Evaluation of Step Feeding Strategy in One-Stage Simultaneous Partial Nitritation, Anammox and Denitrification (SPNAD) Process

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Abstract. Fast start-up of simultaneous partial nitritation, anaerobic ammonium oxidation (anammox) and denitrification (SPNAD) process was achieved within a month by applying step feeding strategy without carbon source addition in the acclimation period. The decreasing cycle number of step feeding strategy successfully improved the SPNAD process performance and enhanced nitrogen and organic removal performance for treating sidestream wastewater. The 3-cycle step feeding strategy obtained higher average values of nitrogen loading rate (NLR), nitrogen removal rate (NRR), ammonium removal rate (ARR) and COD removal rate (CRR) of 0.50, 0.31, 0.45, and 0.18 kg/m³/day, respectively, than 10-cycle and 5-cycle step feeding strategy. The step feeding strategy also contributes in maintaining DO, pH and ORP sensors performance stability even after being operated in long-term duration.

Keywords: sidestream wastewater, step feeding, SPNAD process, sensor stability

1. Introduction

The intention of achieving water-energy nexus for treating wastewater successfully encouraged recent research on autotrophic nitrogen removal which focusing on SPNAD process. The full-scale plants of partial nitritation/anammox (PN/A) are more than 50% with sequencing batch reactors (SBRs) and 88% are being operated in one-stage system [1]. The one-stage SPNAD which combined process of PN, anammox and denitrification in one reactor is recognized as a promising strategy to obtain more efficient nitrogen removal than conventional biological process. The implementation of one-stage SPNAD can significantly reduce the cost of space, operation, and infrastructure compared to two-stage process [2,3]. The operation of SPNAD process as an autotrophic nitrogen removal can decrease up to 50% of energy consumption [4], reduce nearly 100% of carbon source demand and decrease 80% of excess sludge production [5,6].

The main drawback of implementing SPNAD has been its long start-up period due to the long doubling time of anammox bacteria. The use of enriched anammox bacteria as inoculated sludge could reduce the start-up period of anammox process [7]. The one-stage SPNAD implementation is sufficiently challenging to find out favourable conditions for obtaining high removal performance. The complex processes with diverse microbial community existing in one reactor became major challenge of SPNAD application. Favourable conditions are essential to be implemented such as controlling dissolved oxygen at low level for promoting aerobic ammonium oxidizing bacteria (AOB) activity and suppressing the growth of aerobic nitrite oxidizing bacteria (NOB) [8]. The mode of
aeration, length of aeration interval and feeding type also play important role in enhancing SPNAD process performance.

The step feeding strategy in one-stage SBR system offers advanced nitrogen removal with multiple feeding followed by anoxic and aerobic phase. Therefore, step feeding strategy of one-stage SPNAD process was evaluated in this study to treat sidestream wastewater. The SPNAD performance was analyzed based on COD and nitrogen removal of different step-feeding strategies during long-term reactor operation.

2. Materials and Methods

2.1. Reactor configuration and experiment set-up

A 10-L SBR was fabricated from plexiglass and operated with volume exchange ratio (VER) of 25% (2.5 L) (Figure 1a). The reactor configuration was equipped with online monitoring system of pH, DO and ORP sensors to control the stability of SPNAD process (Figure 1b). The temperature of SBR was maintained at 30 ± 1°C.

![Reactor Configuration and Experiment Set-up](image_url)
The SBR was fed with synthetic wastewater that followed real sidestream wastewater from anaerobic digester effluent in Suyoeng WWTP (Busan, South Korea). The wastewater contained 300-350 mg/L NH$_4^+$-N as (NH$_4$)$_2$SO$_4$ with 100-140 mg COD/L as glucose. There were also additions of 54.4 mg/L KH$_2$PO$_4$, 5 mg/L ethylenediaminetetraacetic acid (EDTA), 9 mg/L (0.03 mM) Fe (II) as FeSO$_4$.7H$_2$O, 5.3 mg/L CaCl$_2$.2H$_2$O, and 3.8 mg/L MgSO$_4$.7H$_2$O in the synthetic wastewater.

The total concentration of inoculated biomass was 3.5 g VSS/L (Figure 2). The seeding sludge was composed of enriched AOB about 1.5 g VSS/L. The AOB was enriched within a month from aeration tank at Suyeong sewage treatment plant (Busan, South Korea). Another seeding sludge was granular anammox bacteria which dominated by Ca. Brocadia (2 gVSS/L), that was cultivated in a lab-scale reactor for up to two years.

**Figure 1.** a) A 10-L sequencing batch reactor, b) Experiment set-up configuration

2.2. **SBR operating conditions**

The operating conditions of SBR consisted of two phases: 1) absence of glucose addition and 2) presence of glucose addition. The SBR was operated with step feeding strategy from 10-cycle with absence of glucose addition and presence of glucose addition then decreased to 5-cycle and 3-cycle with glucose addition (Table 1). One batch of SPNAD process consisted of feed, react (anoxic-aerobic), settle and discharge stages. Every cycle had a complete sequence of feeding and reactions (anoxic and aerobic) that ran sequentially until the cycle number completed. The process was followed with settling (30 min) and discharge (1 min) (Figure 3). The NLRs were tested by gradually decreasing aeration time in each cycle until steady state condition was reached.

**Table 1. Operating conditions of a 10-L SBR**

| Phase                  | Number of cycle | Batch Operational Time (min) |
|------------------------|-----------------|-------------------------------|
|                        |                 | Anoxic | Aerobic |
| I (Without glucose addition) | 10              | 0      | 300     |
|                        |                 | 0      | 250     |
|                        |                 | 0      | 200     |
|                        |                 | 0      | 150     |
| Phase                          | Number of cycle | Batch Operational Time (min) |
|-------------------------------|-----------------|-----------------------------|
|                               |                 | Anoxic | Aerobic |
| II (With glucose addition)    | 10              | 0      | 300     |
|                               |                 | 0      | 250     |
|                               |                 | 0      | 200     |
|                               |                 | 0      | 150     |
|                               |                 | 10     | 150     |
|                               |                 | 30     | 150     |
|                               |                 | 50     | 150     |
|                               |                 | 80     | 150     |
|                               |                 | 50     | 120     |
|                               | 5               | 25     | 150     |
|                               | 3               | 15     | 150     |

In the presence of glucose (phase II), anoxic time was introduced prior to aeration stage to see the feasibility of denitrification process occurring in the reactor. Glucose addition also provided sufficient electron donor to support denitrifying bacteria work simultaneously in reducing nitrate production during the anammox process. The SRT control was applied from day 141 with 5-cycle step feeding strategy until the end of operation.

**Figure 3.** One batch of 3-cycle step feeding strategy

### 2.3. Analytical methods

All liquid samples were filtered through 0.2 μm disposable filters (Rephile) before further analysis. The nitrogen compounds (NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N) and COD concentration were monitored regularly at least 1 batch every day. Concentrations of these parameters were measured by spectrophotometry according to water and wastewater examination methods [9] using Humas kit (Humas Co. Ltd. Daejeon, Korea). The biomass concentration was determined as mixed liquor volatile suspended solids (MLVSS) every week according to gravimetric of the Standard Methods [10]. The reactor was equipped with DO, pH, and ORP sensors that monitored every minute, and connected to an advanced SBR controlling program. The sludge retention time (SRT) was controlled at 20-35 days by taking out biomass regularly in order to maintain the stability of biomass growth related to reactor performance.

The SPNAD process performance was evaluated based on nitrogen, ammonium and COD removal performance. The promptness of SPNAD process in SBR was also defined as nitrogen, ammonium and COD removal rate. The hydraulic retention time (HRT) was determined as reaction time of one complete batch divided by VER. Free ammonia (FA) and free nitrous acid (FNA) concentrations were also calculated in this study to monitor SPNAD process performance.
3. Results and Discussion

3.1. SBR performance according to step feeding strategy

The fast start-up phase was obtained within a month by applying 10-cycle step feeding strategy without COD addition and reached the highest nitrogen removal efficiency (NRE) and ammonium removal efficiency (ARE) about 85.81% and 96.6%, respectively. An appropriate operating strategy gives significant effect to attain the acclimation stage. Several SPNAD processes obtained less than 4 months of start-up period without certain biomass inoculation in SBR [11]. Another references reported different starting period about 45 days with SBR working volume of 8 m³ and 50 days in 400 L pilot scale SBR system [12].

The 10-cycle step feeding strategy gave a low initial of NH₄⁺ concentration in each cycle about 8 mg/L with 25% VER. The initial concentration was crucial in determining SPNAD process performance. The different aeration periods of 30, 25, 20, and 15 min/cycle were employed in order to explore the highest performance of one-stage SPNAD process in treating sidestream wastewater. The changing aeration time was based on nitrogen removal stability. A stable performance was obtained when the difference of efficiency was less than 5% for three consecutive days [13] and/or constant results were obtained with experiment lasting at least three times of retention time [14].

The decreasing aeration time from 30 to 20 min/cycle increased nitrate concentration up to 146.61 mg/L. The NOB was estimated to grow in the system with the increase of nitrate concentration in the effluent although the DO level was maintained at about 0.15 mg O₂/L. The increasing of nitrate concentration dropped the NRE until 55% (Figure 4). Low ammonium concentration at each cycle gave insufficient amount of FA concentration to inhibit the growth of NOB. The FA concentration at each cycle of 10-cycle step feeding strategy was as low as 0.13 mg/L with an average pH and temperature of about 7.15 and 33°C, respectively. Inhibitory concentrations of FA were in the range of 1.5-90 mg/L [15]. However, the NOB is also known to be able to acclimatize in high FA levels thus made inhibition control might be ineffective to fully suppressed its existence. Further investigation is still on-going to find the optimum suppression method for completely deprives NOB activity in one-stage SPNAD process.

![Figure 4. Removal performance of nitrogen compounds according to step feeding strategy](image-url)

| I  | 10-Cycle | 5-Cycle | 3-Cycle |
|----|----------|----------|----------|
|    |          |          |          |

| Concentration (mg/L) | Time (d) | Inf. NH₄⁺ | Eff. NH₄⁺ | Eff. NO₂⁺ | Eff. NO₂⁻ | Eff. NO₃⁻ | Nitrogen Removal Efficiency | Nitrogen Removal Rate |
|----------------------|----------|------------|------------|------------|------------|------------|----------------------------|-----------------------|
| 0                    | 0        |            |            |            |            |            |                           |                       |
| 50                   | 50       |            |            |            |            |            |                           |                       |
| 100                  | 100      |            |            |            |            |            |                           |                       |
| 150                  | 150      |            |            |            |            |            |                           |                       |
| 200                  | 200      |            |            |            |            |            |                           |                       |

Legend:
- □ Eff. NH₄⁺  
- △ Eff. NO₂⁻  
- ○ Eff. NO₃⁻  
- Green line: Nitrogen Removal Efficiency  
- Red line: Nitrogen Removal Rate
The decreasing of contact time was implemented by reducing aeration time to 15 min/cycle and this successfully decreased nitrate concentration to 63.5 mg/L after 9 days of operation. The NRE also gradually increased to 79.12% and reached maximum nitrogen loading rate of 0.56 kg N/m³/day. The highest nitrogen removal rate (NRR) was acquired about 0.45 kg N/m³/day (Figure 4).

On day 51, the additional of carbon source as glucose with C/N ratio 0.4 was employed in order to give sufficient electron donor to promote denitrification process occurred simultaneously in the system. The removal performance of COD concentration according to step feeding strategy can be seen in Figure 5. The aeration time was changed back to 30 min/cycle to well-adapt with the microbial community with the presence of additional carbon source then step wise decreased aeration time to 25, 20, and 15 min/cycle like in the previous stage. Nitrate concentration in the effluent was kept in range of 58.41-71.30 mg/L until the end of operation with 15 min aeration/cycle. Only a few amount of ammonium remained in the effluent about 5.9 mg/L and a high ARE of 98.01% was reached.

Short anoxic time was then introduced prior to aeration time for giving adequate time and sufficient carbon source for enhancing denitrification to reduce the remaining nitrate. The variations length of anoxic time tested start from 1, 3, 5, and 8 min/cycle, then subsequently followed with aeration time of 15 min/cycle. No significant change in decreasing nitrate concentration was observed with anoxic time of 1 and 3 min/cycle and aeration time of 15 min/cycle. Nitrate concentration started to decrease in anoxic time of 5 and 8 min/cycle by changing the aeration time to 12 min/cycle. This was done in order to give sufficient FA concentration by controlling residual ammonium to promote NOB suppression.

Residual ammonium concentration controlled AOB growth and could effectively suppress the growth of NOB [16]. High residual ammonium concentration remained about 32.99 mg/L and resulted in a slight increase of NRE about 5% higher than the previous operating condition. The anoxic time was then set to 5 min/cycle with 12 min/cycle of aeration time, which obtained average NRR, ARR and COD removal rate (CRR) about 0.30 kgN/m³/day, 0.45 kgN/m³/day, and 0.15 kgCOD/m³/day, respectively.

Figure 5. Removal performance of COD concentration according to step feeding strategy
The 5-cycle step feeding strategy was started from day 124 with anoxic time 5 min/cycle and total aeration time of 120 min. The feeding strategy with 5-cycle possessed higher ammonium load in each cycle than the 10-cycle step feeding strategy. Air flow rate was changed to 0.6 L/min for giving more oxygen supply to oxidize ammonium. Nevertheless, DO level remained stable in less than 0.1 mgO₂/L concentration. The remaining nitrate concentration could be reduced to 58.45 mgN/L, though the residual ammonium was accumulated until 62.35 mg/L. Aeration time was then extended to 30 min/cycle (with a total duration of 150 min aeration time) and accumulated residual ammonium was successfully decreased to 21.71 mg/L. The obtained NRE and ARE were 71.49 and 95.83%, respectively, with the highest NLR being 0.57 kgN/m³/day.

Decreasing cycle number to 3-cycle step feeding strategy was started from day 175. The 3-cycle step feeding strategy was operated with 5 min anoxic time/cycle and total duration of 150 min aeration time (50 min/cycle). The NRE gradually increased to 73.57% on day 204 and reached an average value of 68.41% in steady state condition. The ARE and CRE still achieved high performance up to 80%, with average value of 91.90% and 83.87%, respectively. The acquired average values of NR, ARR, and CRR were about 0.31 kgN/m³/day, 0.45 kgN/m³/day, and 0.18 kgCOD/m³/day, respectively. Decreasing cycle number of step feeding strategy effectively enhanced one-stage SPNAD performance (Figure 6). It was supported by sufficient substrate load in each cycle and enough contact time in each cycle for pondering microbial reactions that existed in one reactor system.

![Figure 6. SPNAD process performance according to step feeding strategy](image_url)

### 3.2. Performance of biomass concentration based on step feeding strategy

In the absence of COD addition, the slow growth of biomass concentration was analysed (Figure 7). The fast growing of biomass concentration started to occur after the presence of additional carbon source with more diverse microorganisms growing inside the SPNAD system. The amount of flocs biomass also increased inside the reactor due to the growth of nitrifiers and heterotrophic microorganisms. The anammox biomass also increased with the increasing of anammox volume in the reactor. Figure 7 showed the increasing of MLVSS concentration to 4-6 gVSS/L in the presence of COD addition. The increasing of MLVSS was reported due to the increasing of organic matter concentration [17].
The ratio of MLVSS/MLSS should be over than 0.75 as the typical value in a conventional wastewater treatment system [17] and as indicator of microorganism content in the activated sludge concentration [18]. A high ratio of MLVSS/MLSS was obtained almost in all steps of step feeding strategy and was pointed as high activity of microorganisms detected in SPNAD system. The sludge retention time (SRT) was also controlled at 20-35 days in order to control biomass concentration to be less than 5 gVSS/L and bolster NOB out-selection inside the system.

NOB out-selection strategy was employed by taking out biomass and separating flocs and granular biomass using 500 µm sieve. Remaining anammox granules in the sieve were then subsequently washed with tap water to remove nitrifiers especially targeted NOB which were growing and attached on the surface of granules. However, another recovery strategy is essential to be further investigated due to the possibility of NOB might keep growing on the surface of anammox granules even though they have already been passed through the washing step.

3.3. Online monitoring performance of pH, DO and ORP sensor

An online monitoring system was used to check the reactor performance every minute. It helped in controlling nitrogen and COD removal and its relation to pH, DO, and ORP sensors performance. The performance stability of each sensor was also monitored according to step feeding strategy. The DO concentration was counted as prominent parameter for obtaining high performance in a one-stage SPNAD process. Coupling with pH sensor, nitrogen and COD removal performance can be controlled easily as signal of nitrification and denitrification process. ORP sensor gave data on reduction and oxidation reactions that occurred in the system. Regular calibration of sensors was done for preventing sensor contamination in the long-term reactor operation.

Step feeding strategy promotes in maintaining sensors performance on the start-up period owing to low substrate concentration in each cycle. The 10-cycle step feeding strategy as the initial phase of SPNAD process in this study also supported the sensor stability and formed a good pattern based on the cycle number in one batch (Figure 8). In general, the decreasing cycle number did not significantly

![Figure 7. Performance of biomass concentration according to step feeding strategy](image-url)
affect the performance stability of sensors since stepwise decrease gave innocuous condition for microorganisms.

The increasing and decreasing of pH were shown during one batch as the alkalinity production and consumption occurring in the complete process of SPNAD. The pH was maintained in the range of 7 – 7.35 with an alkalinity ratio of 5. The ORP value also revealed continuous ups and downs in the range of -300 to -100 mV. The relation between sensors performance with nitrogen compound and carbon conversion were observed based on tracking conversion during one batch of 3-cycle step feeding strategy (Figure 9).

During anoxic time of feeding, pH increased as alkalinity production of denitrification process and termination of alkalinity addition from substrate. Denitrification process also started to occur as limited oxygen condition during feeding time. The pH started to decrease after denitrification process was completed. In the aeration period, pH kept decreasing due to alkalinity consumption and acid production during nitrification process [19]. Consumed of ammonium concentration was about 14 mg/L in one cycle.

Figure 8. Performance of DO, pH and ORP sensors during one batch according to step feeding strategy a) 10-cycle (without COD), b) 10-cycle (with COD), c) 5-cycle (with COD), d) 3-cycle (with COD)

The last cycle of tracking showed that nitrification process happened during aeration time and ammonium was consumed around 2 mg/L in every 10 minutes. The decrease of ammonium was followed by well-maintained DO at less than 0.1 mgO₂/L. The consumption of nitrate concentration was about 3.76 mg/L and 9.78 mg/L during feeding time of second and third cycle. The decreasing of COD concentration was detected during anoxic time about 5.7 mg/L as electron donor for supporting denitrification process. However, no significant decreasing of nitrate was observed within 5 minutes of anoxic time for each cycle. ORP sensor remained showing insignificant changed during aeration time of last cycle with values between -461 and -462 mV and started to decreased in the anoxic time of settling period.

The nitrate concentration increased about 4.62 mg/L during 50 minutes of aeration time. Theoretically, nitritation of anammox process produced approximately 13% of nitrate from total...
ammonium supply based on stoichiometry of reaction equation [20]. The stoichiometry calculated about 5.96 mg/L nitrate will be produced, which is more than the observed produced nitrate in this study. The tracking result (Figure 9) revealed the ratio of NO$_3$/NH$_4$ was less than 13% as the threshold for indicating the appropriate microorganism activity in SPNAD system [21].

The high ratio of NO$_3$/NH$_4$ more than 13% might reveal the overgrowth of NOB and oxygen competition with AOB that generated nitrate concentration in the system. In this study, the observed ratio of NO$_3$/NH$_4$ was less than the threshold line can be seen as a triumph of suppressing NOB activity by applying the 3-cycle step feeding strategy, employing additional carbon source as glucose with C/N 0.4 and controlling the SRT at 20-35 days. The addition of anoxic time was also promoted denitrification to occur simultaneously in one-stage SPNAD process. However, further investigations are essential for effectively suppressing NOB activity and observing the role of carbon source in reducing residual nitrate in SPNAD system.

![Graph](image)

**Figure 9.** The relation of DO, pH and ORP sensors performance with nitrogen and COD conversion during one batch of 3-cycle step feeding strategy (day 203)

4. Conclusions

Fast start-up was achieved in one-stage SPNAD process by applying 10-cycle step feeding strategy. The 3-cycle step feeding strategy obtained higher values of NLR, NRR, ARR, and CRR of 0.50 kgN/m$^3$/day, 0.31 kgN/m$^3$/day, 0.45 kgN/m$^3$/day and 0.18 kgCOD/m$^3$/day, respectively, than 10-cycle and 5-cycle step feeding strategy. Less cycle number of step feeding strategy provided more sufficient load or concentration of substrate and enough contact time in each cycle to completely balance the complex bacteria reactions in one-stage SPNAD system. However, further investigation of less cycle number in long-term operation is needed for full-scale practical application with more effective strategies for suppressing NOB.
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