

*Full Length Research Paper*

# Removal of nitrogen from ammonium-rich synthetic wastewater in an upflow column type anammox reactor

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**The aim of this study is to illustrate the efficiency of nitrogen removal from synthetic wastewater using a fixed-bed anaerobic ammonium oxidation (anammox) reactor. A continuous fed flow reactor was inoculated with upflow anammox and fed with synthetic wastewater. The reactor was operated for 75 days without pH control. The maximum ammonium and nitrite removal efficiencies achieved were 95 and 97.5%, respectively. The maximum nitrogen conversion capacity was 3.2 kg NH<sub>4</sub><sup>+</sup>-N/m<sup>3</sup>/day, 1.89 kg NO<sub>2</sub><sup>-</sup>-N/m<sup>3</sup>/day and 4.6 kg total N/m<sup>3</sup>/day with maximum loadings of 3.9 kg NH<sub>4</sub><sup>+</sup>-N/m<sup>3</sup>/day, 2.00 kg NO<sub>2</sub><sup>-</sup>-N/m<sup>3</sup>/day and 5.64 kg total-N/m<sup>3</sup>/day, respectively. The experimental results suggest that a fixed-bed anammox reactor can be operated under high ammonium and nitrite concentration with no pH control.**

**Key words:** Ammonium, anammox, nitrogen removal, nitrite, reactor, wastewater.

## INTRODUCTION

Anammox, an abbreviation of anaerobic ammonium oxidation, is an almost completely unexplored part of the biological nitrogen cycle (Arrigo, 2005) and this process opens a new era for wastewater treatment. Anaerobic ammonium oxidation (Anammox) is the biological conversion of ammonium and nitrite to dinitrogen gas. Nitrite and nitrate are subsequently reduced to nitrogen gas by denitrifying bacteria under anoxic conditions.

The anammox process consists of two separate processes for the removal of ammonium in wastewater. The first step is partial nitrification (nitritation) of half of the ammonium to nitrite by ammonia oxidizing bacteria:



The resulting ammonium and nitrite are converted in the anammox process to dinitrogen gas:



Both processes can take place in one reactor where two

guilds of bacteria form compact granules (Kartal et al., 2010; Helen, 2010).

However, a sustainable development of this process requires a more cost-effective and less time-consuming technologies for growing market competition and advancements in technology. Nitrification demands very efficient oxygen supply coupled with adjustment for changes in alkalinity of the wastewater due to formation of hydrogen ions. In addition, heterotrophic denitrifying bacteria essentially need a carbon source as an electron donor. Therefore, it is imperative to develop a process with reduced oxygen and external carbon requirements for treating ammonium-rich wastewater with low carbon to nitrogen (C/N) ratios.

Mulder et al. (1995) discovered anammox process about 15 years ago where dinitrogen gas was produced from directly combining ammonium and nitrite. Anammox process is considered to have high potentials for high nitrogen removal rate (NRR) from wastewaters containing low C/N ratio or high ammonium concentration, such as landfill leachate and sludge digester liquor (Strous et al., 1998). A number of studies have been reported on nitrogen removal from livestock manure digester liquor (LMDL) using conventional nitrification–denitrification process (Bernet et al., 2000; Obaja et al., 2003, 2005;

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**Table 1.** Composition of synthetic wastewater.

Component	Unit	Value
pH		8.5 to 9.2
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	mg/L	200
NaNO <sub>2</sub>	mg/L	100 to 200
KHCO <sub>3</sub>	g/L	1.25
KH <sub>2</sub> PO <sub>4</sub>	mg/L	25
Fe <sup>2+</sup>	mg/L	18
EDTA	mg/L	10

\* and also added Ca<sup>2+</sup>, Mg<sup>2+</sup>, Co<sup>2+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup> as trace elements.

Yang et al., 2003; Shin et al., 2005; Vanotti et al., 2007; Dosta et al., 2008; Waki et al., 2008). Among them, Obaja et al. (2005), showed relatively higher nitrogen removal rate which was approximately 1.03 kg-N/m<sup>3</sup>/day at HRT of 0.87 days but providing to keep C/N ratio equal to or higher than 1.7 to complete denitrification. It is obvious from these results that relatively longer hydraulic retention time (HRT) and lower nitrogen removal rate reflects limitation of conventional nitrification–denitrification process.

To develop more cost-effective and less time consuming technology for biological nitrogen removal from wastewater, efforts were made, such as simultaneous nitrification denitrification (SND) (Chen et al., 1991; Yoo et al., 1999), high ammonium removal over nitrite (SHARON) (Jetten et al., 1997), anaerobic ammonium oxidation (ANAMMOX) (Furukawa et al., 2002; Strous et al., 1997; Graff et al., 1995), oxygen limited autotrophic nitrification denitrification (OLAND) (Kuai and Verstraete, 1998; Pynaert et al., 2003). Another effort was made by combining partial nitrification (PN) and anammox processes. The PN process proceeds as a pretreatment step in order to supply ammonium and nitrite to anammox biomass. The combined process would not only reduce the operational cost significantly, but also decrease the post treatment cost due to the lower sludge production rate in anammox process (Fux et al., 2002). A report showed that anammox process in a lab-scale up flow anaerobic sludge blanket (UASB) reactor removed nitrogen from actual piggery waste digester liquor by adding synthetic nitrite (NaNO<sub>2</sub>) (Ahn et al., 2004). An average nitrogen removal rate (NRR) was obtained which, in terms of total nitrogen (T-N) was 0.66 kg T-N/m<sup>3</sup>/day (0.3 kg NH<sub>4</sub><sup>+</sup>-N/m<sup>3</sup>/day). Hwang et al. (2005) and Yamamoto et al. (2008), also reported that nitrogen was successfully removed from piggery waste by combined PN–anammox processes, but the NRRs only reached 0.72 and 0.22 kg T-N/m<sup>3</sup>/day for combined PN–anammox and anammox reactors, respectively. The relatively lower NRRs reported in these two studies did not illustrate realistic advantages of anammox process for nitrogen removal from high ammonium or low C/N ratio

wastewaters. pH plays important roles in the anammox process. Firstly, it directly influences the growth rates of the bacteria. Secondly, pH is closely related to the available substrate forms and as a result to the nitrification process. Several research reports were found on the effect of pH on biological wastewater treatment process (Fux et al., 2002; Lu et al., 2006). The hydraulic retention time (HRT) is an important parameter for wastewater treatment process. The operation time of the reactor had a significant role in anammox process. The study is tried to find out a favorable HRT where anammox reactor shows the maximum efficiency. Several reports also discussed about the role of HRT in nitrification process (van Kempen et al., 2001; Galí et al., 2007). A number of reports also showed that anammox population growth is largely depended on ammonium concentrations (Kawagoshi et al., 2010; Hao et al., 2002; Jetten et al., 2005). In this study, one of the most important investigations is to operate the anammox process without pH control and examining the efficiency of the reactor. The study is also attempted for understanding the efficiency of anammox bacteria to remove nitrogen from ammonia rich synthetic wastewater using two different ammonium and nitrite concentrations and hydraulic retention time (HRT).

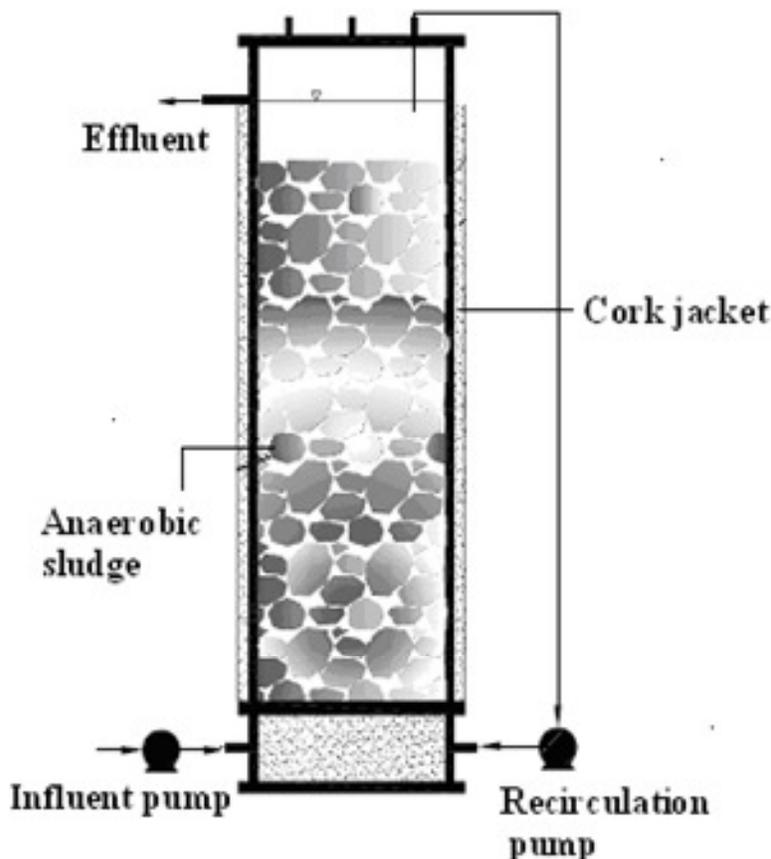
## MATERIALS AND METHODS

### Synthetic wastewater

Synthetic wastewater contained mainly nitrite and ammonium to support anammox activity. It was prepared by dissolving an appropriate amount of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and NaNO<sub>2</sub> reagents to make the desired influent concentrations of nitrogen in NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup>. Other reagents were added to the synthetic wastewater as nutrient for the anammox bacteria. A detailed composition of the synthetic wastewater is illustrated in Table 1. Sodium bicarbonate was added to the feed as an inorganic carbon source for cell growth. The dissolved oxygen concentration of the feed solution was reduced to 0.1 mg/L or lower after deoxygenation by stripping with nitrogen gas in the feed tank. Oxygen intrusion via the influent was not completely prevented but the oxidation-reduction potential (ORP) was regulated to 215 ± 55 mV (Ag/AgCl reference) as a result of approximately 15 min of purging with N<sub>2</sub>. Acetic acid was added to adjust COD/NO<sub>2</sub><sup>-</sup>-N ratio 1 (Strous et al., 1998).

### Anammox reactor

A rectangular column type up flow reactor was inoculated with 4g MLSS/L of the enriched anammox sludge. The enriched sludge was cultivated at 36°C in a 22 L up-flow fixed-bed reactor filled with Biofix as the support material used for the anammox treatment in this study. The biofix biomass carrier was made of acrylic resin with a specific surface area discussed by Qiao et al., (2009). The biomass carrier was used to enhance the anammox sludge attachment performance through its remarkable biomass retention property. The schematic diagram of the fixed-bed anammox reactor for nitrogen removal from synthetic wastewater is shown in Figure 1. The volume of the reactor was 18.5 L and it was constructed with PVC plastic and equipped with sampling pots that allowed the extraction of gas and liquid samples. All tubing and connectors



**Figure 1.** Shows schematic diagram of upflow fixed-bed anammox reactor.

were of butyl rubber and PVC to limit oxygen diffusion into the system. Peristaltic pumps were used to control recirculation rate and influent feed rate to the reactor. A recycle was applied to dilute the influent, because high nitrite concentrations could be toxic to anammox bacteria. The anammox reactor was maintaining the anaerobic conditions during the operation. The reactor and feed vessels were covered to protect them from light and algal growth. The synthetic wastewater was fed through the bottom of the reactor from the feed tank. The reactor was operated during the summer in Japan and the temperature was between 28 to 32 °C, hence no heating element was installed inside the reactor or in the feed tank. The fixed-bed anammox (FBA) reactor was fed continuously with synthetic wastewater (as influent) from the feed tank containing constant concentration of ammonia (200 mg/L) and varying various nitrite nitrogen concentrations (100 to 200 mg/L) during the operation. Higher nitrite concentration was used after 40 days operation. The entire operation of the FBA reactor was carried out with no pH control. The hydraulic retention time (HRT) was maintained at 24 h for the first 40 days and then it was increased to 48 h for the remaining period of the operation. At this stage nitrite concentration was also increased from 100 mg/L to 200 mg/L to evaluate the reactor performance at higher nitrite concentration. The influent and effluent samples were collected three times a week in sampling pots and the total number samples were 150. Samples were filtered through a glass fiber filter (GF/C, 0.45 µm). pH and alkalinity of the collected samples were determined regularly. The ammonium, nitrite and nitrates concentrations were measured calorimetrically by Spectrophotometer (Hitachi U-1900) in accordance with the standard methods for the examination of

water and wastewater (Apha et al., 1998).

## RESULTS AND DISCUSSION

The influent (synthetic wastewater) pH values were observed to be neutral to slightly alkaline (7.2 ~ 8.0), reflecting the presence of ammonium and minerals that suited for anammox bacteria activity.

Figure 2 shows the pH profiles for both the influent and effluent. In the beginning, effluent pH was around 7.6 and it was gradually increased for the first 28 days and then almost the same for the remaining period at HRT of 1 day and similar observation was found at HRT of 2 days, where pH increased in the beginning and finally decreased with time. Hence the difference between the effluent and influent pH was narrow down with time. The highest pH for the effluent was reached at 9.5 in 52 days. In the beginning of the operation, the increase of pH in the effluent is essentially explained by the production of alkalinity due to denitrification reaction. The effluent pH decreased after ammonium consumption was detected in the first anammox process study reported by Mulder et al. (1995). Initially, the denitrification process in the FBA reactor could increase the pH of the effluent, where

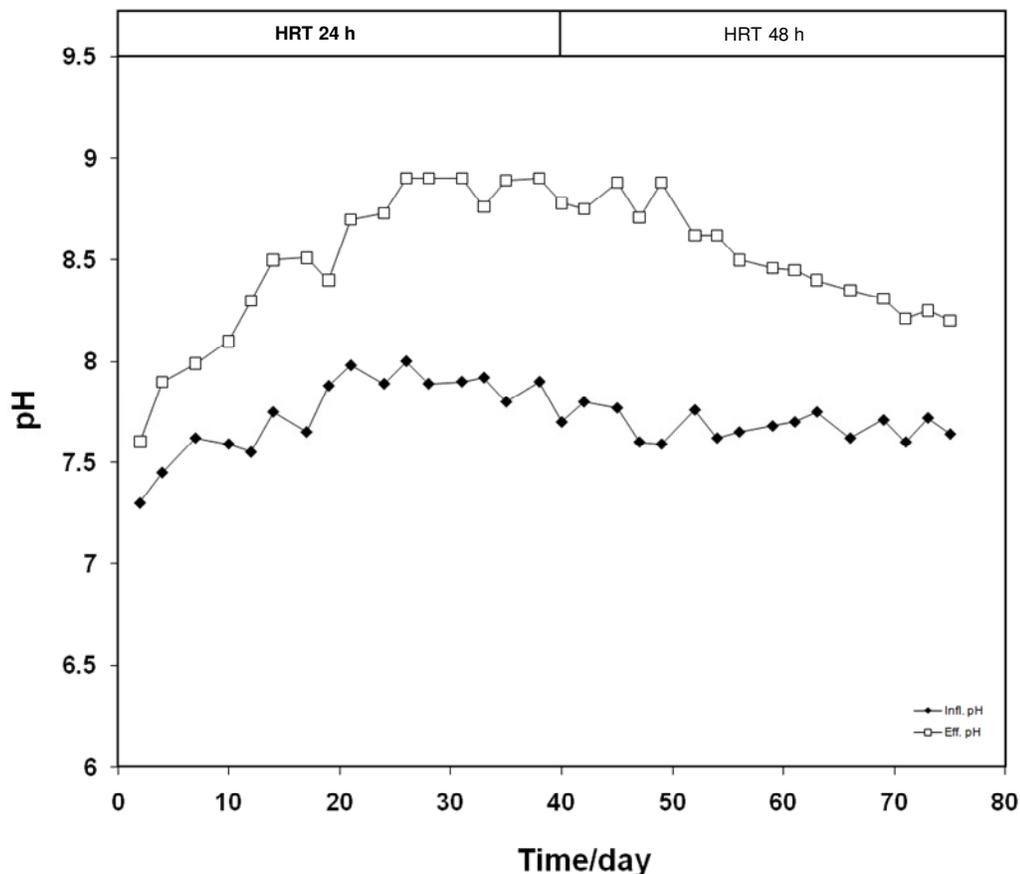


Figure 2. pH profile of the influent and effluent of upflow fixed-bed anammox reactor.

nitrogen oxides were reduced to nitrogen gas. Initially pH of the effluent was slightly increased with time and then showed almost the same trend during the remaining operation period. Effluent pH values were always found to be higher by 0.2 -1.0 than influent pH throughout the operation period and even the effluent pH was higher by 0.4 -0.6 units than the influent pH during the latter period of the experiment.

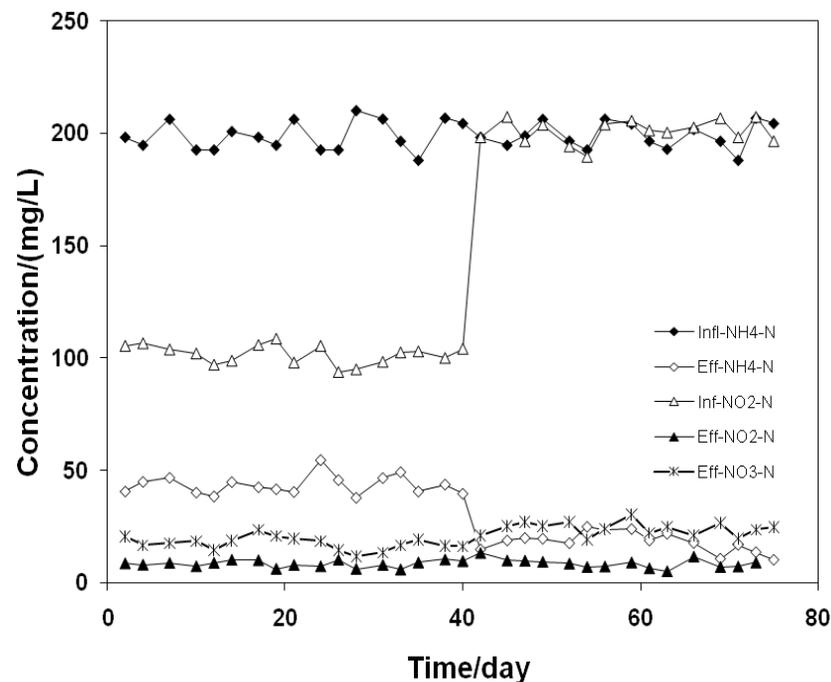
The effluent pH decreased after ammonium consumption was detected in the first anammox process study as reported (Mulder et al., 1995). A similar trend of pH was also observed in this study. The pH of the effluent was decreased after 49 days of the operation period as the ammonium concentration was decreased. This tendency of pH was still observed at that time, even when the reactor was operated under the same feeding conditions (influent nitrite concentration, HRT and with adding of acetic acid). During whole experiment, it is evident that conventional denitrification (denitritation) and anammox reactions were simultaneously occurred in the reactor as the subsequent anaerobic ammonia was oxidized to nitrogen gas by anammox bacteria with nitrate as the electron acceptor under anaerobic condition.

Figure 3 shows the ammonium and nitrite nitrogen

concentrations both in the influent and effluent and nitrate nitrogen concentrations only in the effluent. Initially, maximum ammonium removal rate was 2.9 kg-N/m<sup>3</sup>/day. The effluent ammonium concentration was also showed lower than that of the influent concentration. Ammonia and nitrite removal rates were highly dependent on HRT. With increasing HRT, removal rate for both the ammonium and nitrite nitrogen were decreased, but the removal percentages (%) in all above cases were increased with HRT (Table 2). Nitrate production rate was also decrease with increasing HRT. The figure illustrates that effluent ammonium and nitrate-nitrogen concentrations were always lower than those of influent concentrations.

The summarized results were shown in Table 2. The average ammonium removal of 78 and 90.2% were achieved at HRT of 1 and 2 day, respectively with a maximum of 95%. The average nitrate removal of 91.88 and 95.5% were achieved at HRT of 1 and 2 day, respectively with a maximum of 97.5%.

The total nitrogen (T-N) removal efficiency ranged from 76.86 to 86.82% at different load and HRT conditions. The average ammonium and nitrite removal rate at HRT for one day were about 2.78 and 1.67 kg/m<sup>3</sup>/day with



**Figure 3.** Variation of nitrogen concentrations in the influent and effluent of upflow fixed-bed anammox reactor.

**Table 2.** A summarized analysis results of average nitrogen removal from ammonium, nitrite and nitrate at different hydraulic retention time (HRT) with varying N-load.

HRT (day)	Total nitrogen (TN) loading (kg/m <sup>3</sup> /d)	Total nitrogen (TN) removal (kg/m <sup>3</sup> /d)	Removal rate of NH <sub>4</sub> <sup>+</sup> -N (kg/m <sup>3</sup> /d)	Removal rate of NO <sub>2</sub> <sup>-</sup> -N (kg/m <sup>3</sup> /d)	Removal rate of NO <sub>3</sub> <sup>-</sup> -N (kg/m <sup>3</sup> /d)	T-N removal rate (kg/m <sup>3</sup> /d)	Removal (%) of NH <sub>4</sub> <sup>+</sup> -N	Removal (%) of NO <sub>2</sub> <sup>-</sup> -N	Removal (%) of total nitrogen (TN)
1	5.4	4.15	2.78	1.67	0.314	4.15	78.15	91.83	76.97
2	3.7	3.23	1.67	1.78	0.222	3.23	90.84	95.84	87.35

ammonia and nitrite-nitrogen loading of 3.57 and 1.82 kg-N/m<sup>3</sup>/day (not shown in the table),

respectively and for two days these values were 1.67 and 1.78 kg/m<sup>3</sup>/day with ammonia and

nitrite-nitrogen loading of 1.84 and 1.86 kg-N/m<sup>3</sup>/day, respectively. The total nitrogen removal

rate at HRT for 1 and 2 days were 4.15 and 3.23 kg/m<sup>3</sup>/day with nitrogen loading of 5.4 and 3.7 kg-N/m<sup>3</sup>/day (not shown in the table), respectively. The most interesting observation during this study was that pH did not cause any influence on the anammox activity of removing nitrogen from the water and the reactor was operated even at high ammonium and nitrite concentration. The overall performance for the nitrogen removal was satisfactory in comparison to other processes with removal efficiency of 0.72, 1.03 and 0.66 kg-N/m<sup>3</sup>/day according to Yamamoto et al. (2008), Obaja et al. (2003) and Ahn et al. (2004). Hence the fixed-bed anammox reactor could remove nitrogen efficiently even in higher pH (9 or above). Thus the results of this study illustrate that the fixed-bed anammox (FBA) reactor was an efficient tool for biological nitrogen removal, capable of achieving effluents with very low nitrogen concentrations from highly concentrated wastewaters.

## Conclusions

The results of this study demonstrated that stable and high treatment performance was attained in a fixed-bed anammox reactor for nitrogen removal using a synthetic wastewater without pH control. The average and the maximum total nitrogen removal rates were 3.71 and 4.6 kg-N/m<sup>3</sup>/day in 75 days of continuous operation. The average ammonium and nitrate nitrogen removal rate were 2.26 and 1.72 kg-N/m<sup>3</sup>/day, respectively. The results of this study show that effluent ammonium and nitrate concentrations were always lower than those of influent concentrations. The low effluent nitrate concentrations show that there was no accumulation of nitrates and suggesting that the fixed-bed anammox reactor can be operated without pH control feed with high ammonium and nitrite concentrations. The obtained results during this study may suggest that the fixed-bed anammox reactor could be operated in high ammonia and nitrite concentrations without pH control and could be applied to the treatment of livestock wastewater.

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