



## Effect of organic matter strength on single-stage nitrogen removal using anammox and partial nitritation (SNAP) for treatment of high strength ammonia wastewater

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### ABSTRACT

*In this study, long-term effect of organic matter strength on SNAP (single-stage nitrogen removal using anammox and partial nitritation) process treating high strength ammonia wastewater was investigated. The concentrations of ammonium and COD (chemical oxygen demand) in the wastewater were  $2,000 \pm 20$  and  $500-2,000$  mg/L, respectively. Stable simultaneous nitrogen and COD removal were observed in the SNAP process with the TN (total nitrogen) and COD removal efficiencies of 92% and 80%, corresponding the loading of  $0.5 \text{ kg N m}^{-3} \text{ d}^{-1}$  and  $0.38 \text{ kg COD m}^{-3} \text{ d}^{-1}$ , respectively. This process may have applications for treating high strength ammonia and low C/N wastewater such as mature landfill leachate.*

**Key words:** Anaerobic ammonium oxidation; Nitrogen removal; Organic matter; Partial nitritation; Sequencing batch biofilm reactor (SBBR)

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### INTRODUCTION

Ammonium pollution, which can cause eutrophication and be toxic to aquatic species, is becoming a serious environmental problem [1,2]. In a tradition treatment process, ammonium ( $\text{NH}_4^+\text{-N}$ ) is converted into nitrogen gas via a two-step process starting with nitrification, which is the aerobic oxidation of  $\text{NH}_4^+\text{-N}$  to nitrite ( $\text{NO}_2^-\text{-N}$ ) to nitrate ( $\text{NO}_3^-\text{-N}$ ), followed by heterotrophic denitrification under anaerobic condition. While exogenous carbon sources are required to achieve complete denitrification [3], which not only makes full-scale denitrification quite expensive but also causes secondary pollution, limiting its applications in wastewater treatment with low C/N.

The Anammox (anaerobic ammonium oxidation) had been recognized as a promising process to treat wastewater devoid of organic carbon [4]. Generally, major nitrogen compound in wastewater is ammonium, which must be nitrified partially to nitrite, but not to nitrate. Then the remaining ammonium together with the produced nitrite is converted to dinitrogen gas in Anammox process. This partial nitritation and Anammox process can be performed in two-stage reactors as the SHARON-ANAMMOX process [5] or in a single-stage reactor such as CANON (completely autotrophic nitrogen removal over nitrite) process [6,7] and SNAP (single-stage nitrogen removal using anammox and partial nitritation) process [8]. However, the nitrate is always inevitable in the stage of partial nitritation, since stable operation of partial nitritation process could not achieve 100% of the nitrite accumulation rate for long time even under the optimized conditions [9,10].

Moreover, Mature landfill leachate contains a relatively high concentration of ammonium and organic matter. Previous studies have demonstrated that the presence of organic matter could negatively interfere with

Anammox reaction [11], Ni *et al.* [12] demonstrated that low organic matter concentration did not affect ammonium removal significantly but improved the total nitrogen (TN) removal via denitrification. Chamchoi *et al.* [13] found that the COD concentration was a control variable for process selection between Anammox and denitrification.

Thus, this study was performed to evaluate the effect of organic matter strength on the SNAP process for treatment of high strength ammonia wastewater in a single SBBR (sequencing batch biofilm reactor). Starch and peptone which simulated the organic matter in actual landfill leachate were added to create different influent concentration of COD to ammonium ratio (C/N) for nitrogen and COD removal investigation. Some controlling strategies were optimized and the reactor performance was examined in the SNAP process.

## EXPERIMENTAL SECTION

### 2.1 Reactor and operational strategy

The SBBR (sequencing batch biofilm reactor) was a plexiglas cylinder, the height of which was 500 mm and internal diameter 200 mm, with height to diameter ratio being 2.5. Semi-soft fibre fill was used as the biomass carrier and the packing rate was 50% (V/V). The reactor had a working volume of 10 L feeding with artificial wastewater. During the experiment period, the reactor was placed in a thermostatic chamber, in order to maintain the temperature constant at  $30\pm 1^\circ\text{C}$ .

The SBBR was operated sequentially in 8 h-cycle, with intermittent aeration (aeration 4h / aeration stop 4h). Discharging and feeding were carried out during the last 10 min of each 3 cycles (24h) and the water filling ratio was 0.25. The aeration was controlled using air pumps to regulate the DO concentration of the reactor. The SBBR was run at limited aeration stage, with the concentration of DO strictly controlled around  $2.5\text{ mg L}^{-1}$  by adjusting the air flow rate. At the aeration stop stage, the concentration of DO was at  $0.1 - 0.2\text{ mg L}^{-1}$ .

The strategy of limited aeration was adopted to inhibit (nitrite oxidizing bacteria) NOB activity and prompt (anaerobic ammonium oxidation bacteria) AnAOB proliferation, achieving autotrophic (ammonium oxidizing bacteria) AOB and AnAOB simultaneous growth. During the experiment, different concentration strength COD (500, 1,000, 1,500 and 2,000 mg/L) were introduced in the influent, respectively, resulting in an influent C/N of 0.25, 0.5, 0.75 and 1.

### 2.2 Seed sludge and synthetic wastewater

The SNAP biomass was derived from an ongoing lab-scale SBBR using Anammox and partial nitrification for treatment of high strength ammonium wastewater. The original SBBR was operated for 2 years with influent  $\text{NH}_4^+\text{-N}$  concentration of  $2,000\pm 20\text{ mg/L}$ .

The composition of the synthetic inorganic media was as follows (g/L),  $\text{NH}_4\text{HCO}_3$ : 1700-13540 mg;  $\text{KH}_2\text{PO}_4$ : 25 mg; EDTA: 25 mg;  $\text{FeSO}_4$ : 6.25 mg;  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ : 200 mg;  $\text{CaCl}_2$ : 300 mg; trace nutrient solution: 1.25 ml and the right amount of  $\text{KHCO}_3$  to regulate pH to 8.0. The trace nutrient solution contained (g/L),  $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ : 0.43;  $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ : 0.24;  $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ : 0.99;  $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ : 0.25;  $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$ : 0.22;  $\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ : 0.19;  $\text{H}_3\text{BO}_3$ : 0.014;  $\text{Na}_2\text{WO}_4\cdot 2\text{H}_2\text{O}$ : 0.05. Starch and peptone as the organic carbon source was mixed by equivalent COD ratio of 1:1. Starch and peptone was complex organic substrate which could be representative to the biodegradable organics in wastewater.

### 2.3 Analysis methods

The influent and effluent samples were collected on a daily basis and were analyzed immediately. The concentrations of COD,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , TN and MLSS were measured according to standard methods for the examination of water and wastewater [14]. The system was equipped with suitable submerged probes, such as dissolved oxygen (DO) (Hach, HQ30d, USA), pH (Hach, sension2, USA) and oxidation reduction potential (ORP) (Hach, sension2, USA).

## RESULTS AND DISCUSSION

### 3.1 Autotrophic nitrogen removal performance using anammox and partial nitrification

Experiments were carried out without addition of organic matter in phase 1 (days 1-35). The profiles of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  in effluent, as well as nitrogen removal rate were presented in Figure 1. The  $\text{NH}_4^+\text{-N}$  in influent was kept at  $2,000\pm 20\text{ mg/L}$ , which simulated the ammonium concentration in mature landfill leachate [15,16]. It could be seen that a persistent, stable partial nitrification and Anammox were achieved in the SBBR. A maximal total nitrogen (TN) removal rate of  $0.46\text{ kg N m}^{-3}\text{ d}^{-1}$  was achieved for the SNAP process with the  $\text{NH}_4^+\text{-N}$  and TN removal efficiencies of 99.9% and 91.3%.

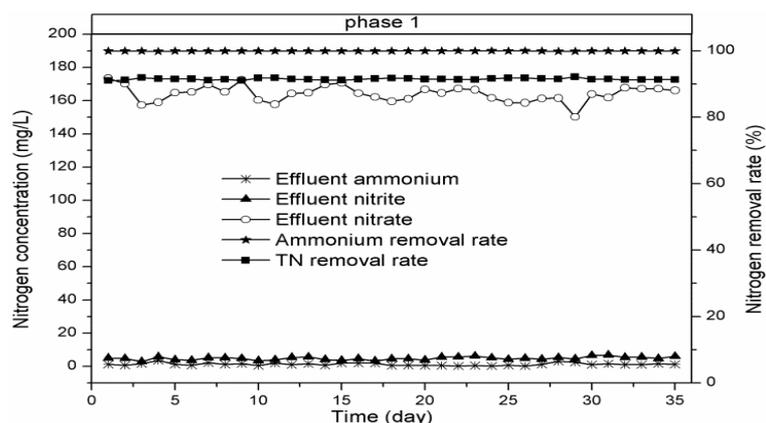


Figure 1 Profiles of nitrogenous compounds concentration and removal rate during phase 1 (without organic matter)

It should be noted that the SBBR biofilm of the SNAP process was reddish. The SEM was used to visualize the surface of aggregated biomass (Figure 2). The sludge sample was taken from the SBBR on the 30th day. The dominant microorganisms in the sludge were dense coccoid cells which were supposed to be Anammox bacteria [17,18].

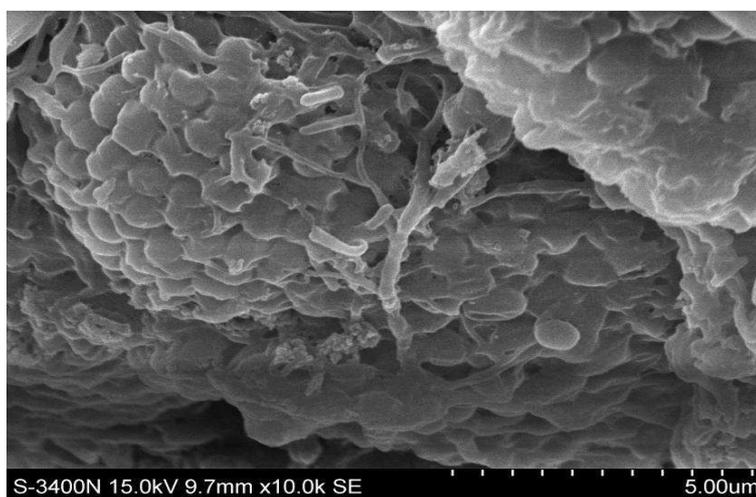
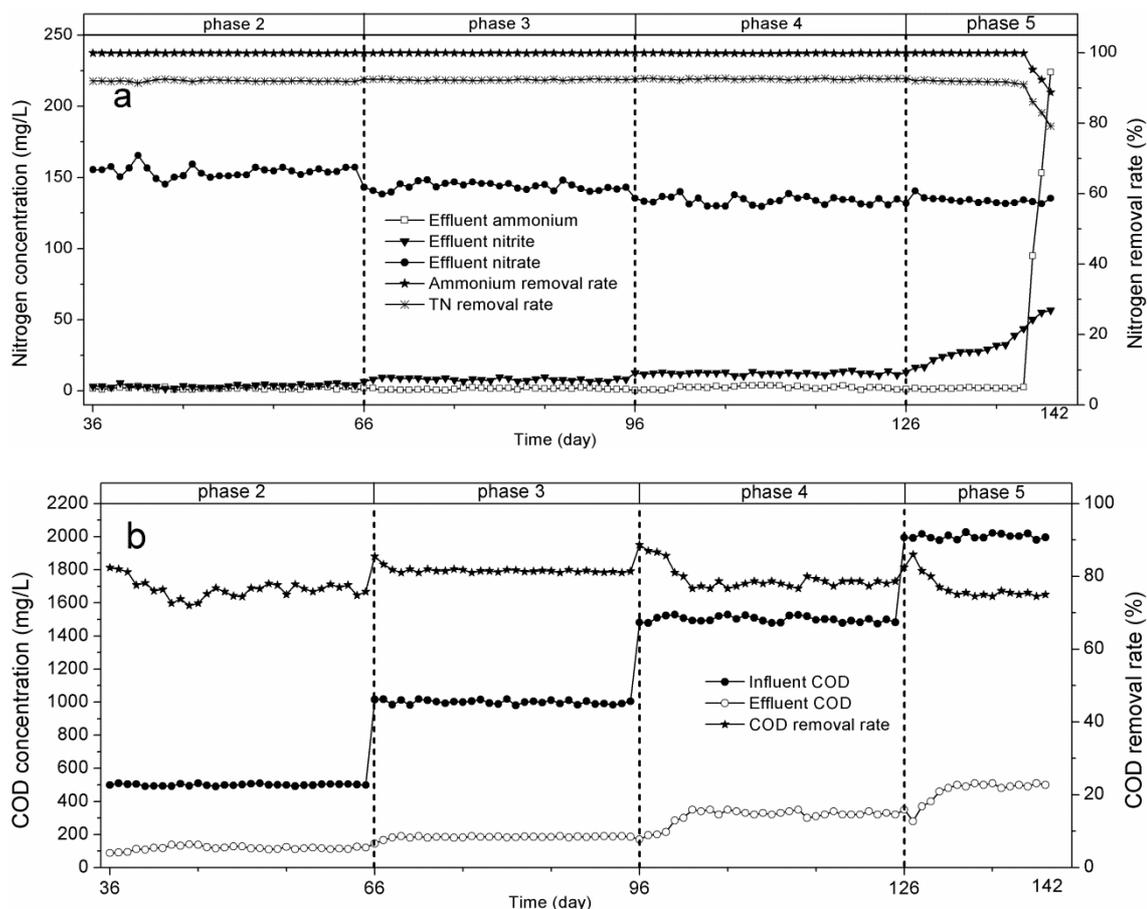


Figure 2 Scanning electron photomicrographs of microorganism on the biofilm

### 3.2 Nitrogen and COD removal performance of the SNAP process at different C/N

Wastewater such as mature landfill leachate contains high concentration of organic matter, which poses a great threat to the surroundings and needs to be treated before discharge. However, to the autotrophic nitrogen removal process, the presence of organic matter might result in excessive growth of heterotrophic denitrification bacteria and inactivating or eradicating Anammox communities with the operation of the SBBR [19]. Different concentration of COD was conducted to investigate nitrogen removal performance, respectively.

The organic matter of different concentration were introduced to the SBBR on day 36, 66, 96 and 126 with the concentration of COD 500 mg/L, 1,000 mg/L, 1,500 mg/L and 2,000 mg/L in the phase 2, 3, 4, and 5 (days 36-65, 66-95, 96-125 and 126-142), respectively. As shown in Figure 3, there was no pronounced change in the  $\text{NH}_4^+\text{-N}$  removal process in the phase 2, 3 and 4. At an applied load of  $0.5 \text{ kg N m}^{-3} \text{ d}^{-1}$  and  $0.375 \text{ kg COD m}^{-3} \text{ d}^{-1}$ , high rate simultaneous nitrogen and COD removal were observed with the TN and COD removal efficiencies of 92% and 80%, respectively. Interestingly, the organic matter or COD was removed sharply by the SBBR. It demonstrated that there were another way such as heterotrophic bacteria or autotrophic bacteria which have the capacity of facultative heterotrophism to remove the organic matter in this SNAP process.



**Figure 3** Profiles of nitrogenous compounds (a) and COD (b) concentration and removal rate during phase 2 (C/N=0.25, COD=500 mg/L), phase 3 (C/N=0.5, COD=1,000 mg/L), phase 4 (C/N=0.75, COD=1,500 mg/L) and phase 5 (C/N=1, COD=2,000 mg/L)

The nitrite clearly started to accumulate and the effluent COD concentration increased suddenly when the influent concentration of COD was increased from 1,500 mg/L to 2,000 mg/L on the 126th day. Which indicating a gradual inhibition of Anammox activity by the high strength organic matter [11] and accumulated nitrite [20]. And the ammonium removal efficiency decreased rapidly, corresponding with the ammonium removal rate from 99.9% on the 139th day to 88.8% on the 142th day. Thus, influent concentration of COD was reduced to 1,500 to avoid the inhibition of Anammox reaction. Otherwise, the system would collapse.

Overall, it could be concluded safely that the system had the capacity of resisting the shock of high organic matter concentration. Therefore, the simultaneous partial nitrification and Anammox SBBR process could be applied for nitrogen and COD removal in treating low C/N wastewater such as mature landfill leachate, even though the high organic matter occurred unconsciously at some time. Nevertheless, some unexpected factors might affect the stability of the system in treating the actual landfill leachate, which would be further investigated in the future.

## CONCLUSION

In a long-term study, the SNAP process demonstrated that advanced simultaneous nitrogen and COD removal could be achieved under low organic matter in a SBBR. A maximal total nitrogen (TN) and COD removal rates of  $0.46 \text{ kg N m}^{-3} \text{ d}^{-1}$  and  $0.3 \text{ kg COD m}^{-3} \text{ d}^{-1}$  were achieved in the SNAP process with TN and COD removal efficiencies of 92% and 80%, respectively. Thus, the SNAP process can be used to treat low C/N wastewater such as mature landfill leachate.

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