

Synchronous rapid start-up of the methanation and anammox processes in two-stage ASBRs

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Abstract. The "methanation + anaerobic ammonia oxidation autotrophic denitrification" method was adopted by using anaerobic sequencing batch reactors (ASBRs) and realized a satisfactory synchronous removal of chemical oxygen demand (COD) and ammonia-nitrogen ($\text{NH}_4^+\text{-N}$) in wastewater after 75 days operation. 90% of COD was removed at a COD load of 1.2 kg/(m³·d) and 90% of TN was removed at a TN load of 0.14 kg/(m³·d). The anammox reaction ratio was estimated to be 1: 1.32: 0.26. The results showed that synchronous rapid start-up of the methanation and anaerobic ammonia oxidation processes in two-stage ASBRs was feasible.

1. Introduction

Nowadays, the main pollutants are organic matters and nitrogenous substances in wastewater, which not only intensified the contradiction of water shortage in our country, but also seriously restricted the implementation of the sustainable development strategy. Therefore, efficient removal of organic matters and nitrogenous substances in wastewater would be one of the most important research directions in the field of controlling water pollution and improving water environment.

At present, the treatment processes for the wastewater contained organic matters and nitrogenous substances mainly focus on the removal of COD and $\text{NH}_4^+\text{-N}$ separately. More and more pollutants like organics, ammonium were discharged together in wastewater from pharmacy [1], food [2], papermaking industry [3] etc. A renewable process that might be capable of simultaneously removing organics and ammonium was received a primary attention. Among all the biological technology in the field of wastewater treatment, the methanation and anaerobic ammonia oxidation methods [4, 5] were more efficient ways. These biochemical processes were produced by different functional biological flora under the anaerobic conditions [6, 7]. As we all know, the doubling time of anammox bacteria was reported to be 11 days with maximum specific growth rate of $0.0027\cdot\text{h}^{-1}$ [1,8]. A disadvantage of this low growth rate was the long start-up period required for anammox process which limited its widespread application. Therefore, reducing the duration of methanation and anammox start-up processes was one of the main challenges for environmental engineering. Yinyin [9] achieved the simultaneous anaerobic ammonia oxidation, methanation and denitrification in UASB reactor. In her study, various activated sludge was inoculated in the same UASB reactor and the reactor which started successfully after about 48 days. The denitrification bacteria and anaerobic ammonium oxidation bacteria could be cultured and enriched in the UASB reactor. However, the results showed that the removal rates of COD and $\text{NH}_4^+\text{-N}$ were not ideal. Zubo [10] studied on integration of methanogenesis with simultaneous anaerobic ammonium oxidation and denitrification in EGSB reactor, and the



granular sludge was formed in EGSB reactor inoculated with the aerobic activated sludge for 120 days' start-up operation. Then, denitrification bacteria and anaerobic ammonium oxidation bacteria were cultured and enriched for 270 days in EGSB reactor with nitrite and ammonium. In Zubo's study, the removal rate was increased, but the start-up time was a little long, which was up to 120 days. There were various wastewater treatment technologies such as anaerobic baffled reactor (ABR [11]), membrane bioreactor (MBR [12]), upflow anaerobic sludge blanket (UASB [13]), biological aerated filter (BAF [14]) and anaerobic sequencing batch reactor (ASBR[1]) et al., which had been used for the cultivation of methanogenic and anammox bacteria. As the third generation of all anaerobic reactors, anaerobic sequencing batch reactor (ASBR) had some advantages: the rapid start-up time, the simple structure, low operation cost, good biological flocculation ability and solid-liquid separation efficiency, little demand for the alkalinity, and efficient anti-shock loading with the formation of A granular sludge, etc [15,16].

The experiment, which aimed at reducing the COD and $\text{NH}_4^+\text{-N}$ in two-stage ASBRs by cultivating the methanogenic and anammox activated sludge, achieved synchronous rapid start-up of methanation and anaerobic ammonia oxidation.

2. Materials and methods

2.1 ASBR start-up and operation

Two identical anaerobic sequencing batch reactors (R1 and R2) were utilized to investigate the rapid start-up processes in this study. The working volume of each reactor was all approximately 2.5 L with an internal diameter of 15 cm and a height of 28.5 cm. The two reactors were operated at 35°C, and the initial pH of R1 and R2 was 7.4 and 8.1, respectively. When the two reactors were separately operated, the fill and draw were operated with 1000 mL influent and 1000 mL effluent every cycle for hydraulic retention time (HRT) 24 hours. Every ASBR operation cycle was consisted of five steps: filling (4 min), reaction (1200 min), settling (120 min), discharge (4 min) and idle (112 min). Each device was intermittently stirred for 8 minutes per hour [17] by biogas produced by itself, the amount of biogas was collected by a biogas bag and measured by a glass syringe. The whole reaction process was controlled by time switch and miniature vacuum pump. When the two reactors were combined by a latex drainage tubing, the effluent of R1 was as the influent of R2, the the fill and draw flow of linked-up state was the same as the separate state. The HRT of R1 and R2 was respectively reduced to 12 hours in every cycle and the HRT of the whole cycle was 24 hours. In the two-stage anaerobic reactors, the influent concentration could be increased after the effluent concentrations of COD and $\text{NH}_4^+\text{-N}$ met the requirements of the National Discharge Standard of China (GB8978-1996). An opaque, black plastic film enclosure was served to inhibit the growth of photosynthetic bacteria [18].

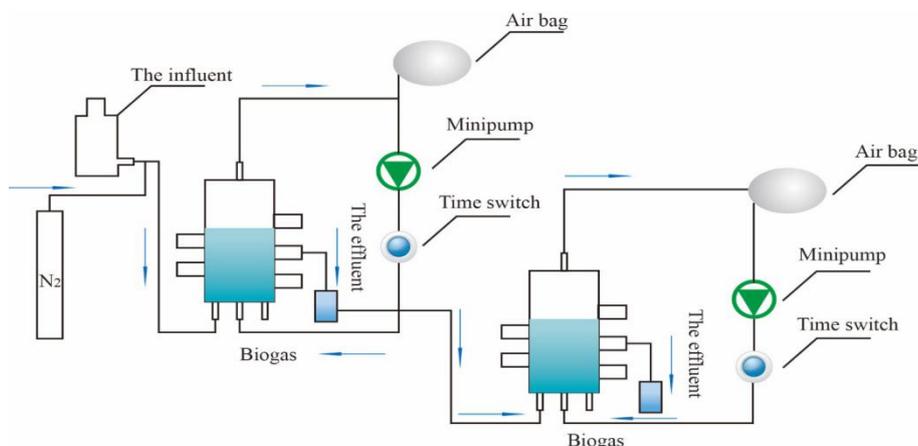


Figure 1. Schematic diagram of anaerobic sequenced batch reactor.

2.2 Wastewater and inoculum

Anaerobic sludge obtained from a wastewater treatment plant in Jinzhong city was used as the inoculum. The anaerobic methanogenic bacteria were sourced from a lab-scale mature anaerobic reactor and anammox bacteria were sourced from an upflow microaerobic sludge reactor cultured for over 2 years. That $C_6H_{12}O_6$ was added into the wastewater to enhance COD loading rate, NH_4Cl was added to enhance NH_4^+ -N loading rate, and $NaHCO_3$ was added to keep the pH of methanation process at 7.4 and the pH of anammox process at 8.1. The initial biomass concentration (expressed as volatile suspended solids, SS) was 13.6g SS/L and VSS/SS was 80%. The methanogenic and anammox bacteria were 15.14 g/L and 16.89 g/L, respectively. R1 was seeded with 1000 mL anaerobic sludge and 250 mL methanogenic bacteria, R2 was also seeded with 1000 mL anaerobic sludge and 250 mL anammox bacteria. The biomass ratios of anaerobic sludge and anaerobic bacteria for R1 and R2 were all 4:1.

The reactors were fed with synthetic wastewater containing (per liter) showed in Table 1: $C_6H_{12}O_6$ 300-1500mg, NH_4Cl 50-120mg, $NaNO_2$ 65-120mg, $NaHCO_3$ 1500mg, KH_2PO_4 30mg, $MgSO_4 \cdot 7H_2O$ 300mg, $CaCl_2 \cdot 7H_2O$ 230mg, trace element solution I (including EDTA 5000mg and $FeSO_4$ 5000mg) 2ml and trace element solution II (including $ZnSO_4 \cdot 7H_2O$ 430mg, $CuSO_4 \cdot 5H_2O$ 250mg, $MnCl_2 \cdot 4H_2O$ 990mg, $NiCl_2 \cdot 6H_2O$ 190mg, $CoCl_2 \cdot 6H_2O$ 240mg, H_3BO_4 14mg) 2ml. Filling the N_2 in the synthetic wastewater to remove dissolved oxygen (DO), which kept the DO below 0.3mg per liter.

Table 1. Components of synthetic wastewater.

Components	Concentration(mg/L)
$C_6H_{12}O_6$	300-1500
NH_4Cl	50-120
$NaNO_2$	65-120
$NaHCO_3$	1500
KH_2PO_4	30
$MgSO_4 \cdot 7H_2O$	300
$CaCl_2 \cdot 7H_2O$	300
Trace element I ^a	2ml/L
Trace element II ^b	2ml/L

^a Trace element I (mg/L) : EDTA 5000 $FeSO_4$ 5000

^b Trace element II (mg/L) : $ZnSO_4 \cdot 7H_2O$ 430, $CuSO_4 \cdot 5H_2O$ 250, $MnCl_2 \cdot 4H_2O$ 990, $NiCl_2 \cdot 6H_2O$ 190, $CoCl_2 \cdot 6H_2O$ 240, H_3BO_4 14

2.3 Analytical methods

COD, NH_4^+ -N, NO_2^- -N, NO_3^- -N, MLSS and MLVSS were detected according to the Standard Methods [19]. DO and pH were measured with a dissolved oxygen meter (Taiwan Hengxin, AZ8403) and a pH meter (Switzerland Mettler Toledo, DELTA320), respectively.

3. Results and discussion

3.1 The rapid start-up of the methanation process

In this stage, the synthetic organic wastewater was added into the sludge-feeding reactor. The initial COD concentration of influent was 300mg/L and the COD removal rate was 65%. In the 3rd day, the COD concentration of influent was increased to 350mg/L, and the removal rate was slightly decreased. The reason was that the activity of the initial sludge was low and the ability adapted to new environment was weak, which resulted in the accumulation of volatile fatty acids. Then the COD concentration of influent was remained at 350mg/L for a period of time (approximately one week), the activity of the sludge was gradually recovered and the removal rate of COD was increased. The COD concentration of the influent was increased at the rate of 50mg/L during the 11th day to the 33rd day.

When the COD concentration of the influent was 1000mg/L, the removal rate of COD reached to 80%. In order to shorten the start-up time and improve the anti-shock load, the COD concentration of the influent was increased at the rate of 100mg/L from the 35th day. When the COD concentration of the influent was 1500mg/L, the removal rate reached to 85%. In the 43rd day, the designed COD load and removal rate were reached ahead of time. The COD concentration of the influent and the effluent in the methanation reactor were shown in Figure 2.

In the 45th day, the actual wastewater contained organic matter and ammonia-nitrogen was simulated. An appropriate amount of NH_4Cl was added into the influent to maintain the $\text{NH}_4^+\text{-N}$ concentration of 50mg/L, when the COD concentration was still 1500mg/L. Then, the COD removal rate was slightly decreased. It might be that the $\text{NH}_4^+\text{-N}$ reacted with a small amount of dissolved oxygen, and the ability that methanogenesis consumed organic matter was decreased. In order to shorten the start-up time and improve the removal load of COD, the hydraulic retention time was reduced to 12 hours from the 51st day. After 4 days, the COD concentration of influent was remained at 1500 mg/L and the removal rate of COD reached to 85% in 55th day. The removal rate of COD was not inhibited for the addition of $\text{NH}_4^+\text{-N}$ in the influent, on the contrary, it was slightly increased. It was estimated that the COD was consumed not only by the methanogenic bacteria, but also the partial nitrifying bacteria and denitrifying bacteria. In the 55th day, the COD concentration of influent was increased to 1500mg/L, while the COD concentration of effluent was less than 50mg/L with the COD removal rate at 95%. Finally, the methanation reactor was in stable operation and the anarobic sludge was in good state.

3.2 The rapid start-up of the anammox process

The anammox reactor was started up with a low $\text{NH}_4^+\text{-N}$ load. A right amount of NaNO_2 was added into the wastewater contained $\text{NH}_4^+\text{-N}$, and the substrates were provided for the anammox reaction. In order to ensure the anaerobic reaction condition, filling the N_2 into the influent for 20 minutes and controlling the pH of R2 at 8.1 with NaHCO_3 . The anammox process could be divided into three stages, and the variations of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$ in the anammox reactor were shown in Figure 3, 4 and 5, respectively.

The first stage (approximately 11days) was the sludge adaptation stage. The $\text{NH}_4^+\text{-N}$ concentration of influent was 50mg/L and the $\text{NO}_2^-\text{-N}$ concentration was 65mg/L. After an anaerobic reaction cycle (24 hours), the $\text{NH}_4^+\text{-N}$ concentration of effluent was 45-55 mg/L and the $\text{NO}_2^-\text{-N}$ concentration was 1-3 mg/L. With the organic matter utilized by a number of denitrifying bacteria [20] from the sludge, $\text{NH}_4^+\text{-N}$ was produced, which caused higher $\text{NH}_4^+\text{-N}$ concentration of effluent than that influent. In the period of this stage, the anammox reaction was not discovered.

The second stage (approximately 20 days) was that the sludge showed anammox activity stage. In this stage, the $\text{NH}_4^+\text{-N}$ concentration of the influent was 60 mg/L and the $\text{NO}_2^-\text{-N}$ concentration was 80 mg/L. After an anaerobic reaction cycle (approximately 24 hours), the $\text{NH}_4^+\text{-N}$ concentration of effluent was 20-30 mg/L and the $\text{NO}_2^-\text{-N}$ concentration was 10-20 mg/L. In this stage, the removal rate of $\text{NH}_4^+\text{-N}$ concentration was significantly increased and a small amount of $\text{NO}_2^-\text{-N}$ was produced, which indicated that the anammox bacteria appeared in the sludge. However, the anammox bacteria was not dominant in the activated sludge [21], the TN load should be further improved to enrich the anammox bacteria in the following stage.

In the third stage (approximately 20 days), the activity of the anammox sludge was improved and the reactor was more stably operated. The $\text{NH}_4^+\text{-N}$ concentration of influent was 80 mg/L and the $\text{NO}_2^-\text{-N}$ concentration was 100 mg/L. During the first 10 days of the stage, the $\text{NH}_4^+\text{-N}$ concentration of effluent was 25 to 30 mg/L, the $\text{NO}_2^-\text{-N}$ concentration was 10 to 20 mg/L, and the $\text{NO}_3^-\text{-N}$ concentration was 15 to 20 mg/L. The removal rate of $\text{NH}_4^+\text{-N}$ concentration was slightly increased, but the $\text{NO}_2^-\text{-N}$ was significantly improved. Then the hydraulic retention time was reduced to 12 hours, but the $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ concentration of influent were remained. After an anaerobic reaction cycle (12 hours), the removal rates of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ were close to 100%, respectively. At the same time, the $\text{NO}_3^-\text{-N}$ [22] concentration of effluent was reached to 20 mg/L. Finally, the removal ratio of

$\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$ concentration was approximately to 1:1.32:0.26 [23, 24] in the anammox reactor.

3.3 Treatment for wastewater in two-stages ASBRs

From the 55th day, the methanation reactor (R1) and anammox reactor (R2) were linked up. The simulative wastewater contained COD and $\text{NH}_4^+\text{-N}$ was filled to the methanation reactor, and then was discharged into the anammox reactor. In this operation, the effluent of R1 was as the influent of R2. The whole anaerobic reaction cycle was 24 hours, including the methanation period (12 hours) and the anammox period (12 hours). During the first 10 days of the linked-up state, the COD concentration of influent was constant 1500mg/L, and the $\text{NH}_4^+\text{-N}$ concentration was 100mg/L. After 12 hours, the COD removal rate of R1 was 95%, but the $\text{NH}_4^+\text{-N}$ concentration in the effluent of R1 was nearly invariable. Then, the effluent of R1 with nearly 50mg/L COD concentration and 100mg/L $\text{NH}_4^+\text{-N}$ flowed into the R2. Over a period of anammox process (12 hours), the effluent COD concentration of R2 was about 0mg/L, and the effluent $\text{NH}_4^+\text{-N}$ concentration was about 1-3 mg/L. In order to improve the removal load of $\text{NH}_4^+\text{-N}$, the influent $\text{NH}_4^+\text{-N}$ concentration of the system was increased to 120mg/L, while the influent COD concentration of R1 was still remained 1500mg/L, to prevent higher COD concentration from influencing the mechanism of removing process of TN in R2. An appropriate amount of NaNO_2 (100mg/L-120mg/L) matched with the $\text{NH}_4^+\text{-N}$ concentration was added into the R2 by sampling opening, which provided the substrates for the anammox reaction. Finally, when the COD, $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ concentration of the two-stage reactors were remained 1500mg/L, 120mg/L and 120mg/L. The removal rates of COD, $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ were respectively as high as 95%, 90% and 100%. Thus, the steady-state condition was reached and the desired data were obtained after approximately 20 days. The removal ratio of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentration was approximately close to 1:1.32:0.26[23,24]. According to some relevant references[20,22,24,25], the synchronous start-up of methanation and anammox processes were successfully achieved after 75 days.

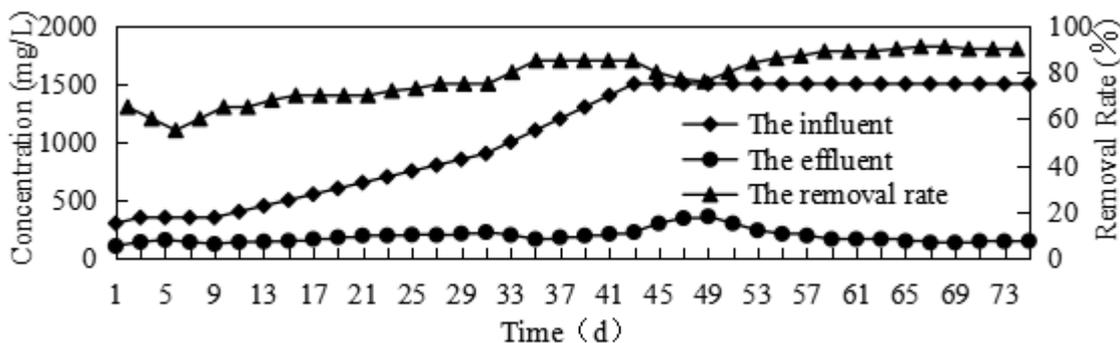


Figure 2. The variation of COD concentration.

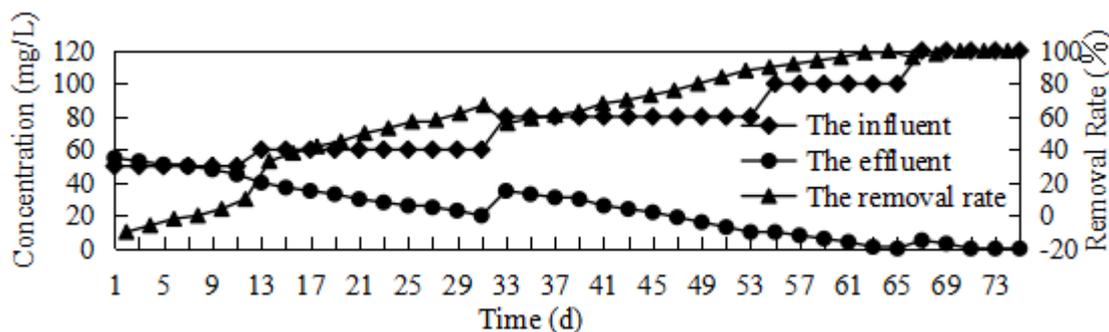


Figure 3. The variation of $\text{NH}_4^+\text{-N}$ concentration.

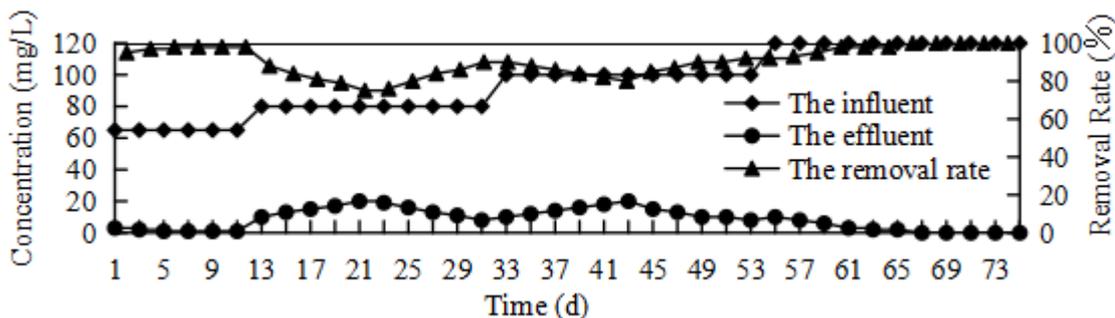


Figure 4. The variation of NO_2^- -N concentration.

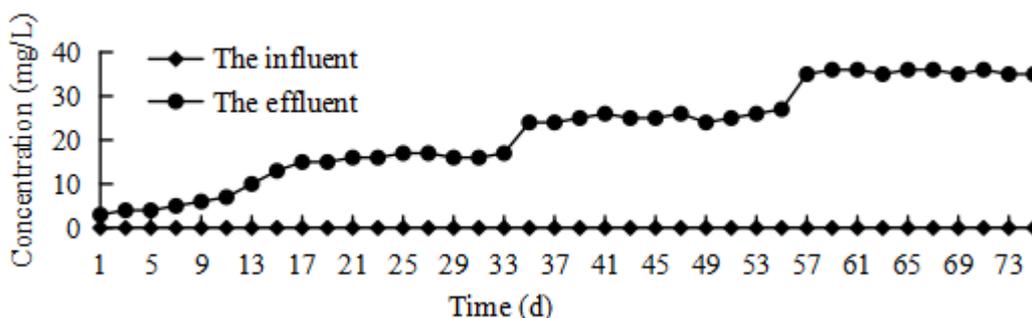


Figure 5. The variation of NO_3^- -N concentration.

4. Conclusion

The "methanation + anaerobic ammonia oxidation autotrophic denitrification" method was adopted by using anaerobic sequencing batch reactors (ASBRs) and realized a satisfactory synchronous removal of chemical oxygen demand (COD) and ammonia-nitrogen (NH_4^+ -N) in wastewater after 75 days operation. The linked-up anaerobic reactors were consisted of methanogenic reactor (R1) and anammox reactor (R2). The chemical oxygen demand (COD) and ammonia-nitrogen (NH_4^+ -N) was in form of $\text{C}_6\text{H}_{12}\text{O}_6$ and NH_4Cl which were added into the synthetic water. During the first 54 days of whole start-up period, R1 and R2 were separately operated and the methanation process achieved the COD removal rate at 95% in R1. When the R1 and R2 were in linked-up station, the synthetic wastewater which contained COD and NH_4^+ -N was discharged into the two-stage anaerobic reactors in sequence. Designed synchronous removal rates of COD and NH_4^+ -N in the two-stage anaerobic reactors was reached after 75 days. It achieved that 95% of COD was removed with a COD load of $1.2\text{kg}/(\text{m}^3\cdot\text{d})$ and over 90% of TN was removed at a TN load of $0.14\text{ kg}/(\text{m}^3\cdot\text{d})$. The removal ratio of NH_4^+ -N, NO_2^- -N and NO_3^- -N concentration was approximately close to 1:1.32:0.26. Compared with other anaerobic reactors, the stably operated ASBRs had many advantages in the field of controlling water pollution and protecting water environment.

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