ANAMMOX and partial denitritation in anaerobic nitrogen removal from piggery waste

Y.-H. Ahn *, I.-S. Hwang ** and K.-S. Min ***

*School of Civil, Urban and Environmental Engineering, Yeungnam University, Kyungsan, 712-749, Korea (E-mail: yhahn@yu.ac.kr)
**Livestock Wastewater Treatment Plant, Sangju, Kyungpook, Korea (E-mail: enviluck@hanmail.net)
***Department of Environmental Engineering, Kyungpook National University, Daegu, 702-701, Korea (E-mail: ksmin@knu.ac.kr)

Abstract
The anaerobic ammonium removal from a piggery waste with high strength (56 g COD/L and 5 g T-N/L) was investigated using a lab-scale upflow anaerobic sludge bed reactor at a mesophilic condition. Based on the nitrogen and carbon balance in the process, the contribution of autotrophic and heterotrophic organisms was also evaluated in terms of the influent NO₂-N/NH₄-N ratio (1:0.8 and 1:1.2 for Phase 1 and Phase 2, respectively). The result of this research demonstrates that the anaerobic ammonium removal from the piggery waste, using the UASB reactor, can be performed successfully. Furthermore, it appears that by using granular sludge as the seed biomass, the ANAMMOX reaction can start more quickly. Average nitrogen conversion was 0.59 kg T-N/m³ reactor-day (0.06 kg T-N/kg VSS/day) and 0.66 kg T-N/m³ reactor-day (0.08 kg T-N/kg VSS/day) for Phase 1 and Phase 2. The NO₂-N/NH₄-N removal ratio by the ANAMMOX was 1.48 and 1.79 for Phase 1 and Phase 2. The higher nitrite contents (about 50%) in the substrate resulted in higher nitrite nitrogen removal by the partial denitritation, as well as the ANAMMOX reaction, implying higher potential of partial denitritation. However, the result reveals that the ANAMMOX reaction was influenced less by the degree of partial denitritation, and the ANAMMOX bacteria did not compete with denitritation bacteria. The colour of the biomass at the bottom of the reactor changed from dark gray to dark red, which was accompanied by an increase in cytochrome content. At the end of the experiment, red-coloured granular sludge with diameter of 1–2 mm at the lower part of the reactor was also observed.

Keywords Anaerobic nitrogen removal; ANAMMOX; denitritation; granular sludge; piggery waste

Introduction
In recent years, there have been several reports stating that nitrogen removal can be observed in an anaerobic process, which is called ANAMMOX (ANAerobic AMMonium OXidation). This “new” biochemical process may represent a sustainable method for removing nitrogen from wastewater containing a large amount of nitrogen. Compared with the conventional nitrogen removal process, the ANAMMOX process operated with a partial nitrification process such as the SHARON (Single reactor system for High Ammonium Removal Over Nitrite) process, or a combined autotrophic process such as the CANON (Completely Autotrophic Nitrogen removal Over Nitrite) has many advantages: 1) 40% less oxygen (= energy) is necessary; 2) organic carbon source is not required; 3) sludge production is negligible (van Dongen et al., 2001; Sliekers et al., 2003). The ANAMMOX reaction was observed in a denitrifying-fluidized bed reactor treating the effluent from a methanogenic reactor (Mulder et al., 1995) but Broda (1977) had postulated two thermodynamically possible stoichiometries for ammonium oxidation in the absence of oxygen, in which the molar ratios of ammonium to nitrite were 1:1 and 1:1.67. The basic examinations to describe the ANAMMOX process have been run with a synthetic enriched culture (van de Graaf et al., 1996; Strous et al., 1997, 1999; van Dongen et al., 2001; Sliekers et al., 2003) and with digester sludge liquor (Strous et al., 1997; van...
Dongen, et al., 2001; Fux, et al., 2002). Jetten et al. (2001) reported the physiological information of the ANAMMOX biomass, stating that at least two genera in Planctomycetales can catalyze the ANAMMOX process. The researchers used a fluidized (or fixed) bed, SBR or gas-lift reactor. Because the ANAMMOX bacteria are characterized by a low maximum growth rate, a reactor with high biomass retention such as immobilization process (biofilm or UASB) is required.

The ANAMMOX is a biological conversion process of ammonium and nitrite to dinitrogen gas under anaerobic conditions without the need to add an external carbon source. From several basic studies, the stoichiometry of the ANAMMOX reaction was represented by the following reactions (Strous et al., 1998).

\[
\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{HCO}_3^- + 0.13\text{H}^+ \\
\rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- + 0.066\text{CH}_2\text{O}_{0.5}\text{N}_{0.15} + 2.03\text{H}_2\text{O}
\] (1)

Because the ANAMMOX process requires nitrite as an electron donor, a pre-partial nitritation process should be initiated, but this situation could result in competition between autotrophic ANAMMOX bacteria and heterotrophic denitrification bacteria in the process of biological nitrogen removal while treating carbonaceous waste. Actually, this will be a common problem in the ANAMMOX system because most of the wastes contain carbon compounds as well as nitrogen. Toh and Ashbolt (2002) reported that ANAMMOX activity was greatly affected by the addition of phenol but recovered by using chloramphenicol, which inhibited denitrification. But in an exploration of ANAMMOX using poultry manure, Dong and Tollner (2003) suggested that the ANAMMOX was less competitive with denitrification.

In this study, the anaerobic ammonium removal of the really strong nitrogenous waste, such as piggery waste, was investigated using a lab-scale UASB reactor at a mesophilic condition. In the anaerobic nitrogen removal, the contribution of autotrophic and heterotrophic organisms, based on nitrogen and carbon balance, was also evaluated in terms of the influent NO$_2$-N/NH$_4$-N ratio.

**Materials and methods**

**Laboratory reactor setup**

A lab-scale 1 L upflow anaerobic sludge bed (UASB) reactor with a 0.5 L settling tank was operated at mesophilic (35 °C) condition. The reactor was operated using a semi-continuous feeding system. The substrate was fed four times daily in a fill-and-draw mode. During the overall operating period, the reactor was constantly operated with 5 days of hydraulic retention time (HRT). The sludge settled in the settler was recycled into the bottom of the UASB reactor, resulting in a recycle ratio of about 0.5Q. For steady-state operation, excess biomass (about 0.1 L/d) was wasted daily from the bottom of the settling tank. 0.7 L of granular sludge (18.6 g VS/L and 65% VS/TS) from a full-scale UASB reactor, treating brewery wastewater, was inoculated as seed biomass.

**Substrate**

A slurry-type piggery waste with high strength (56 g COD/L and 5 g T-N/L) was used as the substrate for this study. The waste has a high ammonium fraction (about 90% of NH$_4$-N/T-N). Ammonium nitrogen loading of the UASB reactor was 0.43 kg NH$_4$-N/m$^3$-day for both phases. To induce the ANAMMOX reaction, nitrite stock solution (NaNO$_2$) with the same flowrate was fed to the separated feed line with the substrate. Nitrite nitrogen loading was 0.36 kg NO$_2$-N/m$^3$-day for Phase 1 and 0.5 kg NO$_2$-N/m$^3$-day for Phase 2, corresponding to a total nitrogen loading and influent NO$_2$-N/NH$_4$-N ratio of 0.83 kg T-N/m$^3$-day and 0.8:1 for Phase 1, and 1.02 kg T-N/m$^3$-day and 1.2:1 for Phase 2,
respectively. The pH of the influent was 8.2–8.5 and was not controlled throughout the reactor operation. The characteristics of the piggery waste and the influent mixture are given in Table 1.

Analysis

Analytical procedures were conducted according to Standard Methods (1998). In the operation of the reactor, the pH, alkalinity (total-TA, bicarbonate-BA), volatile acids (Buchauer, 1998) and gas production (Wet-test gas meter, Sinagawa Model W-NK-0.5A, Japan) were monitored daily. Volatile fatty acids (VFAs) were measured using a HPLC (Shimadzu Model LC-10AD, Japan) equipped with a UV detector and an organic acid analysis column (Aminex HPX-87H, Bio-Rad, Inc., USA). Samples were prepared by centrifuging (Hanil Instruments) at 4000 rpm for 15 min and then filtering through 0.45-µm of filter paper (Micron Separations, Inc.). A gas chromatograph (Tremetrics Model 9000, USA) with a TCD detector and a Hayesap Q (80/100) column was utilized to measure gas composition (N₂O, N₂, CO₂, NH₃ and CH₄). Temperature for the column was kept at 35 °C, 120 °C for the injector, and 120 °C for the detector. The helium carrier gas had a flow rate of 30 mL/min. Data integration was accomplished using a Varian 4270 Integrator.

Results and discussion

Nitrogen removal

During the overall operating period, the nitrogen concentration and removal in the UASB reactor are shown in Figure 1. After day 26 from the reactor startup, the reactor reached a stable condition. At stable operation for Phase 1, average T-N and NH₄⁻-N concentration in the effluent were respectively about 740 mg/L and 570 mg/L, which means that the average T-N and NH₄⁻-N removal were 82% and 74%, based on the effluent concentration, respectively. The wasting sludge contained 1.7 g T-N/L and 0.56 g NH₄⁻-N/L; this means that actual T-N and ammonium removal were 71% and 74% in the reactor. At stable operating period for Phase 2 (after 80 days), average T-N and NH₄⁻-N concentration in the effluent were respectively about 1,000 mg/L and 830 mg/L, this means 80% and 62% of T-N and

Table 1  Characteristics of piggery waste

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Phase 1 Raw waste</th>
<th>Influent mixture*</th>
<th>Phase 2 Raw waste</th>
<th>Influent mixture*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.4 ± 0.1</td>
<td>8.4 ± 0.1</td>
<td>8.6 ± 0.1</td>
<td>8.6 ± 0.1</td>
</tr>
<tr>
<td>TCOD</td>
<td>56,200 ± 1,370</td>
<td>30,100 ± 690</td>
<td>55,500 ± 3,520</td>
<td>30,600 ± 1,760</td>
</tr>
<tr>
<td>SCOD</td>
<td>47,000 ± 1,760</td>
<td>25,600 ± 880</td>
<td>30,500 ± 3,960</td>
<td>18,100 ± 1,980</td>
</tr>
<tr>
<td>T-N</td>
<td>4,760 ± 400</td>
<td>4,150 ± 200</td>
<td>5,230 ± 440</td>
<td>5,110 ± 220</td>
</tr>
<tr>
<td>TKN</td>
<td>4,710 ± 400</td>
<td>2,350 ± 200</td>
<td>5,180 ± 440</td>
<td>2,590 ± 220</td>
</tr>
<tr>
<td>NH₄⁻-N</td>
<td>4,300 ± 330</td>
<td>2,150 ± 170</td>
<td>4,320 ± 170</td>
<td>2,160 ± 80</td>
</tr>
<tr>
<td>NO₂⁻-N</td>
<td>0</td>
<td>1,800</td>
<td>0</td>
<td>2,500</td>
</tr>
<tr>
<td>NO₃⁻-N</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>420</td>
<td>210</td>
<td>420</td>
<td>210</td>
</tr>
<tr>
<td>T-P</td>
<td>249 ± 5</td>
<td>249 ± 5</td>
<td>249 ± 5</td>
<td>249 ± 5</td>
</tr>
<tr>
<td>S-P</td>
<td>53 ± 3</td>
<td>53 ± 3</td>
<td>53 ± 3</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>TS</td>
<td>14,300 ± 420</td>
<td>14,300 ± 420</td>
<td>14,300 ± 420</td>
<td>14,300 ± 420</td>
</tr>
<tr>
<td>VS</td>
<td>10,800 ± 630</td>
<td>10,800 ± 630</td>
<td>10,800 ± 630</td>
<td>10,800 ± 630</td>
</tr>
<tr>
<td>VSS</td>
<td>8,890 ± 190</td>
<td>8,890 ± 190</td>
<td>8,890 ± 190</td>
<td>8,890 ± 190</td>
</tr>
<tr>
<td>TA</td>
<td>8,150 ± 140</td>
<td>8,150 ± 140</td>
<td>8,150 ± 140</td>
<td>8,150 ± 140</td>
</tr>
<tr>
<td>BA</td>
<td>5,150 ± 770</td>
<td>5,150 ± 770</td>
<td>5,150 ± 770</td>
<td>5,150 ± 770</td>
</tr>
<tr>
<td>VA (as HAc)</td>
<td>229 ± 44</td>
<td>229 ± 44</td>
<td>229 ± 44</td>
<td>229 ± 44</td>
</tr>
</tbody>
</table>

* after adding nitrite stock solution (3.6 g NO₂⁻-N/L for Phase 1, 5 g NO₂⁻-N/L for Phase 2)
Unit: mg/L, except pH
NH₄-N removal based on the effluent concentration, respectively. Because the wasting sludge contained 2.63 g T-N/L and 0.45 g NH₄-N/L, the actual T-N and ammonium removal were 65% and 70%. From day 26 until the end of the operation, 100% of nitrite was removed and the total NO₂-N/NO₃-N removal ratio was 1.13 for Phase 1 and 1.65 for Phase 2. A little by-product of nitrate nitrogen was produced (i.e. < 5 mg/L for Phase 1 and <15 mg/L for Phase 2) during the overall operating period.

As shown in Figure 2, average nitrogen conversion was 0.59 kg T-N/m³ reactor-day (0.32 kg NH₄-N/m³ reactor-day) for Phase 1, and 0.66 kg T-N/m³ reactor-day (0.30 kg NH₄-N/m³ reactor-day) for Phase 2. Also, the average specific nitrogen conversion rate was 0.06 kg T-N/kg VSS/day for Phase 1 and 0.08 kg T-N/kg VSS/day for Phase 2. In spite of the higher nitrogen loading in Phase 2, the specific nitrogen conversion rate was 33% higher than that in Phase 1. Gas production in the reactor was 0.45 L/d and 0.57 L/d (STP) for Phase 1 and Phase 2, respectively. The gas produced from the reactor, respectively, contained 95% of dinitrogen gas and 5% of CO₂ for Phase 1 and 100% of dinitrogen gas for Phase 2, which means that the large amount of ammonium removal in the reactor was not due to ammonia stripping at the operating condition of a high pH. Van Dongen et al. (2001) reported that 100–180 days of the operating time was required to reach approximately 1 kg N/m³ reactor/day of loading rate although they used the enhanced ANAMMOX biomass as seed biomass. The result of this research demonstrates that, in the UASB reactor, the anaerobic ammonium removal from the strong nitrogenous waste such as piggery waste can be performed successfully. Furthermore, it appears that by using granular sludge as the seed biomass, the ANAMMOX reaction can start more quickly, implying that a UASB reactor has many advantages; such as an easy start-up and operation.

**pH and alkalinity**

Figure 3(a) and (b) represent the pH and BA profiles in both influent and effluent. According to the possible ANAMMOX stoichiometry (Eq.1), the pH in an ANAMMOX reactor may be increased because bicarbonate (HCO₃⁻) serves as the carbon source. It was reported that the physiological pH range of the ANAMMOX bacteria was 6.7–8.3 (Strous et al., 1999). Egli et al. (2001) observed the activity even at pH 8.5–9. In this research, the pH of the effluent was constantly maintained at 9.3 for Phase 1 and 9.5 for Phase 2 while it was kept at 8.2–8.5 for the influent for both phases, implying that the anaerobic ammonium removal in the reactor was not inhibited in spite of the high pH condition.
According to (Eq.1), HCO$_3^-$ consumption of 0.066 mol could be expected. The average BA in the effluent was 6,060 mg CaCO$_3$/L (6,440 mg CaCO$_3$/L of TA) for Phase 1 and 16,500 mg CaCO$_3$/L (16,800 mg CaCO$_3$/L of TA) for Phase 2, which were 2.3 and 2.7 times higher than those of the influent, respectively. The results demonstrate that a large amount of alkalinity (particularly BA) was produced due to organic reduction in the anaerobic treatment of the piggery waste, implying no alkalinity deficiency problem in the ANAMMOX treatment for the piggery waste.

COD removal

As shown in Figure 3(c), COD removal in the reactor was observed and slightly increased with the ammonium removal. During the stable period, the average total and soluble COD removal based on effluent quality was, respectively, about 40% and 41% (5.2 kg COD/kg NH$_4$-N removal) for Phase 1 and about 47% and 55% (4.7 kg COD/kg NH$_4$-N removal) for Phase 2, indicating that most of the COD removal was from a soluble fraction. The average COD loading rate during the overall operation period was about 6 kg COD/m$^3$.day.

In the ANAMMOX reaction, which is an autotrophic process, organic removal cannot be expected and in this research about 6.8% of COD removal for Phase 1 (9.3% for Phase 2) could be expected, which is the equivalent of nitrite reduction (1.14 kg COD/kg NO$_2$-N), because nitrite is inherently an unstable COD inducing substance. Therefore, the remaining COD removal could be explained by heterotrophic microbial activation in the reactor. Three types of heterotrophic organisms such as nitrite/nitrate denitrifying bacteria, acido-gens and sulfate reducing bacteria could be considered due to the substrate and process characteristics. However, contribution of methanogens in the reactor could not be expected because the methane gas was not produced.

Carbon can be partially consumed by nitrite-denitrification as well as sulfate reduction.
The production of by-product nitrate nitrogen in the ANAMMOX process can result in the denitrification. The organic acid analysis on the effluent revealed that the main organic acids were butyric (45% of COD) and acetic acid (6.3% of COD), while a little acetic acid is contained only in the soluble organics of the substrate. This result demonstrates that acid fermentation (particularly butyric acid) would occur in the reactor. The possible butyrate fermentation could be expressed as (Eq. 2) which is analogous with ethanol fermentation (Rittmann and McCarty, 2001). From this equation, COD consumption will be 11.4 g/g NH₄-N removal due to the biomass synthesis in acid fermentation. Consequently, it could be conjectured that acid fermentation was followed by denitrification and sulfate reduction (even quite small) using the butyric acid as a carbon source for the following.

\[
\begin{align*}
C_{6}H_{12}O_{6} + 0.2 \text{NH}_{4}^{+} + 0.2 \text{HCO}_{3}^{-} & \rightarrow 0.2 \text{C}_{2}\text{H}_{3}\text{O}_{2}\text{N} + \text{CH}_{3}\text{CH}_{2}\text{CH}_{2}\text{COOH} + 1.2 \text{CO}_{2} + 1.8 \text{H}_{2}\text{O} \\
\text{NO}_{2}^{-} + 0.19 \text{CH}_{3}\text{CH}_{2}\text{CH}_{2}\text{COOH} + \text{H}_{2}\text{CO}_{3} & \rightarrow 0.037 \text{C}_{2}\text{H}_{3}\text{O}_{2}\text{N} + \text{HCO}_{3}^{-} + 1.14 \text{H}_{2}\text{O} + 0.585 \text{CO}_{2} + 0.481 \text{N}_{2} \\
\text{NO}_{3}^{-} + 0.29 \text{CH}_{3}\text{CH}_{2}\text{CH}_{2}\text{COOH} + \text{H}_{2}\text{CO}_{3} & \rightarrow 0.034 \text{C}_{2}\text{H}_{3}\text{O}_{2}\text{N} + \text{HCO}_{3}^{-} + 1.54 \text{H}_{2}\text{O} + 0.986 \text{CO}_{2} + 0.483 \text{N}_{2} \\
\text{SO}_{4}^{2-} + 0.4 \text{CH}_{3}\text{CH}_{2}\text{CH}_{2}\text{COOH} + 1.5 \text{H}^{+} & \rightarrow 1.6 \text{CO}_{2} + 1.6 \text{H}_{2}\text{O} + 0.5 \text{H}_{2}\text{S} + 0.5 \text{HS}^{-} 
\end{align*}
\]

Material balance

Based on the possible stoichiometries (Eq. 1–5) and the experimental results for both phases, mass balance of the nitrogen excepted organic nitrogen fraction is summarized in Figure 4. In the constant ammonium nitrogen loading (0.43 g/L-day for both phases), the ammonium removal by the ANAMMOX was higher for Phase 2 (0.219 g/d, 51%) than that of Phase 1 (0.202 g/d, 46%). The nitrite removal for the ANAMMOX reaction as well as the partial denitritation also increased at Phase 2, while the acid fermentation decreased at Phase 2. This result demonstrates that the ANAMMOX activity at Phase 2 was higher than that for Phase 1. Although it is not clear whether the higher nitrite uptake for the ANAMMOX reaction was due to the higher ANAMMOX activity or the higher nitrite contents in the substrate, it appears that the higher nitrite content may enhance the potential...
of partial denitrification. By the ANAMMOX and partial denitrification in the reactor, total nitrogen conversion to dinitrogen gas was 0.56 g/d (67% of T-N) for Phase 1 and 0.708 g/d (69% of T-N) for Phase 2, respectively. Also, total biomass production in Phase 1 (16.8%) was a little higher than that (10.5%) for Phase 2, due to higher fermentation activity at Phase 1.

Figure 5 represents the soluble COD balance for both phases. With the organic loading of about 6 kg COD/m³ reactor/day (0.65 kg COD/kg VSS/day), the removal of SCOD, which the COD equivalent of nitrite excepted, was 35.4% (1.66 g/day) for Phase 1 and 46.8% (1.43 g/day) for Phase 2, respectively. COD removal by sulfate reduction (Eq. 5) was about 0.016 g/d for both phases. Carbon consumptions for partial denitrification (Eq. 3) and nitrate denitrification (Eq. 4) were 2.9% and 4% of COD removal for Phase 1 and 7.9% and 6.6% of COD removal for Phase 2, respectively.

As shown in Table 2, which summarized the comparative results for both phases, a large fraction of COD removal was mainly due to the biomass synthesis in the acid fermentation. The ammonium nitrogen removal by the ANAMMOX reaction for Phase 1 and Phase 2 were 47% and 51%, respectively, and 27% and 19% by fermentation. The higher nitrite
content (about 50%) resulted in a higher nitrite nitrogen removal by the partial denitrification (Phase 2), which led to higher bicarbonate alkalinity production as shown in Table 2. Contrary to that of Phase 2, the production of bicarbonate alkalinity for Phase 1 was lower than the calculated value. This may be due to CO₂ gas solubility and HCO₃⁻/H₂CO₃ fraction in the reactor (CO₂ fraction was 5% for Phase 1 but 0% for Phase 2). Also, in spite of the higher nitrite denitrification, the NO₂⁻/NH₄⁺ removal ratio by the ANAMMOX was higher for Phase 2, implying a higher ANAMMOX activity. This result demonstrates that the ANAMMOX reaction was less influenced by the degree of partial denitrification, and the ANAMMOX bacteria did not compete with denitrification bacteria. It was observed that the colour of the biomass at the bottom of the reactor changed from dark gray to dark red, which was accompanied by an increase in cytochrome content (van de Graaf et al., 1996). At the end of the experiment, red-coloured granular sludge with diameter of 1–2mm was observed at the lower part of the reactor, meaning that granulation of the ANAMMOX sludge was occurring. By the ANAMMOX reaction, the NO₂⁻/NH₄⁺ removal ratio was 1.48 and 1.79 for Phase 1 and Phase 2, while the total NO₂⁻/NH₄⁺ removal ratio was 1.13 and 1.65 for Phase 1 and Phase 2, respectively. As shown in the literature (Table 3), the NO₂⁻/NH₄⁺ removal in the various ANAMMOX reactors is in the range 0.5–4 depending on the operating condition of the process. Strous et al. (1999) demonstrated that the ratio increased at higher nitrite concentration, and the ANAMMOX reaction was inhibited under 0.2 g/L of NO₂⁻ concentration. In this research, the nitrogen removal was performed successfully and the nitrite inhibition was not observed in spite of the high NO₂⁻ concentration in the influent substrate. This might be due to the feeding type (semi-continuous) of the substrate. Specific nitrogen removal was 0.06 g N total/g VSS-d for Phase 1 and 0.08 g N total/g VSS-d for Phase 2, implying the gradual accumulation of the ANAMMOX biomass according to the reactor operation.

Conclusions
- The result of this research demonstrates that, in the UASB reactor, anaerobic ammonium removal from strong nitrogenous waste, such as piggery waste, can be performed successfully. Furthermore, it appears that by using granular sludge as the seed biomass, the ANAMMOX reaction can start more quickly, implying that a UASB reactor has many advantages such as an easy start-up and operation.
- In the constant ammonium nitrogen loading (0.43 g/L-day), average nitrogen conversion was 0.59 kg T-N/m³ reactor-day and 0.66 kg T-N/m³ reactor-day for Phase 1 (1:0.8 of influent NO₂⁻/NH₄⁺) and Phase 2 (1:1.2 of influent NO₂⁻/NH₄⁺). From nitrogen mass balance, the NO₂⁻/NH₄⁺ removal ratio by the ANAMMOX reaction

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reactor</th>
<th>NH₄⁺-N mg/L</th>
<th>N conversion g N/g L-d</th>
<th>NO₂⁻-N/NH₄⁺ (g/g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic</td>
<td>FBR</td>
<td>70</td>
<td>4.8</td>
<td>1.31 ± 0.06</td>
<td>van de Graaf et al. (1996)</td>
</tr>
<tr>
<td>FBR</td>
<td>70–840</td>
<td>1.8</td>
<td>1.4–1.5</td>
<td>1.0–1.2</td>
<td>Strous et al. (1997)</td>
</tr>
<tr>
<td>Fixed bed</td>
<td>70–840</td>
<td>1.1</td>
<td>1.3–4</td>
<td>1.3–4</td>
<td>Strous et al. (1999)</td>
</tr>
<tr>
<td>SBR</td>
<td>–</td>
<td>0.75–0.96</td>
<td>1.03–1.05</td>
<td>1.34</td>
<td>Sliekers et al. (2003)</td>
</tr>
<tr>
<td>Gas-lift</td>
<td>1,360</td>
<td>8.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digester effluent</td>
<td>FBR</td>
<td>1,100–2,100</td>
<td>1.5</td>
<td>0.06–0.55</td>
<td>Strous et al. (1997)</td>
</tr>
<tr>
<td>SBR</td>
<td>550±10</td>
<td>0.75</td>
<td>1.03</td>
<td>1.3–4</td>
<td>van Dongen et al. (2001)</td>
</tr>
<tr>
<td>SBR</td>
<td>620–657</td>
<td>0.6–2.4</td>
<td>1.29–1.47</td>
<td></td>
<td>Fux et al. (2002)</td>
</tr>
<tr>
<td>Piggery waste</td>
<td>UASB</td>
<td>2,150</td>
<td>0.6–0.7</td>
<td>1.5–1.8</td>
<td>This research</td>
</tr>
</tbody>
</table>

* FBR, fluidized bed reactor; SBR sequencing batch reactor; UASB, upflow anaerobic sludge bed reactor
was 1.48 and 1.79 for Phase 1 and Phase 2. The colour of the biomass at the bottom of the reactor changed from dark gray to dark red, which was accompanied by an increase in cytochrome content. Granulation of the ANAMMOX sludge at the lower part of the reactor was also observed.

- A large amount of alkalinity (particularly BA) was produced due to organic reduction in the anaerobic treatment of the piggery waste, implying no alkalinity deficiency problem in the ANAMMOX treatment for the piggery waste. The unexpected carbon consumption (about 35–47% of influent SCOD) in the process could explain the microbial activation of three types of heterotrophic organisms such as nitrite/nitrate denitrifying bacteria, acidogens and sulfate reducing bacteria.

- The higher nitrite contents (about 50%) in the substrate resulted in higher nitrite nitrogen removal by the partial denitrification as well as the ANAMMOX reaction, implying higher potential of partial denitrification. However, the result reveals that the ANAMMOX reaction was less influenced by the degree of partial denitrification, and the ANAMMOX bacteria did not compete with denitrification bacteria.

References


