Effects of carbon sources, COD/NO$_2$-N ratios and temperature on the nitrogen removal performance of the simultaneous partial nitrification, anammox and denitrification (SNAD) biofilm

Zhaoming Zheng, Yun Li, Jun Li, Yanzhuo Zhang, Wei Bian, Jia Wei, Baihang Zhao and Jingyue Yang

ABSTRACT

The aim of the present work was to evaluate the effects of carbon sources and chemical oxygen demand (COD)/NO$_2$-N ratios on the anammox–denitrification coupling process of the simultaneous partial nitrification, anammox and denitrification (SNAD) biofilm. Also, the anammox activities of the SNAD biofilm were investigated under different temperature. Kaldnes rings taken from the SNAD biofilm reactor were operated in batch tests to determine the nitrogen removal rates. As a result, with the carbon source of sodium acetate, the appropriate COD/NO$_2$-N ratios for the anammox–denitrification process were 1 and 2. With the COD/NO$_2$-N ratios of 1, 2, 3, 4 and 5, the corresponding NO$_2$-N consumption via anammox was 87.1%, 52.2%, 29.3%, 23.7% and 16.3%, respectively. However, with the carbon source of sodium propionate and glucose, the anammox bacteria was found to perform higher nitrite competitive ability than denitrifiers at the COD/NO$_2$-N ratio of 5. Also, the SNAD biofilm could perform anammox activity at 15°C with the nitrogen removal rate of 0.071 kg total inorganic nitrogen per kg volatile suspended solids per day. These results indicated that the SNAD biofilm process might be feasible for the treatment of municipal wastewater at normal temperature.

Key words | anammox, carbon source, COD/NO$_2$-N ratio, SNAD biofilm, temperature

INTRODUCTION

The release of excessive nitrogen into aquatic systems would cause eutrophication problems. Biological nitrification–denitrification is commonly used to remove the nitrogen from wastewater. However, these practices usually lead to the need for a large volume reactor and high operating costs (Bagchi et al. 2012). Recently, the simultaneous partial nitrification, anammox and denitrification (SNAD) process has been developed to remove nitrogen and organic matter from wastewater (Chen et al. 2009; Daverey et al. 2015). In the SNAD process, ammonium is firstly oxidized to nitrite by ammonia-oxidizing bacteria (AOB). Then the anammox bacteria would convert ammonium with nitrite to nitrogen gas and produce small amounts of nitrate. Afterwards, the denitrifiers would convert nitrite or nitrate to nitrogen gas with the electron donor of the carbon source. Although the SNAD process is recognized as an energy saving and cost-effective biological nitrogen removal method, it is mainly used to treat wastewaters with high ammonium concentration (>500 mg N/L) and high temperature (>30°C) (Wang et al. 2010; Zhang et al. 2015). So far, there is little information about the SNAD process for treating domestic wastewater.

Previous studies reported that both chemical oxygen demand (COD) concentrations and COD/N ratios influenced the anammox activity (Chamchoi et al. 2008; Tang et al. 2010). The heterotrophic denitrifiers (yield coefficient of 0.27–0.3) possess a higher growth rate than anammox bacteria (yield coefficient of 0.066) (Strous et al. 1998; Molinuevo et al. 2009). Therefore, the anammox bacteria could not compete with denitrifiers for nitrite under high COD concentration or COD/N ratios. Several studies reported that the anammox and denitrification coupling process could perform efficient nitrogen removal performance with the COD concentration...
below 400 mg/L (COD/NO\textsubscript{2}-N ratios of 2–4) (Chamchoi et al. 2008; Tang et al. 2010). Meanwhile, other studies suggested that the SNAD process was suitable for the treatment of wastewater with the COD concentration below 300 mg/L (COD/N ratio < 1.2) (Chen et al. 2009; Li et al. 2012). Recently, several studies described that, when the SNAD process was operated with intermittent aeration and high dissolved oxygen (DO) condition, it was possible to achieve good nitrogen removal performance with high COD concentration of 600–1,400 mg/L (COD/N ratios of 1–5) (Zekker et al. 2014; Yang et al. 2015). In previous studies, the effect of COD concentrations or COD/N ratios on the SNAD process was mainly evaluated with the existence of oxygen. Under this circumstance, the organic matter was partially degraded by the heterotrophic bacteria, which made it difficult to evaluate the exact effect of COD concentrations or COD/N ratios on the cooperative mechanism of denitrifiers and anammox bacteria.

Generally, the denitrification process largely depends on the COD/N ratios (Henze et al. 1994). If the growth of biomass is not included, this ratio would be 2.86 kg COD/kg NO\textsubscript{3}-N for complete denitrification. If the growth of biomass is considered, this ratio would be about 3.5 to 4.5 kg COD/kg NO\textsubscript{3}-N. Various carbon sources were used to enhance the denitrification process, such as methanol, acetate, ethanol, glucose and volatile fatty acids (VFAs) (Constantin & Fick 1997; Foglar & Briski 2005; Ge et al. 2012; Yang et al. 2012). Naturally, VFAs are produced by the acid-phase anaerobic digestion process. The use of VFAs as external carbon source would save the total operating and maintenance costs of the municipal wastewater treatment facility. In the SNAD process, an external carbon source could also be introduced to improve the nitrogen removal performance. Therefore, it is necessary to investigate the effect of carbon sources on the SNAD process.

Additionally, the optimum temperature of the anammox bacteria has been reported to be 30–37 °C (Strous et al. 1999; Isaka et al. 2008). Lowering the temperature resulted in a large decrease in the anammox activity and growth rate (Isaka et al. 2008). Most anammox processes were operated with biofilm or granule carriers to improve the reactor performance at low temperature (Isaka et al. 2008; Gilbert et al. 2015). The SNAD process was reported to perform anammox activity at temperature of 10–20 °C in the rotating biological contactor (RBC) or biofilm reactor (Cema et al. 2007; Gilbert et al. 2015). In our previous study, the SNAD biofilm reactor showed good performance at 30 °C with the influent of domestic wastewater (Zheng et al. 2016a). However, the municipal wastewater has a lower temperature (10–25 °C). In order to apply the SNAD process to the treatment of municipal wastewater, it is necessary to investigate the effect of temperature on the anammox activity of the SNAD biofilm.

Based on the discussion above, the aim of this study was to develop an efficient SNAD biofilm reactor for treating domestic wastewater. The effects of carbon sources, COD/NO\textsubscript{2}-N ratios and temperature on the anammox activity of the SNAD biofilm were investigated without the existence of oxygen. It is expected that the knowledge obtained in this study will be critical for the application of the SNAD process to the treatment of municipal wastewater.

**MATERIALS AND METHODS**

**SNAD biofilm reactor**

The configuration scheme of the SNAD biofilm reactor is shown in Figure 1(a). The SNAD biofilm reactor was operated in sequencing batch mode with a working volume of 89.5 L. The height and the inner diameter of the reactor were 79 cm and 38 cm, respectively. The reactor contained 77.7 L water and the exchange ratio was fixed at 81%. Kaldnes rings (K3 carriers, AnoxKaldnes, Beijing) were placed in the reactor to the apparent volume of 54 L. The carriers had a cylindrical shape (diameter of 25 mm and grid of 4 mm) (Figure 1(b)). The reactor was operated at 30 °C and without pH control. Oxygen was supplied by an air compressor through an air diffuser. During the non-aerobic period, liquid mixing was provided by a hydraulic agitator (HJ1841, Sensen, China). The reactor was operated and controlled by a programmable logic controller (PCL-812, Advantech, USA).

**Operation of the SNAD biofilm reactor**

The detailed operation condition of the SNAD biofilm reactor was described by Zheng et al. (2016a). The reactor performed stable SNAD performance for more than 60 days. The cycle time of the reactor was 286 min. Each cycle consisted of feeding (5 min), intermittent aeration condition (aeration 20 min/mixing 20 min), aeration (20 min), settling (10 min), decanting (10 min) and idling (1 min). The intermittent aeration condition consisted of six aerobic periods and six mixed periods. During the aerobic period, the aerator worked and the hydraulic agitator stopped. The aeration rate was 500 L/hr. During the mixing periods, the aerator stopped and the hydraulic agitator worked to increase the mass transfer rate. The average DO concentrations during aerobic and mixed periods were 5.6 and 0 mg/L, respectively.
Domestic wastewater from a residential area near to the laboratory was fed to the SNAD biofilm reactor. The main wastewater characteristics are as follows: pH 7.1–8.0, COD 150–250 mg/L, total nitrogen (TN) 70–120 mg/L, NH₄⁺-N 60–90 mg/L, NO₂⁻-N < 1 mg/L, NO₃⁻-N < 3 mg/L, alkalinity (as CaCO₃) 300–400 mg/L. The average effluent total inorganic nitrogen (TIN) concentration of the SNAD biofilm reactor was 11 mg/L with the average TIN removal efficiency of 85%. Batch tests showed that the anammox, denitrification and nitrite-oxidizing bacteria (NOB) activities of the SNAD biofilm were 0.267 kg TIN/(kg VSS·d), 0.211 kg NO₂⁻/C₀-N/(kg VSS·d) and 0.053 kg NO₂⁻-N/(kg VSS·d), respectively (VSS: volatile suspended solids).

**Batch tests**

Batch tests were performed to determine the nitrogen removal rates of the SNAD biofilm. All batch tests were repeated three times in beakers (working volume of 1,000 mL). In the beginning, Kaldnes rings taken from the biofilm reactor were carefully rinsed with tap water (30 °C) to remove residual substrate. Afterwards, 50 Kaldnes rings were transferred to beakers together with 1,000 mL synthetic wastewater. The ammonium and nitrite in the synthetic wastewater were in the form of NH₄Cl and NaNO₂. The initial pH value in batch tests was maintained at 7.0 with the addition of NaOH or HCl. Except for the temperature experiments, all the batch tests were conducted at 30 °C.

The measurement of NOB, anammox and denitrification activities

The NOB, anammox and denitrification activities were determined in batch tests according to Zheng et al. (2016a). The composition of synthetic wastewater is described in Table 1. During the measurement of specific anammox and denitrification activity, the beakers were flushed with N₂ gas (99.99%) to remove the oxygen at the first 10 minutes. To avoid the introduction of oxygen, the beakers were covered with plastic wrap. During the measurement of NOB activity, the DO concentration was maintained above 5 mg/L with the aeration rate of 250 mL/min. In order to keep the biomass in suspension and increase the mass transfer rate, the magnetic stirrer worked (200 r/min). Samples were periodically taken from the beakers for analysis.

The sludge activity was expressed according to Equation (1). The terminal time was selected when the NH₄⁺-N or NO₂⁻-N concentration was less than 10 mg/L during the sampling process. If no substrate concentration was below

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Anammox activity</th>
<th>NOB activity</th>
<th>Denitrification activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺-N (mg/L)</td>
<td>70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO₂⁻-N (mg/L)</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>NaCH₃COO (mg/L)</td>
<td>0</td>
<td>0</td>
<td>520</td>
</tr>
</tbody>
</table>

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10 mg/L at the end time of the sampling process, then the end time was selected as the terminal time.

\[
\text{sludge activity} = \frac{1.44 \times (C_{\text{initial}} - C_{\text{terminal}})}{\text{Terminal time} \times \text{VSS}} \quad (1)
\]

where \( C_{\text{initial}} \) and \( C_{\text{terminal}} \) represent substrate concentrations at initial and terminal time (mg/L); The terminal time was expressed in min; the VSS is the biomass in kg N/(kg VSS·d); The measurement of mixed liquor volatile suspended solids (MLVSS) of biomass on the carriers was as follows: 50 Kaldnes rings were placed in water solution and treated with ultrasonic apparatus (VCX105PB, Sonics & Materials Incorporated Company, USA) to separate the biomass from the carriers. Then the mixture of biomass and water solution was filtered with a 0.45 μm filter to obtain mixed liquor suspended solids (MLSS) and MLVSS (APHA 2005).

**COD/NO\textsubscript{2}-N** ratios, carbon sources and temperature experiments

The effect of COD/NO\textsubscript{2}-N ratios on the nitrogen removal rates of the anammox–denitrification coupling process was evaluated with the carbon source of sodium acetate. The initial NH\textsubscript{4}-N and NO\textsubscript{2}-N concentrations were both 70 mg/L; sodium acetate was added to adjust the COD/NO\textsubscript{2}-N ratios to 0, 1, 2, 3, 4 and 5, respectively. The effect of carbon sources on the anammox–denitrification coupling process was evaluated with sodium formate, sodium acetate, sodium propionate and glucose, respectively. The initial NO\textsubscript{2}-N and NH\textsubscript{4}-N concentrations were both 70 mg/L; The COD/NO\textsubscript{2}-N ratio was 5. The effect of temperature on the anammox activity was evaluated with the temperature of 30, 25, 20, 15 and 10 °C, respectively. The initial NO\textsubscript{2}-N and NH\textsubscript{4}-N concentrations were both 70 mg/L. The procedures for the batch tests and the determination of the nitrogen removal rates were similar to those given for the measurement of anammox and denitrification activities.

**Analytical methods**

All the samples were filtered with a 0.45 μm filter before analyzing. The determination of NH\textsubscript{4}-N, NO\textsubscript{2}-N, NO\textsubscript{3}-N, COD and alkalinity were performed according to Standard Methods (APHA 2005). DO, pH, and temperature (T) were monitored by a WTW Multi 3420i meter (WTW Company, Germany). The summation of NH\textsubscript{4}-N, NO\textsubscript{2}-N and NO\textsubscript{3}-N concentration is expressed as the total inorganic nitrogen concentration. In previous study, the \( \Delta \text{NO}_\text{2}-\text{N}/\Delta \text{NH}_\text{4}-\text{N} \) ratio was 1.45 during the anammox process (Zheng et al. 2016a). Therefore, in the present study, the nitrite consumed by the anammox bacteria was calculated as the ammonium consumption multiplied by 1.45 during the anammox–denitrification coupling process; the nitrite consumed by the denitrifiers was calculated by the total nitrite consumption minus the nitrite consumed by the anammox bacteria.

**Calculation methods**

The COD discussed in this study was calculated as Equation (2) (Liang & Liu 2007).

\[
\text{COD} = \text{COD}_{\text{measured}} - \frac{8}{7} C_{\text{NO}_2-N\text{effluent}} \quad (2)
\]

where \( c \) is the concentration (mg/L).

**RESULTS AND DISCUSSION**

**Effect of COD/NO\textsubscript{2}-N ratios on the anammox–denitrification coupling process of the SNAD biofilm**

Figure 2 shows the effect of COD/NO\textsubscript{2}-N ratios on the nitrogen removal rates of the SNAD biofilm via the anammox–denitrification coupling process in batch tests. Sodium acetate was added as the sole carbon source. With the COD/NO\textsubscript{2}-N ratio of 0, the SNAD biofilm mainly performed anammox activity. As a result, the NH\textsubscript{4}-N and NO\textsubscript{2}-N concentrations decreased while the NO\textsubscript{3}-N concentration increased. The NO\textsubscript{3}-N concentration reached 13.6 mg/L at the end of the sampling process. However, with the COD/NO\textsubscript{2}-N ratios above 0, the SNAD biofilm was able to perform anammox and denitrification activities simultaneously. Consequently, the NH\textsubscript{4}-N and NO\textsubscript{2}-N concentrations decreased while the NO\textsubscript{3}-N concentration was always below 2 mg/L. With the COD/NO\textsubscript{2}-N ratios of 0, 1, 2, 3, 4 and 5, the corresponding NH\textsubscript{4}-N removal rates were 0.121, 0.145, 0.101, 0.079, 0.062 and 0.040 kg N/(kg VSS·d), respectively; Meanwhile, the NO\textsubscript{2}-N removal rates were 0.180, 0.242, 0.281, 0.392, 0.379 and 0.358 kg N/(kg VSS·d), respectively. During the anammox–denitrification coupling process, the NH\textsubscript{4}-N concentration was removed by anammox bacteria; the NO\textsubscript{2}-N concentration was removed...
by anammox bacteria and denitrifiers together. It was observed that with the increase of COD/NO$_2$-N ratio, the NH$_4^+$-N removal rate deceased while the NO$_2$-N removal rate increased, which indicated that the anammox activity decreased while the denitrification activity increased. This result can be explained as follows. It is expected that AOB tend to locate on the surface of the biofilm, while anammox bacteria and denitrifiers tend to grow in the inner part (Volcke et al. 2010; Winkler et al. 2012). Also, substrate penetration into the deeper layers could be greatly reduced as the biofilm performs high mass transfer resistance (Vazquez-Padin et al. 2011). With the increase of COD/NO$_2$-N ratio, the denitrification activity rose significantly, which might lead to the decrease of nitrite concentration inside the biofilm. Consequently, the anammox activity decreased due to the shortage of nitrite concentration. However, the NH$_4^+$-N removal rate at COD/NO$_2$-N ratio of 1 was higher than that at COD/NO$_2$-N ratio of 0. This result might be attributed to the higher nitrite concentration in the biofilm at COD/NO$_2$-N ratio of 1. Normally, the anammox process would produce a small amount of nitrate. At COD/NO$_2$-N ratio of 1, the denitrifiers might reduce some nitrate to nitrite rather than nitrogen directly due to the insufficiency of COD amount. Consequently, the nitrite concentration in the biofilm might be higher at COD/NO$_2$-N ratio of

![Figure 2](https://iwaponline.com/wst/article-pdf/75/7/1712/453914/wst075071712.pdf)

Figure 2 | The effect of COD/NO$_2$-N ratios on the nitrogen removal rate via anammox-denitrification coupling process.
1 than that at COD/NO₂-N ratio of 0, which led to a higher anammox activity at COD/NO₂-N ratio of 1.

Table 2 describes the nitrogen balance in batch tests via the anammox–denitrification coupling process under different COD/NO₂-N ratios. With the COD/NO₂-N ratios of 1, 2, 3, 4 and 5, the corresponding NO₂-N consumption via anammox were 87.1%, 52.2%, 29.3%, 23.7% and 16.3%, respectively. The NO₂-N consumption via anammox decreased with the increase of COD/NO₂-N ratio. With the COD/NO₂-N ratios of 0 to 2, the NO₂-N consumption via anammox was above 50%, which indicated that the anammox bacteria performed greater nitrite competitive ability than denitrifiers. Under this circumstance, the SNAD biofilm could achieve good nitrogen removal performance via the anammox–denitrification coupling process. However, with the COD/NO₂-N ratios above 2, the NO₂-N consumption via anammox was below 50%, which indicated that the anammox bacteria were weaker in the competition for nitrite than denitrifiers. Some researchers have investigated the effect of COD/N ratios on the anammox–denitrification coupling reactor with long-term experiments (Chamchoi et al. 2008; Tang et al. 2010). Chamchoi et al. (2008) reported that the anammox–denitrification coupling reactor performed good anammox activity with the influent COD/NOₓ-N ratio of 2 to 3; however, the anammox activity was largely inhibited with the influent COD/NOₓ-N ratio above 3. The result of their study was similar to the present study. Tang et al. (2010) also found the similar result in an anammox–denitrification coupling granular reactor. The NO₂-N consumption via anammox reached 55.3% with the influent COD/NO₂-N ratio of 1.25, while it decreased to 2.1% with the influent COD/NO₂-N ratio of 2.92. Moreover, several studies have investigated the effect of COD/N ratios on the nitrogen removal performance of the SNAD process (Chen et al. 2009; Li et al. 2012). Chen et al. (2009) described that the SNAD biofilm reactor achieved the TN removal efficiency of 70% with the influent COD/NH₄-N ratio of 0.5, while it decreased to 40% with the influent COD/NH₄-N ratio of 0.75. Furthermore, Li et al. (2012) reported that the NO₂-N consumption via anammox in the SNAD biofilm reactor was only 49.22% with the influent COD/NH₄-N ratio of 1.2. Based on the discussion above, it seems that the SNAD process was more sensitive to the organic matter than was the anammox–denitrification coupling process. The difference might be related to the DO concentration and the anammox bacteria abundance. Compared with the anammox–denitrification coupling process, the biomass of anammox bacteria in the SNAD process was relatively lower since the SNAD sludge mainly consisted of heterotrophic bacteria, AOB, anammox bacteria and denitrifiers. Also, the DO in the SNAD process might inhibit the anammox activity (Strous et al. 1997). Furthermore, the anammox bacteria might be washed out from the reactor since high COD concentration favors the growth of heterotrophic bacteria.

Recently, other studies found that when the SNAD process was operated with intermittent aeration and high DO condition, it was possible to achieve good anammox activity under high COD/N ratios (Zekker et al. 2014; Yang et al. 2015). Zekker et al. (2014) described the nitrogen removal performance of a SNAD floccular biomass reactor with intermittent aeration condition (aeration 30 min/non-aeration 30 min). The COD/NH₄-N ratio of the influent was above 5 and the DO concentration during the aerobic period was 3.18 mg/L. As a result, the COD and TN removal efficiencies were both above 50%. In order to avoid the adverse effect of high DO concentration on the anammox bacteria, most studies operated the SNAD process with a biofilm or granule reactor (Yang et al. 2015). As for the biofilm or granule, the DO on the outer layer could be largely consumed by AOB and heterotrophic bacteria, providing an anoxic environment in deeper layers (Siegrist et al. 2008; Li et al. 2012). Moreover, the biofilm or granule performs high mass transfer resistance of DO

### Table 2 | Nitrogen balance in batch tests via anammox–denitrification coupling process under different COD/NO₂-N ratios

<table>
<thead>
<tr>
<th>C/N ratio</th>
<th>Terminal time (min)</th>
<th>NH₄-N removal (mg N)</th>
<th>NO₂-N removal (mg N)</th>
<th>Total</th>
<th>Anammox</th>
<th>Denitrification</th>
<th>NO₂-N consumption via anammox (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>160</td>
<td>34.0</td>
<td>56.6</td>
<td>49.3</td>
<td>7.3</td>
<td></td>
<td>87.1</td>
</tr>
<tr>
<td>2</td>
<td>140</td>
<td>20.7</td>
<td>57.6</td>
<td>30.1</td>
<td>27.5</td>
<td></td>
<td>52.2</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>11.6</td>
<td>57.4</td>
<td>16.8</td>
<td>40.6</td>
<td></td>
<td>29.3</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>9.1</td>
<td>55.6</td>
<td>13.2</td>
<td>42.4</td>
<td></td>
<td>23.7</td>
</tr>
<tr>
<td>5</td>
<td>110</td>
<td>6.5</td>
<td>57.7</td>
<td>9.4</td>
<td>48.3</td>
<td></td>
<td>16.3</td>
</tr>
</tbody>
</table>
concentration (Vazquez-Padin et al. 2011; Rathnayake et al. 2013; Zheng et al. 2016b). Yang et al. (2015) operated the SNAD biofilm reactor with intermittent aeration condition (aeration 15 min/non-aeration 45 min). The DO concentration during the aerobic period was 3.5 mg/L. The TN removal efficiency reached 88% with the influent COD/\(\text{NH}_4\)-N ratio of 0.69. In the present study, the SNAD biofilm reactor also achieved good nitrogen removal performance with the average influent COD/\(\text{NH}_4\)-N ratio of 2.57. The present reactor was operated with intermittent aeration condition (aeration 20 min/non-aeration 20 min) and the DO concentration during the aerobic period was as high as 5.6 mg/L. The reason why the SNAD process could tolerate higher COD/\(\text{NH}_4\)-N ratio with intermittent aeration and high DO condition was analyzed as follows. As high DO and COD concentrations favor the growth of heterotrophic bacteria, a large amount of COD would be degraded by the heterotrophic bacteria during the aerobic period. Therefore, the growth of denitrifiers might be partially suppressed due to the insufficiency of COD. Also, with the intermittent aeration condition, the anammox bacteria were able to recover anammox activity in the non-aerobic period.

**Effect of carbon sources on the anammox–denitrification coupling process of the SNAD biofilm**

Figure 3 shows the effect of carbon sources on the nitrogen removal rates of the SNAD biofilm via the anammox–denitrification coupling process (COD/\(\text{NO}_2\)-N ratio of 5). With the carbon sources of sodium formate, sodium acetate, sodium propionate and glucose, the corresponding \(\text{NH}_4\)-N removal rates were 0.040, 0.057, 0.070 and 0.082 kg N/(kg VSS·d), respectively. Meanwhile, the \(\text{NO}_2\)-N removal rates were 0.358, 0.222, 0.136 and 0.154 kg N/(kg VSS·d), respectively. The \(\text{NH}_4\)-N removal rates of different carbon sources increased in the order of sodium formate < sodium acetate < sodium propionate < glucose. Table 3 describes the nitrogen balance in batch tests via the anammox–denitrification coupling process under different carbon sources. With the carbon sources of sodium formate, sodium acetate, sodium propionate and glucose, the corresponding \(\text{NO}_2\)-N consumption via anammox was 16.3%, 37.1%, 74.1% and 76.8%, respectively. The anammox bacteria performed greater nitrite competitive ability than denitrifiers with the carbon sources of sodium propionate or glucose at the COD/\(\text{NO}_2\)-N ratio of 5. It is the different nitrite utilization capacities of denitrifiers that leads to different nitrite competitive abilities of anammox bacteria with different carbon sources. Previous studies suggested that a more complex carbon metabolic pathway might result in a lower denitrification rate (Eleftheriou & Li 2006; Yang et al. 2012). Sodium formate and sodium acetate have a simpler pathway, while the degradation of sodium propionate and glucose is under a more intricate pathway (Cherchi et al. 2009; Ge et al. 2012). Consequently, in the present study, the anammox bacteria performed greater nitrite competitive ability than denitrifiers with the carbon sources of sodium propionate and glucose.

**Effect of temperature on the anammox activity of the SNAD biofilm**

Figure 4 shows the anammox activities of the SNAD biofilm under different temperature. With the temperature of 30, 25, 20, 15 and 10°C, the corresponding anammox activities were 0.250, 0.193, 0.107, 0.071 and 0.002 kg TIN/(kg VSS·d), respectively. Compared with the anammox activity at the temperature of 30°C, the anammox activities at the temperature of 25, 20, 15 and 10°C decreased by 16.1%, 53.6%, 69.0% and 99.2%, respectively. Moreover, the anammox activity almost ceased at 10°C. Several studies reported the nitrogen removal performance of anammox bacteria at low temperature (Cema et al. 2007; Isaka et al. 2008; Gilbert et al. 2015). Cema et al. (2007) demonstrated that the anammox bacteria could perform high activity at 15–19°C in an RBC reactor. Also, Gilbert et al. (2015) investigated the effect of temperature on the anammox activity with different forms of sludge aggregate. They found that the anammox activity of the floc sludge stopped at 10°C whereas the reactors filled with Kaldnes or granules still performed anammox activity. Moreover, Isaka et al. (2008) reported that the anammox bacteria were able to perform activity below 10°C when the anammox bacteria were entrapped in a gel carrier. They found that the nitrogen removal rates of the anammox reactor were 6.2, 2.8, 1.4, 0.7 and 0.36 kg N m\(^{-3}\) d\(^{-1}\) at the temperature of 32, 22, 17, 12 and 6°C, respectively. The reason why the anammox activity in the present study ceased at 10°C can be analyzed as follows. Firstly, the biomass concentration of anammox bacteria on the SNAD biofilm was relatively lower than the special anammox biofilm. Also, the anammox bacteria used in the present study were not acclimated to low temperature because the SNAD biofilm reactor was always operated at 30°C. However, the SNAD biofilm was observed to perform anammox activity at 15°C, which was critical for the application of the SNAD process to the treatment of municipal wastewater.
Table 3 | Nitrogen balance in batch tests via anammox-denitrification coupling process under different carbon sources (COD/NO₂-N = 5)

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Terminal time (min)</th>
<th>NH₄⁺-N removal (mg N)</th>
<th>NO₂⁻-N removal (mg N)</th>
<th>NO₂⁻-N removal via anammox (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium formate</td>
<td>110</td>
<td>6.5</td>
<td>57.7</td>
<td>9.4</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>180</td>
<td>15.0</td>
<td>58.5</td>
<td>21.7</td>
</tr>
<tr>
<td>Sodium propionate</td>
<td>280</td>
<td>28.5</td>
<td>55.7</td>
<td>41.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>260</td>
<td>31.1</td>
<td>58.7</td>
<td>45.1</td>
</tr>
</tbody>
</table>

Figure 3 | The effect of carbon sources on the nitrogen removal rate via anammox-denitrification coupling process (COD/NO₂-N = 5).
The carbon sources and COD/NO₂⁻N ratios played a significant role in the anammox–denitrification coupling process of the SNAD biofilm. As for the carbon source of sodium acetate, the nitrite competitive ability of denitrifiers rose with the increase of COD/NO₂⁻N ratio, which led to the decrease of anammox activity. With the COD/NO₂⁻N ratios of 0, 1, 2, 3, 4 and 5, the corresponding NH₄⁺ removal rates of anammox bacteria were 0.121, 0.145, 0.101, 0.079, 0.062 and 0.040 kg N/(kg VSS·d), respectively. The anammox bacteria exhibited higher nitrite competitive ability than denitrifiers with the COD/NO₂⁻N ratios below 3.0. As for other carbon sources, the nitrite competitive ability of anammox bacteria increased in the order of sodium acetate < sodium formate < sodium propionate < glucose. Moreover, the SNAD biofilm was able to perform anammox activity at 15 °C. However, this study mainly evaluated the short-term effect of carbon sources, COD/NO₂⁻N ratios and temperature. As the denitrifiers grow faster than anammox bacteria when organic matter coexists with ammonium and nitrite, a long-term experiment needs to be conducted for further study.

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