-N accumulation was achieved in this study. Queiroz et al.\textsuperscript{7} also determined the accumulation of high concentration of NO$_2^-$-N at the temperature of 25 °C and oxidation to NO$_3^-$-N was not occurred under the operational condition.

NO$_2^-$-N build-up can be controlled by changing the aerobic phase period. Although the removal efficiency of NH$_4^+$-N was decreasing at a short reaction time, it was observed that NO$_2^-$-N accumulation was higher in the short aerobic periods compared to longer.\textsuperscript{37} As it was known, the activity of organism can be improved by increasing the temperature up to the optimum level depends on the organism type. Due to the operating temperature of 19±1 °C, long aeration times were required to achieve higher than 90% NH$_4^+$-N removal efficiency.

It was reported that successful NO$_2^-$-N accumulation could be possible when the pH and temperature was higher than 8.0 and 35 °C, respectively. Although the previous experimental studies support that the NO$_2^-$-N accumulation could be achieved by controlling the FA concentration, the FA concentration is not only factor for the NO$_2^-$-N accumulation in this study. It was thought that higher than 80% of NO$_2^-$-N/NO$_x^-$-N ratio was achieved for all the initial concentrations of NH$_4^+$-N in the SBR because of combined inhibition of DO and the FA on the NOB.

As expected, the highest nitrification rates of 0.69, 0.95, and 0.81 mg NH$_4^+$-N/mg MLVSS.hour was determined at the beginning of reaction for the initial concentrations of 100, 150, and 200 mg NH$_4^+$-N, respectively. It can be seen in Figure 5 that the nitritation rate was high at high concentrations of NH$_4^+$-N and the oxidation rate decreased gradually throughout the experimental period due to the low concentration of NH$_4^+$-N remaining in the SBR.

After the nitritation process, denitrification was carried out by keeping the COD/NO$_x^-$-N ratio constant at 3.0, taking into account the NO$_x^-$-N amounts for each initial NH$_4^+$-N concentration in the same SBR. The overall stoichiometric equation, which includes biosynthesis, characterizing of denitrification process by using methanol as a C-source (Eq 5)\textsuperscript{38} can be written as;

$$\text{NO}^-_3 + 1.08 \text{CH}_3\text{OH} + \text{H}^+ \rightarrow 0.065\text{C}_3\text{H}_7\text{O}_2\text{N} + 0.47\text{N}_2 + 0.76\text{CO}_2 + 2.44\text{H}_2\text{O}$$  (5)
For the inlet concentrations of 100, 150 and 200 mg NH$_4$-N/L, at the end of oxic phase, average 91, 142 and 180 mg/L NO$_x$-N produced, which were consumed at reaction times of 96, 120 and 264 hour in the denitrification process, respectively (Figure 5).

Through the use of nitritation/denitrification processes, more than 90% inorganic nitrogen removal could be achieved from the synthetic wastewater. Increasing the influent NH$_4$-N concentrations from 100 to 200 mg/L increased the biological reaction time of nitrogen elimination approximately two times in the SBR.

4. Conclusion

In particular, recycling the side stream wastewater containing high N concentration to the beginning of WWTPs cause the decrease in treatment efficiency. In order to treat like this wastewater, nitritation followed by denitrification processes has been widely investigated in the last decades because of the lower operational cost compared to the classical BNR process.

In this study, NH$_4$-N removal by nitritation and denitrification in the SBR was studied for the concentrations between 100 mg/L and 200 mg/L. Almost complete removal of NH$_4$-N by nitritation/denitrification reactions were occurred at the total reaction time of about 264, 360, and 576 hours for the initial concentrations of 100, 150 and 200 mg/L, respectively.

The SBR was operated at lower pH and temperature than the optimal value, which was reported in the literature for nitritation. The results showed the possibility of achieving stable conversion of NH$_4$-N to NO$_2$-N by operating the SBR under operational conditions. During the experimental study, the calculated initial FA concentrations were higher than the threshold concentration of inhibition level for the nitrifiers. However, the FA concentration decreased to about 0.08 mg/L which was below the lowest concentration causing inhibition of the NOB. It was assumed that the FA concentration was not the only factor for the NO$_2$-N accumulation. The results support that the NO$_2$-N accumulation was achieved by controlling the FA and DO.

Results indicated that the SBRs could be successfully applicable for the nitritation and denitrification processes. Compared to the results of study with NH$_4$-N oxidation at equal concentration in SBR under the same operating conditions, in this experimental study, nitritation occurred at a longer bioreaction time, which will cause the elevation of reactor volume.

References

33. Kim J H, Guo X, Park H S. Comparison study of the effects of temperature and free ammonia concentration on nitrification

**Declaration of Competing Interest**

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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