Effect of mixed liquor suspended solids (MLSS) on simultaneous nitrification and denitrification in a sequencing batch reactor

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Abstract. This study investigated the effect of MLSS on simultaneous nitrification and denitrification in a sequencing batch reactor treating domestic wastewater. The average removal efficiencies of COD and TN increased from 93 and 68.21% to 97 and 74.20% with the increase in MLSS from 3.5-4.0 to 7.5-8.0 g/L, respectively. However, the average removal efficiency of TN decreased to 39.45% when MLSS increased to 9.5-10.0 g/L. Simultaneous nitrification and denitrification (SND) was carried out in high MLSS (7.5-8.0 and 9.5-10.0 g/L), and SND efficiencies were 43.4-52.0% and 72.1-80.7%, respectively. Batch tests suggested that there was a strong potential to apply high MLSS for treating wastewater containing high-strength ammonia nitrogen.

1. Introduction
In recent years, the requirements for wastewater discharge have become stricter due to the problem of wastewater eutrophication. It is essential and compulsory for nitrogen removal to an appropriate concentration before wastewaters are being discharged into the environment. Biological treatment has been proven to be an economical and feasible process for nitrogen removal. Biological nitrogen removal is usually achieved through traditional nitrification–denitrification process, by which nitrification and denitrification are operated in the separation of aerobic and anoxic zones.

Simultaneous nitrification and denitrification (SND), as one of the economically effective and space-saving technologies, has attracted the interests of many researchers. Compared with traditional biological nitrogen removal processes, the SND system represents a significant advantage. First, SND eliminates the need for a separate anoxic zone, inducing simplified operating procedures and a smaller footprint. It is estimated that the SND system reduces 40% of COD demand during denitrification and saves 25% of aeration energy [1]. Simultaneously, the SND system can complete the nitrification and denitrification process under the neutral pH with less demand for alkalinity. Because of these advantages, increasing attentions have been paid to the research of SND. Therefore, it was found that there are a lot of factors which influence SND, including C/N ratio [2], temperature [3], MLSS [4], etc.

In the present study, the performance of three SBRs was investigated under three kinds of sludge concentrations of 3.5-4.0, 7.5-8.0 and 9.5-10.0 g/L feeding with domestic wastewater. The changes of organic carbon and nitrogen removal rate, SND rate under different MLSS were detected. The main
objective of this study was to investigate the effect of MLSS on SND in a sequencing batch reactor, and provided useful guidance for its operation.

2. Material and Methods

2.1. Reactors setup and operating conditions
The three SBRs correspond to the sludge concentrations of 3.5-4.0, 7.5-8.0 and 9.5-10.0 g/L, which were called them SBR1, SBR2 and SBR3. Three identical reactors were made of Plexiglas, with an effective volume of 36 L. The exchange volume ratio of three reactors was 12 L/cycle, and the SBR had three cycles a day. Each cycle (8 h) consisted of the following steps: feeding (0.5 h), aeration (5 h), settling (1.5 h), and decanting (0.5 h) and idling (0.5 h). Aeration rate was controlled at 2 L/min for each reactor. The operation of the three reactors was controlled by a programmable logic controller (PLC), and their temperature varied with ambient temperature (25 ± 2 °C).

2.2. Wastewater and seeding sludge
The synthetic wastewater fed to the reactors was as follows: COD, 405.4-592.7 mg/L; \( \text{NH}_4^+ \)-N, 27.8-39.7 mg/L; Organic nitrogen, 3.5-13.0 mg/L; TN, 36.0-46.8 mg/L; TP, 3-5 mg/L.

2.3. Analytical methods
COD, TN, \( \text{NH}_4^+ \)-N, TP, mixed liquor suspended solid (MLSS) were analyzed according to the Chinese NEPA standard methods (Chinese NEPA 2002). The concentrations of nitrate and nitrite were measured by using an ion chromatography (881 Compact IC pro-Cation, Metrohm, Switzerland).

2.4. Batch tests
Three 500 mL measuring cylinders were operated in batch tests to study the utilization characteristics of different high-strength ammonia nitrogen under different MLSS, called RUN1, RUN2 and RUN3. The activated sludge samples were taken from the three reactors (SBR1, SBR2 and SBR3) at the end of the aeration phase on day 100 and settled for 30 min, and the supernatant was drained, washed three times with deionized water.

Test1: The initial \( \text{NH}_4^+ \)-N concentrations was 64 mg/L in RUN1, RUN2 and RUN3, which were aerated for 8 h and had the same aeration rate of 0.1 m³/h.

Test2: The initial \( \text{NH}_4^+ \)-N concentrations was 122 mg/L in RUN1, RUN2 and RUN3, which were aerated for 10 h and had the same aeration rate of 0.1 m³/h.

Test3: The initial \( \text{NH}_4^+ \)-N concentrations was 284 mg/L in RUN1, RUN2 and RUN3, which were aerated for 10 h and had the same aeration rate of 0.1 m³/h.

Ammonia nitrogen utilization rate (R) can be calculated using the following formula:

\[
R = \frac{(C_1 - C_2) \times V}{V_{MLSS} \times t}
\]

where C1 and C2 was the influent and effluent concentration of ammonia nitrogen. V is the volume of influent and t is the aeration time.

3. Results and Discussion

3.1. Organic carbon and Nitrogen removal
The time courses of COD concentrations and removal efficiencies under different MLSS in three reactors are shown in Figure 1. The average COD removal efficiencies slightly increased from 93 to 97% with the increase in MLSS from 3.5-4.0 to 9.5-10.0 g/L at steady states. It is clear that the higher MLSS operated the higher removal efficiencies of COD achieved. The increase in COD removal efficiency could be attributed to a large number of microorganisms in the higher sludge concentrations.

The time courses of \( \text{NH}_4^+ \)-N and TN concentrations and removal efficiencies under different MLSS in three reactors are shown in Figure 2. The average removal efficiency of \( \text{NH}_4^+ \)-N was 98.52, 98.69
and 35.74%, and the average removal efficiency of TN was 68.21, 74.20 and 39.45%, respectively, at the MLSS of 3.5-4.0, 7.5-8.0 and 9.5-10.0 g/L. The higher MLSS of 7.5-8.0 g/L promoted the growth of nitrification and denitrification bacteria since the generation time of these bacteria is long. Although SBR3 had a sludge concentration of 10.0 g/L, previous literatures reported that excessive sludge concentrations might affect oxygen transfer efficiency and hinder nitrification [5].

![Figure 1. COD concentrations and removal efficiencies under different MLSS](image1)

![Figure 2. NH\textsubscript{4}\textsuperscript{+} and TN concentrations and removal efficiencies under different MLSS](image2)

3.2. Simultaneous nitrification and denitrification (SND) in reactors

The occurrence of SND were generally judged by the loss of N under aerobic conditions in the reactor. In this study, three methods were used to analyze the occurrence of SND in the first stable stage.

The first method, SND efficiency is calculated using equation (1) which does not take into account sludge assimilation. Where \( NO_{\text{produced}} \) represents the total amount of \( NO_{2}^{-} - N \) and \( NO_{3}^{-} - N \) produced during the aerobic period, \( NH_{\text{removal}} \) represents the total amount of \( NH_{4}^{+} - N \) removed in the nitrification reaction.
\[ \text{SND efficiency} = \left( 1 - \frac{\text{NO}_x \text{produced}}{\text{NH}_4 \text{removal}} \right) \times 100\% \]  

(1)

Table 1 provides an example of the nitrogen composition balances related to the tracking research on the 25th day of the stabilization phase. The initial content of various forms of nitrogen was the value at which the aeration began. Since the sludge content and dilution of the three reactors were different, the initial content was also varied. Figure 3 shows the performance of SND efficiency under different MLSS on the 25th, 55th, 85th, 115th, 130th day of the stabilization phase. The SND efficiency of SBR2 varied from 46.2 to 51.2%, while the SND efficiency of SBR3 was above 90%, suggesting that the higher the sludge concentration, the higher the SND efficiency. Compared to the high sludge concentrations, the average SND efficiency of SBR1 with conventional sludge concentration was only 12.4%.

The second method, SND efficiency is calculated using equation (1) which takes into account sludge assimilation. where \( T_{Nassimilation} \) represents the total amount of TN removed by the assimilation of the sludge, \( T_{Nremoval} \) represents the total amount of TN removed.

\[ \text{SND efficiency} = \left( 1 - \frac{T_{Nassimilation}}{T_{Nremoval}} \right) \times 100\% \]  

(2)

In the stable state of activated sludge, the mass of discharged sludge represented the mass of microorganism growth and formation. Nitrogen consumption due to cell assimilation was calculated with the cell formula \((C_5H_7NO_2)\), in which N accounted for 12%. The comparison of SND efficiency which considered cell assimilation under different MLSS is shown in Table 2. TN removal efficiencies in the SBR2 and SBR3 were larger than cell assimilation, illustrating that SND occurred in the reactors. However, in the SBR1, the amount of cell assimilation was substantially equal to the amount of TN removal, indicating that the removal of nitrogen was achieved by cell assimilation.

On the basis of these results, we concluded that the high sludge concentrations had a lower DO concentration as a result of decreasing oxygen transfer efficiency, which led to the formation of the anoxic zone and the occurrence of SND in SBR2 and SBR3. Additionally, the SND efficiency increased with increasing MLSS. These results were consistent with the results obtained by Baeza et al. (2002) [5] that the aerobic reactors achieve the highest SND efficiency under the lowest dissolved oxygen, however, the concentration of ammonia in the effluent increased as low dissolved oxygen that hinders nitrification.

Table 1. Summary of nitrogen composition tracking on the Day 25

| Aeration phase          | SBR1    | SBR2    | SBR3    |
|-------------------------|---------|---------|---------|
| Initial TN (mg/L)       | 15.63   | 16.08   | 31.19   |
| Initial NH\(_4\)-N (mg/L)| 7.43    | 11.49   | 29.22   |
| Initial organic nitrogen (mg/L) | 8.20    | 4.59    | 1.97    |
| Initial NO\(_2\)-N and NO\(_3\)-N (mg/L) | 0       | 0       | 0       |
| Final TN (mg/L)         | 14.01   | 10.25   | 26.50   |
| Final NH\(_4\)-N (mg/L) | 0.20    | 1.47    | 24.90   |
| Final organic nitrogen (mg/L) | 2.18    | 2.00    | 1.29    |
| Final NO\(_2\)-N and NO\(_3\)-N (mg/L) | 11.63   | 6.78    | 0.31    |
| NH\(_4\)-N produced by amination (mg/L) | 6.02    | 2.60    | 0.68    |
| NH\(_4\)-N removed (mg/L) | 13.25   | 12.61   | 5.01    |
| Unaccounted nitrogen loss (mg/L) | 1.62    | 5.83    | 4.70    |
| SND rate (%)            | 12.2    | 46.2    | 93.8    |
3.3 Effect of high ammonia nitrogen concentrations under different MLSS in batch experiments

Three experiments were operated at influent ammonia nitrogen concentrations of 64, 123 and 284 mg/L, respectively. Figure 4 shows the removal efficiency and utilization rate of ammonia nitrogen at three high concentrations of ammonia nitrogen.

In test 1, when the influent NH₄⁺-N concentration was 64 mg/L, from RUN1 to RUN3, effluent NH₄⁺-N concentrations were all close to zero, removal efficiencies basically reached 100%. In test 2-3, as the influent NH₄⁺-N concentrations increased to 123 and 284 mg/L, from RUN1 to RUN3, the effluent NH₄⁺-N concentrations declined from 75 to 10 mg/L and from 250 to 110 mg/L. Simultaneously, the NH₄⁺-N utilization rate in test 2-3 was 5.7 and 9.3 mg NH₄⁺-N/(gMLSS•day) in the RUN2, 4.5 and 7.0 mg NH₄⁺-N/(gMLSS•day) in the RUN3, which were remarkably higher than that of 4.3 and 3.06 mg NH₄⁺-N/(gMLSS•day) in the RUN1, indicating that the higher MLSS had a strong resistance to high concentrations of ammonia nitrogen. Moreover, in the RUN2 and RUN3, effluent NH₄⁺-N concentration of RUN2 was lower than that of RUN3 in test2, and the utilization rate of RUN2 was higher than that of RUN3 in three experiments, implying that SBR2 with MLSS of 7.5-8.0 g/L had the
strongest resistance to high concentrations of ammonia nitrogen. These results showed that the higher MLSS could be used to treat high concentrations of ammonia nitrogen.

![NH₄⁺-N profiles during batch experiments.](image)

Figure 4. NH₄⁺-N profiles during batch experiments.

4. Conclusion
Organic carbon and nitrogen removal performance, SND rate were investigated and compared in three SBR reactors with different MLSS. The average removal efficiencies of COD and TN increased with the increase in MLSS from 3.5-4.0 to 7.5-8.0 g/L. However, the average removal efficiency of TN decreased when MLSS increased to 9.5-10.0 g/L. The SND efficiency increased significantly with the increase in MLSS, although ammonia nitrogen removal efficiency was reduced. Batch tests indicated that high sludge concentrations could resist the high concentrations of ammonia nitrogen.

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