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# NITRITATION-ANAMMOX PILOT SYSTEM FOR NITROGEN REMOVAL FROM EFFLUENT OF UASB REACTOR TREATING SWINE WASTEWATER

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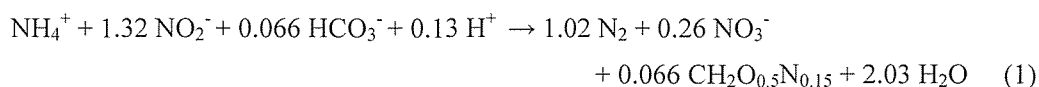
## Abstract

This paper describes the performance of a pilot treatment system for nitrogen removal from an ammonium-rich effluent of UASB reactor treating swine wastewater. The system consisted of two 60-L column nitrification reactors and one 180-L column anammox reactor. Nitrification reactors were seeded with activated nitrifying sludge and anammox reactor was seeded with cultivated anammox sludge. All the reactors were packed with a mineral biomass carrier. The influent ammonium concentrations were from 256 to 424 mg-N/L. Ammonium removal rates have increased with elapsed operational time. The best performance was observed from days 180 to 240 at the influent flow of 0.4 m<sup>3</sup>/d, corresponding to average loading rate of 0.35 kg-N/m<sup>3</sup>/d. The average ammonium removal efficiencies over 60 days of operation were 53.0 ± 6.9%, 89.4 ± 8.6% and 95.3 ± 3.5% for nitrification stage, anammox stage and overall system, respectively. This indicates partial nitrification was achieved and subsequent anammox reaction was highly effective.

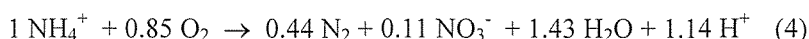
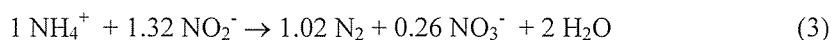
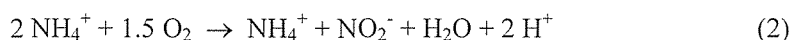
**Keywords:** anammox, nitrification, nitrogen removal, swine wastewater, UASB effluent.

## 1. Introduction

Anaerobic ammonium oxidation (anammox) reaction was detected in 1995 (Mulder *et al.*, 1995) and has become attractive to many researches since then. This is a biological oxidation of ammonium under anaerobic condition with nitrite as an oxidant. The stoichiometric equation of anammox was described by Strous *et al.* (1998) as follows:



For applying anammox into treatment of wastewater containing ammonium, a preceding nitrification is required. A novel nitrogen removal process is based on the combination of partial nitrification and anammox, as summarized in equations 2-4 (Jetten *et al.*, 2001).



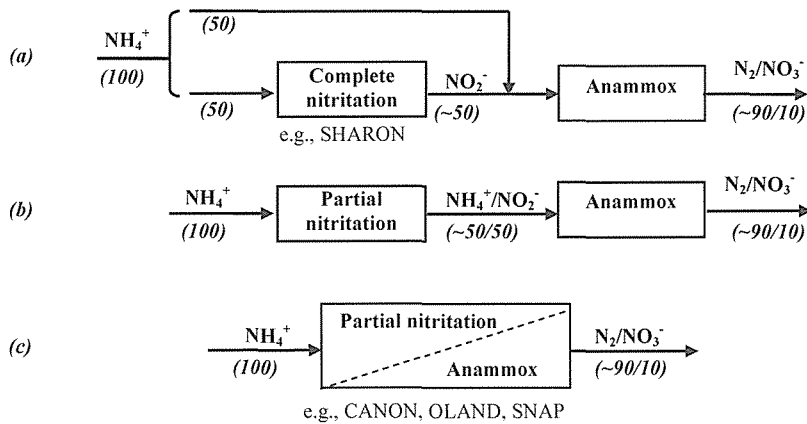
This novel process is therefore a wholly autotrophic nitrogen removal process, different with the conventional process which consists of autotrophic nitrification and heterotrophic denitrification steps. Application of the novel process into treatment of ammonium-rich wastewater has been increasingly concerned due to some advantages over the conventional one. Reduction of oxygen supply and elimination of organic carbon addition are two typical advantages of the novel process.

To combine nitrification and anammox steps, separate systems or single-stage systems can be applied. Principal schemes of these systems can be illustrated in Figure 1. Typical systems with separate

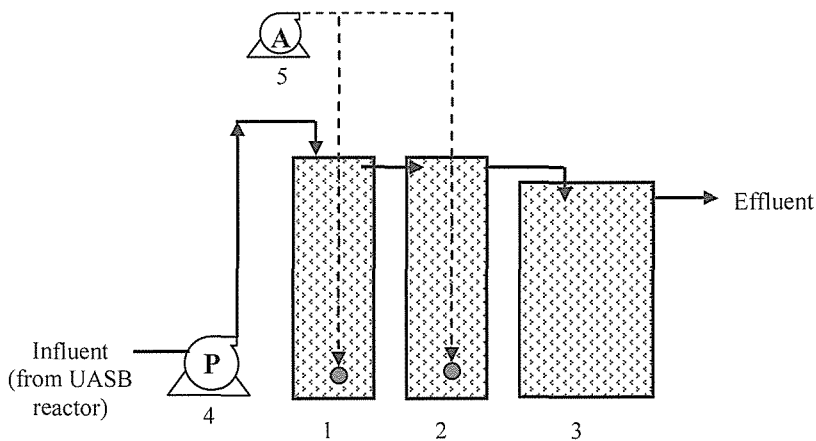
reactors include SHARON<sup>®</sup>-ANAMMOX<sup>®</sup> (van Dongen *et al.*, 2001) or partial nitritation-anammox (Fux *et al.*, 2002). For single-stage processes, OLAND (Kuai and Verstraete, 1998) and CANON (Sliemers *et al.*, 2002) are widely known. A single-stage process was also developed by some of our authors named SNAP (Single-stage Nitrogen removal using Anammox and Partial nitritation) (Furukawa *et al.*, 2006; Lieu *et al.*, 2006).

Principally, optimum condition for the application of the novel process is the avoidance of organic carbon in influent. Wastewaters containing high ammonium but low COD such as supernatant of sludge digester, pre-treated landfill leachate are suitable for novel process application.

Previously, our study was successful in enrichment of anammox bacteria from sludge of an UASB reactor treating swine wastewater. The existence of anammox bacteria was confirmed by chemical evidents (simultaneous consumption of ammonium and nitrite and formation of nitrate) as well as microbiological evidents (isolation and amplification of 16 S-rDNA sequences which are highly similar with sequences of known anammox strains) (Phuong *et al.*, 2005). Using this anammox sludge together with an available nitrifying sludge, a pilot-scale separate system for nitrogen removal was installed to study the treatability on effluent from an UASB treating swine wastewater. This paper describes some results of the pilot system operation.



**Fig. 1.** Optional flux diagrams for the combination of nitritation and anammox processes. (a) and (b): separate reactors, (c): single-reactor.



**Fig.2.** Pilot system for ammonium removal from the effluent of UASB reactor at Dong A Factory of Breed Pigs. (1) and (2): Nitritation reactors; (3): Anammox reactor, (4): Influent pump, (5): Air blower

## 2. Materials and methods

### 2.1. Pilot system description

As shown in Figure 2, the pilot treatment system consists of two identical nitrification reactors (30 cm in diameter and 90 cm in height) and an anammox reactor (50 cm in diameter and 80 cm in height). All reactors were made from PVC and packed with a biomass carrier at package rate of 20% (v/v). The carrier, which has a specific area of 100 m<sup>2</sup>/m<sup>3</sup>, was prepared from an inorganic material as sheet type (100 x 150 x 2 mm) and put vertically into the reactors. The system was installed at Dong A Factory of Breed Pigs, Ho Chi Minh city.

At start-up time, the nitrification reactors were seeded with a nitrifier culture in which *Nitrosomonas* was enriched to 10<sup>9</sup>-10<sup>11</sup> cells/mL, at the rate of 500 g per each reactor. The anammox reactor was seeded with an anammox sludge which was obtained during more than 9-month enrichment with specific medium and anammox strains was detected by DNA analysis. Both microbial culture sources were from the labs of our Institute of Tropical Biology. The influent pump operated at flow range of 0.15- 0.52 m<sup>3</sup>/d. The air blower had a maximum capacity of 15 L/min. After starting-up, the system operated in different phases with changing influent flow, as described in Table 1.

**Table 1.** Different operational phases of the pilot system

Operational phase	I	II	III	IV
Time of operation (days)	1-60	61-120	121-180	181-240
Flow rate (m <sup>3</sup> /d)	0.5	0.2	0.3	0.4

### 2.2. Influent characteristics

The pilot system was fed with effluent from an UASB reactor used for treatment of wastewater from the pig-breeding factory. Characteristics of this UASB effluent are shown in Table 2. High concentrations of ammonium and low concentrations of COD made the wastewater suitable for nitrogen removal by autotrophic processes.

**Table 2.** Characteristics of effluent from UASB reactor treating swine wastewater

Parameter	Unit	Min	Max	<i>Average</i>
pH		7.12	8.05	7.69
COD	mg/L	115	320	184
NH <sub>4</sub> -N	mg-N/L	256	424	320
NO <sub>2</sub> -N	mg-N/L	0.0	1.5	0.5
NO <sub>3</sub> -N	mg-N/L	0.0	3.8	1.2
PO <sub>4</sub> -P	mg-P/L	20	60	36.9
SS	mg/L	112	85	98.5

### 2.3. Analytical methods

Influent and effluent samples of the pilot system were taken and analyzed for such parameters as pH, DO, COD, NH<sub>4</sub>-N, NO<sub>3</sub>-N, NO<sub>2</sub>-N, and TDS. Measurement of pH was done by using pH meter Model 2000 VWR Scientific (USA). DO and TDS were measured using multiparameter water quality checker TOA Model WQC 22 (Japan). Analyses of COD and nitrogenous species were

performed in accordance with “Standard methods for the examination of water and wastewater” (1999). The Thermo Spectronic Model 4001/4 (USA) was used for spectrophotometric measurements.

### 3. Results and discussion

#### 3.1. Ammonium removal during 1<sup>st</sup> operational phase (0.5-m<sup>3</sup>/d flow rate)

During the first 60 days of experiment, the pilot system operated at an inlet flow rate of 0.5 m<sup>3</sup>/d as intended design. Ammonium removal efficiencies of nitrification reactor, anammox reactor and the whole system during this phase are shown in Figure 3. There was a sudden fluctuation in all the removal efficiencies. The overall removal was almost below 60%. This kind of behaviour is due to the high ammonium load were applied while nitrifiers and anammox bacteria were not well adapted. In addition, there might exist a competition between nitrifiers and heterotrophic bacteria that consumed organic carbon in nitrification reactors in the presence of COD and DO.

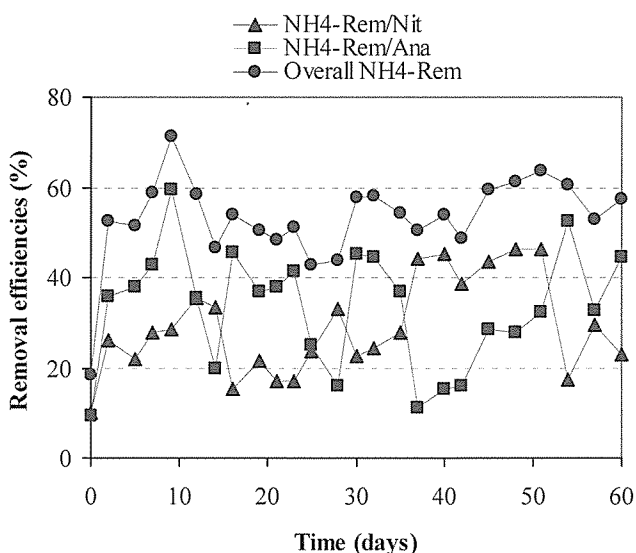


Fig. 3. Ammonium removal during the first phase of operation at flow rate of 0.5 m<sup>3</sup>/d

#### 3.2. Ammonium removal during 2<sup>nd</sup> operational phase (0.2-m<sup>3</sup>/d flow rate)

From days 61 to 120 the inlet flow rate was reduced to 0.2 m<sup>3</sup>/d for better adaptation of the system. Profiles of ammonium concentrations and removal efficiencies during this 2<sup>nd</sup> phase are presented in Figures 4 and 5. It can be seen from these figures that ammonium removal had an upward trend in this phase for both reactors. Removal efficiencies were 42.6 ± 8.7 %, 51.7 ± 15.1 % and 71.7 ± 11.4 % for nitrification reactor, anammox reactor and the whole system, respectively. At low nitrogen loads, nitrosomonas and anammox bacteria have well acclimated and grown. The biomass increase in all three reactors was observed and the brownish red color of anammox sludge in anammox reactor become clearer. The simultaneous ammonium decrease in both nitrification and anammox reactors indicates the occurrence of partial nitrification producing nitrite which used for subsequent anammox reaction.

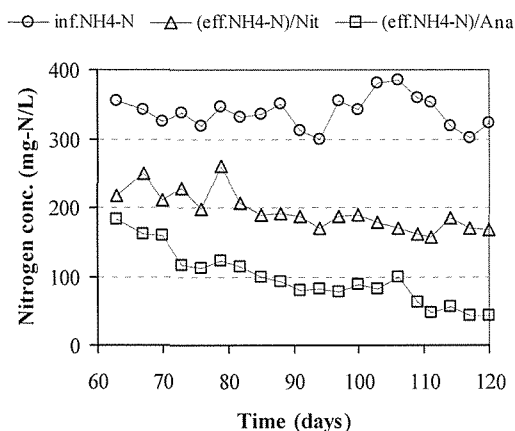


Fig. 4. Ammonium concentrations during the 2<sup>nd</sup> phase of operation at flow rate of 0.2 m<sup>3</sup>/d.

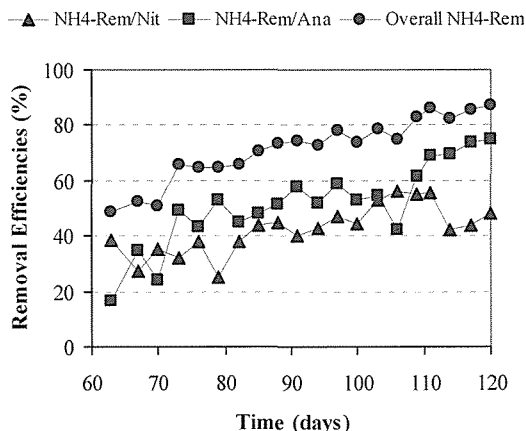


Fig. 5. Ammonium removal during the 2<sup>nd</sup> phase of operation at flow rate of 0.2 m<sup>3</sup>/d.

### 3.3. Ammonium removal during 3<sup>rd</sup> operational phase (0.3-m<sup>3</sup>/d flow rate)

In this phase, the influent flow rate increased to 0.3 m<sup>3</sup>/d. Performance data during this phase are shown in Figures 6 and 7. The system adapted to higher nitrogen loading for about 20 days during which removal efficiencies were still low and unchanged. Thereafter, removal efficiency of the anammox reactor increased sharply and then became quite stable for 20 days. Removal efficiency of the nitrification reactor, however, was not much changed. Anammox stage decided overall removal. Average removal efficiencies were  $54.1 \pm 5.3$  %,  $71.9 \pm 18.5$  % and  $86.6 \pm 9.5$  % for nitrification reactor, anammox reactor and the whole system, respectively. The rapid increase in biomass was observed for both nitrosomonas and anammox bacteria.

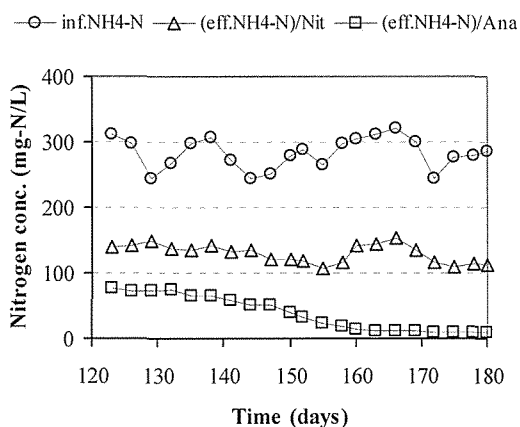


Fig. 6. Ammonium concentrations during the third phase of operation at flow rate of 0.3 m<sup>3</sup>/d.

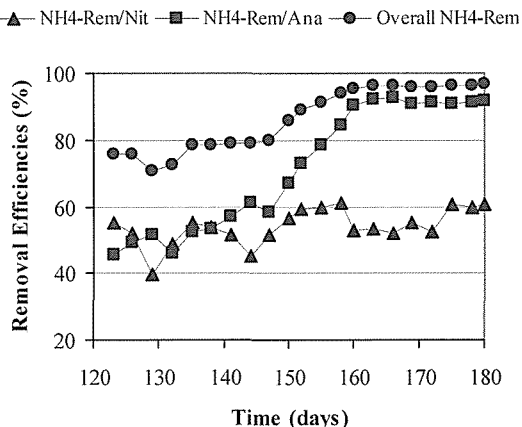


Fig. 7. Ammonium removal during the third phase of operation at flow rate of 0.3 m<sup>3</sup>/d

### 3.4. Ammonium removal during 4<sup>th</sup> operational phase (0.4-m<sup>3</sup>/d flow rate)

In this phase, from day 180 to day 240, the system operated with influent flow of 0.4 m<sup>3</sup>/d. The ammonium removal was nearly stable, especially from day 200 onward. Removal efficiency in nitrification reactor ( $53.0 \pm 6.9$  % in average) was almost same as one of previous phase, while removal efficiency in anammox reactor exhibited a significant increase ( $89.6 \pm 8.5$  % in average). This led to an increase in overall system efficiency up to  $95.4 \pm 3.6$  %. Due to the slow growth of anammox bacteria, it requires long time for accumulating enough biomass concentration. Therefore, the removal efficiency of anammox reactor had increased with elapsed operational time. Profiles of

ammonium concentration and removal during the 4<sup>th</sup> operational phase are shown in Figures 8 and 9. The continuous growth of microorganisms in both reactors could be observed via the increase in biomass.

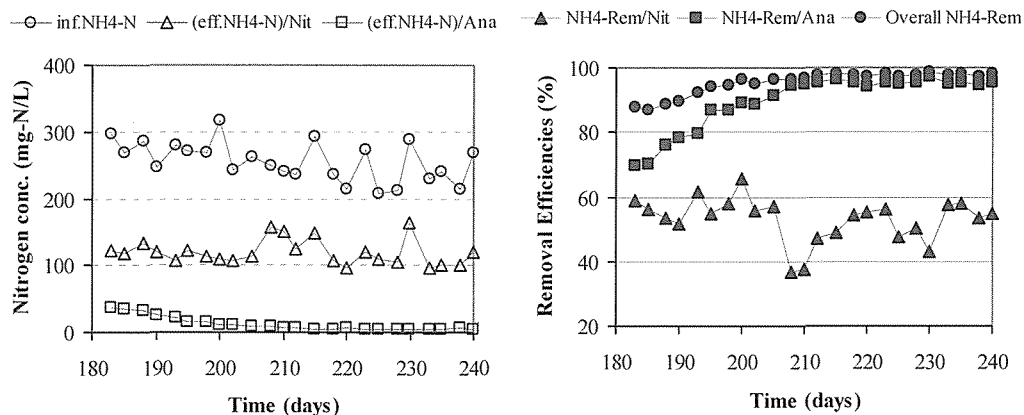


Fig. 8. Ammonium concentrations during the fourth phase of operation at flow rate of 0.4 m<sup>3</sup>/d.

Fig. 9. Ammonium removal during the fourth phase of operation at flow rate of 0.4 m<sup>3</sup>/d.

### 3.5. Variations in DO, pH, COD and phosphorous during 240 days of operation

During 240 days of operation, DO values of system's influent, nitritation reactor's effluent and anammox reactor's effluent were 0.1 - 0.2 mg/L, 0.5 - 0.9 mg/L and 0.3 - 0.6 mg/L, respectively. It is remarked that influent of the pilot system was from UASB reactor; hence DO value was always very low. The range of 0.5 - 0.9 mg/L is a typical range for nitritation reaction in which AOB exhibit a dominant growth over NOB. In experiments, a ratio of 40 - 60 % ammonium converted to nitrite was achieved in nitritation reactor at DO from 0.4 - 0.6 mg/L. Because there was no oxygen stripping after nitritation reactor, condition in anammox reactor was not strictly anaerobic.

The system's influent pH varied from 7.12 to 8.05. After nitritation stage, pH slightly decreased to 6.8 - 7.5 due to a consumption of alkalinity (see equation 2). After anammox stage, pH increased to 8.0 - 8.5, which is one of typical characteristics of anammox reaction (see equation 1).

COD removal of the system over 240 days of operation was about 30 - 60 %. The lowest COD removal was in the initial phase in which high hydraulic loading rate was applied. Regarding to treatment stage, COD removal was higher in nitritation reactor than in anammox reactor. This indicates aerobic oxidation, rather than anoxic denitrification, was the major mechanism of organic removal.

Overall reduction of phosphorous through pilot system was about 55 - 75 %, in which nitritation stage accounted for 25 - 40% and anammox stage accounted for 40 - 60 %. Major phosphorous removal might be due to the synthesis of bacterial cells.

## 4. Conclusions

It was successful in combination of nitritation and anammox into a pilot system that could efficiently remove ammonium from effluent of anaerobic treatment of swine wastewater. After 180 days from starting-up, the system could achieve about 95 % of total ammonium removal at flow rate of 0.4 m<sup>3</sup>/d or loading rate of 0.35 kg-N/m<sup>3</sup>/d. Partial nitritation was quite stable (about 53 %) and anammox reaction was highly effective (about 90 %) during operation. The use of an inorganic biomass carrier was proved to be suitable for attached growth of both nitrosomonas and anammox bacteria groups. The changes in hydraulic or nitrogen loading rates showed a higher effect on anammox reaction than on nitritation.

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