Model-based Evaluation of Effects of Temperature on Nitrogen Removal in Low- and Moderate-temperature Type Anammox Reactors

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Abstract

A mathematical model was developed for predicting effects of temperature on nitrogen removal in continuous-flow reactors via anaerobic ammonium oxidation (anammox). A cardinal temperature equation with inflection for the bacterial growth was incorporated into the anammox model of low-temperature type reactors, Low-R1 and Low-R2 inoculated with activated sludge respectively in Kumamoto and Hokkaido. The maximum specific growth rate ($\mu_{opt}$) was calibrated to 0.052 d⁻¹ at the optimal temperature ($T_{opt}$) of 26°C between the minimum temperature ($T_{min}$) of 4°C and the maximum temperature ($T_{max}$) of 36°C for anammox bacteria in Low-R1 (correlation with measured data, $r = 0.851$). The values of $\mu_{opt}$, $T_{opt}$, $T_{min}$, and $T_{max}$ were calibrated to be 0.089 d⁻¹, 31°C, 0°C, and 36°C, respectively for anammox bacteria in Low-R2 ($r = 0.995$). A typical exponential temperature equation was incorporated into the anammox model of a moderate-temperature type reactor, Mod-R inoculated with activated sludge in Kumamoto. The maximum specific growth rate at 30°C was calibrated to 0.055 d⁻¹ with the temperature coefficient of 0.104°C⁻¹ for anammox bacteria in Mod-R ($r = 0.987$). The mathematical model simulated treatment of ideal wastewater containing ammonium at 50 mg-N/L and nitrate at 60 mg-N/L at hydraulic retention time of 0.5d and sludge retention time (SRT) of 50–150d at 10–30°C. The simulations predicted stable nitrogen removals in Low-R1 and Low-R2 at low temperatures in comparison with Mod-R. Although Low-R1 at short SRTs showed lower nitrogen removals than Mod-R at high temperatures around 35°C, Low-R2 demonstrated robust T-N removals in the whole temperature range. In conclusion, the low-temperature type anammox reactors, especially Low-R2, would have a sufficient potential to develop a versatile nitrogen removal process without any heating system in mild climate conditions.

Key words: anaerobic ammonium oxidation, temperature dependence, mathematical model, nitrogen removal

INTRODUCTION

Anaerobic ammonium oxidation (anammox) is a biologically mediated reaction in which ammonium (NH₄⁻N) is oxidized anaerobically using nitrite (NO₂⁻N) as an electron acceptor to nitrogen gas (N₂) and a small amount of nitrate (NO₃⁻N). Compared to conventional biological processes, nitrogen removal from wastewater using anammox provides benefits such as lower oxygen demand, an external carbon source, and sludge production. However, the anammox process generally requires a heating system to maintain temperatures of 30–37°C for efficient nitrogen removal, which elevates the wastewater treatment cost. Therefore, several trials have been undertaken to operate the anammox...
process at ambient or rather lower temperatures\textsuperscript{85-14}.

Our research group also conducted start-up of lab-scale anammox reactors (Low-R1 and Low-R2) at 20°C\textsuperscript{15}. Seed sludge samples for Low-R1 and Low-R2 were originally collected respectively from a domestic wastewater treatment plant in Kumamoto City and from a livestock wastewater treatment plant in Hokkaido Prefecture. The two anammox reactors achieved high nitrogen removal rates\textsuperscript{15}. The batch assays clarified that the respective specific anammox activities of the Low-R1 sludge and the Low-R2 sludge were higher at 10–25°C and 10–30°C than that of an anammox reactor Mod-R inoculated with activated sludge in Kumamoto operated at a moderate temperature of 35°C (Mod-R)\textsuperscript{16}. The Low-R1 and Low-R2 sludge samples are expected to be applicable for developing the nitrogen removal process with no heating system. Results also clarified that Low-R1 and Low-R2 showed different temperature dependence of the anammox activity: the former had a peak specific anammox activity at 25°C and the latter at 30°C\textsuperscript{16}. Therefore, it is important to evaluate the nitrogen removal in these reactors comparatively under various temperature fluctuations.

However, experimental evaluation for potential scale-up of the anammox reactors takes an extremely long time because of the slow growth rate of anammox bacteria. Several months or even more than 1 year might be necessary to test the anammox reactor performance for each operational condition. Therefore, modeling and simulation studies must be conducted to predict the temperature dependence of the anammox reactors. Model-based studies are helpful to elucidate their design and operation, and are useful for predicting the nitrogen removal of the anammox process. Actually, several model-based studies have been conducted of anammox processes in a sequencing batch reactor\textsuperscript{17}, an up-flow anaerobic sludge blanket reactor\textsuperscript{18}, of the complete autotrophic ammonium removal over nitrite (CANON) process\textsuperscript{19-23}, and the simultaneous anammox and denitrification (SAD) process\textsuperscript{24}. Although only Hao et al\textsuperscript{19} mathematically expressed temperature dependence of the anammox activity in the CANON process, their model incorporated the van’t Hoff exponential equation in which a reaction rate exhibits an exponential increase with temperature.

For this study, a cardinal temperature equation with inflection\textsuperscript{25} for growth of anammox bacteria was incorporated into a mathematical model\textsuperscript{26} for the anammox process. The modified model was used for predicting nitrogen removal and bacterial growth in continuous-flow reactors inoculated with sludge samples of Low-R1, Low-R2, and Mod-R under various temperature of 10–35°C. Advantages of anammox reactors with high activities at low temperatures were assessed using simulation studies.

**MODEL DEVELOPMENT**

**Governing equations** The mass balance was solved for the reactor fed continuously with substrates NH\textsubscript{4}-N and NO\textsubscript{2}-N under anoxic condition (Eqs. 1 and 2). It was assumed that the anammox biomass and the substrates were distributed uniformly in a reactor (e.g. a membrane bioreactor). It was also assumed that heterotroph and nitrifier populations were negligible. Decay of anammox bacteria results in inert biomass. No rate-limiting factor exists on the anammox process except for the substrates and temperature.

\[
\begin{align*}
\frac{dX_i}{dt} &= \sum \nu_{ij}\rho_{j} - \frac{(X_{in} - X_i)}{\theta_i} \\
\frac{dS_i}{dt} &= \sum \nu_{ij}\rho_{j} - \frac{(S_{in} - S_i)}{\theta}
\end{align*}
\]

Eq. 1  Eq. 2

In those equations, \(X_i\) and \(S_i\) respectively denote the concentrations for particulate components and soluble components. This model included anammox bacteria \((X_{AN}, \text{mg-COD/L})\), inert biomass \((X_i, \text{mg-COD/L})\), NH\textsubscript{4}-N \((S_{NH4}, \text{mg-N/L})\), NO\textsubscript{2}-N \((S_{NO2}, \text{mg-N/L})\), NO\textsubscript{3}-N \((S_{NO3}, \text{mg-N/L})\), \(N_2 (S_{N2}, \text{mg-N/L})\), and dissolved oxygen \((S_{O2}, \text{mg/L})\). \(t, \theta_i\), and \(\theta\) respectively represent time (d), sludge retention time (SRT, d), and hydraulic retention time (HRT, d). Subscript “in” denotes the influent concentrations. The anammox model used in our previous study\textsuperscript{26} was applied to this study. Kinetic rate expressions \(\rho_j\) are shown.
Table 1  Kinetic rate expressions for the anammox reactor

<table>
<thead>
<tr>
<th>j</th>
<th>Process</th>
<th>Process rate (mg COD/m$^3$/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Growth of $X_{AN}$</td>
<td>$R_1 = \mu_{AN} \left( \frac{K_{AN}^{NO_2}}{K_{AN}^{NO_2} + S_{NO_2}} \right) \left( \frac{S_{NH_4}}{K_{AN}^{NH_4} + S_{NH_4}} \right) \left( \frac{S_{NO_2}}{K_{AN}^{NO_2} + S_{NO_2}} \right) X_{AN}$</td>
</tr>
<tr>
<td>2</td>
<td>Aerobic endogenous respiration of $X_{AN}$</td>
<td>$R_2 = b_{AN} \left( \frac{S_{NO_2}}{K_{AN}^{NO_2} + S_{NO_2}} \right) X_{AN}$</td>
</tr>
<tr>
<td>3</td>
<td>Anoxic endogenous respiration of $X_{AN}$</td>
<td>$R_3 = b_{AN}o_{AN} \left( \frac{K_{AN}^{NO_2}}{K_{AN}^{NO_2} + S_{NO_2}} \right) \left( \frac{S_{NO_2}}{K_{AN}^{NO_2} + S_{NO_2}} \right) X_{AN}$</td>
</tr>
</tbody>
</table>

Table 2  Stoichiometry for the anammox model

<table>
<thead>
<tr>
<th>j</th>
<th>$X_{AN}$</th>
<th>$X_1$</th>
<th>$S_{NO_3}$</th>
<th>$S_{NO_2}$</th>
<th>$S_{NH_4}$</th>
<th>$S_{O_2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1/1.14</td>
<td>-1/$Y_{AN}$</td>
<td>-1/$i_{NXR}$</td>
<td>-1/$Y_{AN}$ + 2/$Y_{AN}$</td>
</tr>
<tr>
<td>2</td>
<td>-1</td>
<td>$f_{X1}$</td>
<td>$-Y_{X1}$</td>
<td>$-i_{NXR}$</td>
<td>$-i_{NXX}$</td>
<td>$-i_{NXX}$ + $-f_{X1}$</td>
</tr>
<tr>
<td>3</td>
<td>-1</td>
<td>$f_{X1}$</td>
<td>0</td>
<td>$-i_{NXX}$</td>
<td>$-i_{NXX}$</td>
<td>$(1-f_{X1})/2.86$</td>
</tr>
</tbody>
</table>

in Table 1. Every process rate was described by multi-Monod kinetics as a function of substrate concentrations. Stoichiometric parameters $v_j$ are presented in Table 2.

Parameter values used for this study are shown in Tables 3 and 4. The stoichiometric parameters presented in Table 3 were selected commonly for Mod–R, Low–R1, and Low–R2 sludges as typical values according to reports of studies related with the anammox process$^{19,24,26-28}$.

Actually the anammox sludge comprises diverse anammox bacteria with different affinity and rate constant. Characteristics of the bacterial community reflect the parameter values used in this kinetic model. Kinetic parameters of two kinds exist: affinity ($K$) and rate constants ($\mu$ and $b$) in the model. Substrate affinity constants are temperature-independent in a narrow temperature range. The affinity constants of $NH_3$–N and $NO_2$–N were also set as common values for anammox bacteria in the Mod–R, Low–R1, and Low–R2 sludges.

Temperature dependence functions for anammox  Growth and decay values are typical temperature-dependent rate constants. The temperature dependence of the maximum specific growth rate $\mu_{AN}$ (d$^{-1}$) of the Mod–R sludge can be expressed as the van’t Hoff exponential equation (Eq. 3).

$$
\mu_{AN}^{T=30} = \mu_{AN}^{T=30} \exp \left[ \theta_{\mu} (T - 30) \right]
$$

Eq. 3

In this equation, $\mu_{AN}^{T=30}$ is the maximum specific growth rate for anammox bacteria at 30°C. $T$ (°C) stands for temperature (10°C ≤ $T$ ≤ 30°C) in the reactor. $\theta_{\mu}$ is the temperature coefficient (°C$^{-1}$) for the bacterial growth. The values for $\mu_{AN}^{T=30}$ and $\theta_{\mu}$ in an earlier study$^{19}$ were, respectively, about 0.07 d$^{-1}$ and 0.096°C$^{-1}$.

Although the van’t Hoff exponential equation can be applied to the anammox reaction in Low–R1 and Low–R2, its applicable range is limited to 10–25 and 10 –30°C, respectively. The temperature dependence of $\mu_{AN}$ of the Low–R1 and Low–R2 sludges can be expressed by the Rosso equation$^{25}$ (Eq. 4).

$$
\mu_{AN} = \frac{\mu_{AN}^{T_{opt}} (T - T_{min})(T - T_{max})^2}{(T_{opt} - T_{min})(T_{opt} - T_{max})(T_{opt} - T_{min} - 2T)}
$$

$T_{min} \leq T \leq T_{max}$

Eq. 4
Therein, $\mu_{AN}$ is the maximum specific growth rate (d$^{-1}$) at the optimal temperature ($T_{opt}$, °C) between the minimum temperature ($T_{min}$, °C) and the maximum temperature ($T_{max}$, °C). Although $T_{min}$ and $T_{max}$ were defined for Eq. 4, the practical temperature range was $10$°C $\leq T \leq 30$°C for anammox in this study.

The temperature dependence model was calibrated using regression analysis with experimental data of the specific anammox activity$^{16}$, which was determined using the sludge sample at the volatile suspended solid (VSS) concentration of $1400$-$1600$ mg/L and $NH_4^-$-$N$ of $50$ mg-$N/L$ and $NO_2^-$-$N$ of $60$ mg-$N/L$ as the initial condition. The DO concentration in the batch assay was assumed as about $0.1$ mg/L. Anammox sludge was assumed to consist of $70\%$ anammox bacteria biomass ($X_{AN}$) and $30\%$ inert biomass ($X_I$). The unit conversions of $X_{AN}$ from mg-$VSS/L$ to g-$COD/L$ were performed by multiplying a factor of $1.5$ g-$COD/g-VSS$.$^{29}$ Figure 1 and Table 4 show that the values for $\mu_{AN}$ and $\theta$ of the Mod-R sludge were estimated re-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition (Units)</th>
<th>Values</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{AN}^{NH}$</td>
<td>Saturation constant for $NH_4^-$-$N$ (mg-$N/L$)</td>
<td>$0.07$</td>
<td>Strous et al.$^{26}$</td>
</tr>
<tr>
<td>$K_{AN}^{NO2}$</td>
<td>Saturation constant for $NO_2^-$-$N$ (mg-$N/L$)</td>
<td>$0.05$</td>
<td>Hao et al.$^{19}$</td>
</tr>
<tr>
<td>$K_{AN}^{O2}$</td>
<td>Inhibition constant for $O_2$ (mg/L)</td>
<td>$0.01$</td>
<td>Strous et al.$^{26}$</td>
</tr>
<tr>
<td>$b_{AN}^{V30}$</td>
<td>Endogenous respiration rate at 30°C (d$^{-1}$)</td>
<td>$0.003$</td>
<td>Hao et al.$^{29}$</td>
</tr>
<tr>
<td>$\eta_{AN}$</td>
<td>Anoxic reduction factor ($\sim$)</td>
<td>$0.5$</td>
<td>Hao et al.$^{39}$, Koch et al.$^{22}$</td>
</tr>
<tr>
<td>$Y_{AN}$</td>
<td>Anammox bacteria on ammonium (g-$COD/g-N$)</td>
<td>$0.164$</td>
<td>Ni et al.$^{18}$</td>
</tr>
<tr>
<td>$f_{XI}$</td>
<td>Inert content in lysis of biomass (g-$COD/g-N$)</td>
<td>$0.2$</td>
<td>Henze et al.$^{27}$</td>
</tr>
<tr>
<td>$f_{NXI}$</td>
<td>Inert content in lysis of biomass (g-$COD/g-N$)</td>
<td>$0.02$</td>
<td>Henze et al.$^{27}$</td>
</tr>
<tr>
<td>$i_{NXB}$</td>
<td>Nitrogen content in biomass (g-$N/g-COD$)</td>
<td>$0.07$</td>
<td>Hiatt and Grady$^{28}$</td>
</tr>
<tr>
<td>$i_{NXI}$</td>
<td>Nitrogen content in inert biomass (g-$N/g-COD$)</td>
<td>$0.03$</td>
<td>Henze et al.$^{27}$</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Temperature coefficient (°C$^{-1}$)</td>
<td>$0.096$</td>
<td>Hao et al.$^{29}$</td>
</tr>
</tbody>
</table>

Table 3 Kinetic and stoichiometric parameters commonly for Low-R1, Low-R2 and Mod-R sludges

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition (Units)</th>
<th>Low-R1</th>
<th>Low-R2</th>
<th>Mod-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{AN}^{T=30}$</td>
<td>Maximum specific growth rate at 30°C (d$^{-1}$)</td>
<td>$--$</td>
<td>$--$</td>
<td>$0.055$</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Temperature coefficient (°C$^{-1}$)</td>
<td>$--$</td>
<td>$--$</td>
<td>$0.104$</td>
</tr>
<tr>
<td>$\mu_{AN}^{opt}$</td>
<td>Maximum specific growth rate at $T_{opt}$ (d$^{-1}$)</td>
<td>$0.052$</td>
<td>$0.089$</td>
<td>$--$</td>
</tr>
<tr>
<td>$T_{opt}$</td>
<td>Optimum temperature (°C)</td>
<td>$26$</td>
<td>$31$</td>
<td>$--$</td>
</tr>
<tr>
<td>$T_{min}$</td>
<td>Minimum temperature (°C)</td>
<td>$4$</td>
<td>$0$</td>
<td>$--$</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>Maximum temperature (°C)</td>
<td>$36$</td>
<td>$36$</td>
<td>$--$</td>
</tr>
</tbody>
</table>

Fig. 1 Temperature dependence of the specific anammox activity of sludge samples of Low-R1, Low-R2, and Mod-R. Experimental data plots and model lines of the van’t Hoff exponential equation (Eq. 3) for Mod-R and the Rosso equation (Eq. 4) for Low-R1 and Low-R2 are shown. Circles and dotted line, Low-R1; triangles and solid line, Low-R2; squares and dashed line, Mod-R. The model parameters are presented in Table 4.
respectively as 0.055 d\(^{-1}\) and 0.104\(^{\circ}\)C\(^{-1}\). The values for \(\mu_{\text{opt}}^\text{AN}, T_{\text{opt}}, T_{\text{min}}, \) and \(T_{\text{max}}\) of the Low–R1 sludge were estimated respectively as 0.052 d\(^{-1}\), 26\(^{\circ}\)C, 4\(^{\circ}\)C, and 36\(^{\circ}\)C. The values for \(\mu_{\text{opt}}^\text{AN}, T_{\text{opt}}, T_{\text{min}}, \) and \(T_{\text{max}}\) of the Low–R2 sludge were estimated respectively as 0.089 d\(^{-1}\), 31\(^{\circ}\)C, 0\(^{\circ}\)C, and 36\(^{\circ}\)C. Although the model showed low agreement for Low–R1 sludge, values of correlation coefficient between the experimental data and the fitted model data were 0.987, 0.851, 0.995 for Mod–R, Low–R1, and Low–R2 sludges, respectively.

The temperature dependence of the specific decay rate \(b_{\text{AN}}^\text{opt}\) (d\(^{-1}\)) of all anammox sludge can be expressed as the van’t Hoff exponential equation\(^{27}\) (Eq. 5).

\[
\begin{align*}
\mu_{\text{opt}}^\text{AN} & = b_{\text{AN}}^\text{opt} \exp\left\{\theta_b(T-T_0)\right\} \quad \text{Eq. 5}
\end{align*}
\]

In that equation, \(b_{\text{AN}}^\text{opt}\) is the specific decay rate for anammox bacteria at 30\(^{\circ}\)C; \(\theta_b\) is the temperature coefficient (\(^{\circ}\)C\(^{-1}\)) for the bacterial decay. The values for \(b_{\text{AN}}^\text{opt}\) and \(\theta_b\) of the Low–R1, Low–R2, and Mod–R sludges were commonly assumed as typical values of 0.003 d\(^{-1}\) and 0.096\(^{\circ}\)C\(^{-1}\) respectively.

**Model simulation**

The anammox reactor was computed with continuous flow of ideal wastewater containing \(S_{\text{NH4, in}} = 50 \text{ mg-N/L}, S_{\text{NO2, in}} = 60 \text{ mg-N/L}, S_{\text{O2, in}} = 0 \text{ mg/L}, X_{\text{AN, in}} = 0 \text{ mg-COD/L}, \) and \(X_{\text{in}} = 0 \text{ mg-COD/L}. \) HRT was set at 0.5 d. The initial \(X_{\text{AN}}\) value in the reactor was set at 1000 mg-COD/L.

Temperature in the reactor was firstly set constant. After a steady-state was established in the reactor, temperature was increased stepwise by 1\(^{\circ}\)C. Secondly, annual temperature variations in the anammox reactor were given as;

\[
T = T_{\text{avg}} + T_{\text{amp}} \sin \left(\frac{2\pi}{365} t - \frac{\pi}{2}\right) \quad \text{Eq. 6}
\]

where \(T_{\text{avg}}\) and \(T_{\text{amp}}\) respectively denote the average temperature (\(^{\circ}\)C) and amplitude (\(^{\circ}\)C).

Total-nitrogen (T–N) removal (%) was calculated using the following equation:

\[
\text{T–N removal} = \frac{(S_{\text{NO3, in}} + S_{\text{NO2, in}} + S_{\text{NH4, in}}) - (S_{\text{NO3}} + S_{\text{NO2}} + S_{\text{NH4}})}{S_{\text{NO3, in}} + S_{\text{NO2, in}} + S_{\text{NH4, in}}} \times 100\% \quad \text{Eq. 7}
\]

Model simulation was performed using a simulation software package (Berkeley Madonna ver.8.3.18; University of California at Berkeley).

**RESULTS**

**Anammox reactors under constant temperature**

First, the nitrogen removal in the anammox reactor fed with wastewater (\(S_{\text{NH4, in}} = 50 \text{ mg-N/L}, S_{\text{NO2, in}} = 60 \text{ mg-N/L}\)) was simulated at SRT of 80 d at constant temperatures. Time courses of temperature, the bacterial population, the nitrogen concentrations, and the T–N removal are depicted in Fig. 2.

Although anammox bacteria were washed out from Mod–R at < 16\(^{\circ}\)C (data not shown), stable nitrogen removal was achieved at 17\(^{\circ}\)C (Fig. 2A). The aerobic endogenous respiration rate (R2 in Table 1) was negligible. Therefore, the bacterial population was determined mainly by the growth rate (R1 in Table 1), the anoxic respiration rate (R3 in Table 1), and the sludge withdrawal rate (Eq. 1). After a sufficient time of 850 days for establishment of a steady-state, the effluent NH\(_4\)-N, NO\(_2\)-N, and NO\(_3\)-N concentrations and the bacterial population in Mod–R were 0.72 mg/L, 4.25 mg/L, 6.93 mg/L, and 1.24 g–COD/L, respectively, resulting in T–N removal of 89%. The inert biomass in the reactor was about 1% against the anammox biomass. Subsequently, the temperature in Mod–R was increased stepwise by 1\(^{\circ}\)C at 250–d intervals which was more than three times longer than the SRT. NH\(_4\)-N was the rate-limiting substrate for the anammox reaction used instead of NO\(_2\)-N in Mod–R. With increased temperature, the effluent NH\(_4\)-N and NO\(_3\)-N concentrations decreased slightly, but the NO\(_3\)-N concentration increased slightly, resulting in almost constant T–N removals around 90%.

Under the same SRT with Mod–R, the stable nitrogen removal was achieved in Low–R1 at 12\(^{\circ}\)C (Fig. 2B). The nitrogen removal over 90% was achieved in Low–R1 with the stepwise increase of temperature up to 34\(^{\circ}\)C. However, anammox bacteria were washed out from Low–R1 at high temperatures of ≥ 35\(^{\circ}\)C because of the growth inhibition defined as the Rosso model. The T–N removal over 90% was stably achieved in Low–R2 at
widely various temperatures of 12–35°C (Fig. 2C).

Effects of constant temperatures on the steady-states of the anammox reactors at various SRTs are presented in Fig. 3. At longer SRTs, the washout temperature for anammox bacteria in Mod-R decreased, respectively, i.e. 20°C, 16°C, and 10°C at the SRT of 50 d, 80 d, and 150 d. When steady-states were obtained, the T-N removal was around 90%. The bacterial population increased from about 0.75 g-COD/L to 2.0–2.2 g-COD/L with increase in SRT from 50 d to 150 d. In Low-R1, anammox bacteria were washed out both below 13°C and over 32°C at SRT of 50 d because of the growth inhibition at low and high temperatures. At longer SRTs, anammox bacteria were retained in Low-R1 at 10–35°C at SRT of 150 d. In Low-R2, anammox bacteria were retained at temperatures of >12°C at the SRT of 50 d. At longer SRTs, anammox bacteria were able to survive in Low-R2 at 10–35°C at SRT of 150 d.

**Anammox reactors under annually varying temperatures** Effects of annually varying temperatures on the anammox reactors were computed at SRT of 80 d. Time courses of the T-N removal and the bacterial population are depicted in Fig. 4. Periodic steady-states were obtained in all anammox reactors after 2–3 years. The T-N removal and the bacterial population of the anammox reactors under annually varying temperatures are presented.
Fig. 3 Effects of temperature on stable steady-states of the annamox reactors fed with wastewater ($S_{\text{NH}_{4},\text{in}} = 50 \text{ mg/L, } S_{\text{NO}_{2},\text{in}} = 60 \text{ mg/L, } S_{\text{O}_{2},\text{in}} = 0 \text{ mg/L}$) at HRT=0.5 d and various SRT (50, 80, and 150 d): (A) Mod-R, (B) Low-R1, and (C) Low-R2. Solid line, T-N removal; dashed line, anammox bacteria biomass ($X_{\text{AN}}$).

Fig. 4 Periodic states of the annamox reactors, Mod-R, Low-R1, and Low-R2, fed with wastewater ($S_{\text{NH}_{4},\text{in}} = 50 \text{ mg/L, } S_{\text{NO}_{2},\text{in}} = 60 \text{ mg/L, } S_{\text{O}_{2},\text{in}} = 0 \text{ mg/L}$) at HRT=0.5 d and SRT = 80 d against the annual cycle of water temperature ($T = T_{\text{avg}} + T_{\text{amp}} \sin(2\pi t/365 - \pi/2)$). (A) $T_{\text{avg}} = 17.5\degree \text{C}, T_{\text{amp}} = 7.5\degree \text{C}$, (B) $T_{\text{avg}} = 27.5\degree \text{C}, T_{\text{amp}} = 7.5\degree \text{C}$, and (C) $T_{\text{avg}} = 22.5\degree \text{C}, T_{\text{amp}} = 12.5\degree \text{C}$. Solid line, T-N removal; dashed line, anammox bacteria biomass ($X_{\text{AN}}$).
in Fig. 5 as box–whisker plots of the daily values.

Under the temperature variation of 10–25°C ($T_{\text{avg}} = 17.5^\circ\text{C}$ and $T_{\text{amp}} = 7.5^\circ\text{C}$), the T-N removal dropped to 30%, 54%, and 84% at the lowest temperature, respectively, in Mod-R, Low-R1, and Low-R2, although it recovered up to 90% with temperature rise. No phase difference of the annual variation existed between temperature and the T-N removal, which was reflected mainly by the growth rate of anammox bacteria. However, the annual variation of the bacterial population slightly lagged that of temperature.

Given temperature variation of 20–35°C ($T_{\text{avg}} = 27.5^\circ\text{C}$ and $T_{\text{amp}} = 7.5^\circ\text{C}$), the T-N removal rate was higher than 90% through a year in Mod-R and Low-R2 with slightly varying bacterial populations. However, the T-N removal rate in Low-R1 dropped to 68% at the highest temperature. Under large temperature variation of 10–35°C ($T_{\text{avg}} = 22.5^\circ\text{C}$ and $T_{\text{amp}} = 12.5^\circ\text{C}$), the T-N removal dropped to 34% and 83%, respectively, in Mod-R and Low-R2 at the lowest temperature, although it increased up to 90% with temperature rise. The T-N removal rate in Low-R1 was higher than 90% at 22–31°C, dropping respectively to 68% and 53% at the lowest temperature and the highest temperature.

**DISCUSSION**

This report is the first describing a study of the anammox model as a function of temperature (the Rosso equation) except for those using the van’t Hoff exponential equation\(^{(19)}\). The Rosso equation with four parameters ($T_{\text{min}}$, $T_{\text{opt}}$, $T_{\text{max}}$, and $\mu_{\text{AN}}^{\text{opt}}$) fitted the experimental data at 10–35°C. Although the simulation results might not be interpreted as quantitatively correct, they can provide a qualitative prediction of the anammox process.

Simulations in this study predicted more stable nitrogen removal in Low-R1 and Low-R2 at low temperatures than in Mod-R. Although Low-R1 at short SRTs showed lower nitrogen removal rates than Mod-R at high temperatures around 35°C, Low-R2 demonstrated robust T-N removal in the wide temperature range of 10–35°C. The temperature of sewage flowing to domestic wastewater treatment plants is generally 20–30°C in warm regions and 10–20°C in cold regions in Japan. Sewage temperatures are rarely below 10°C, with some exceptions such as during winter months in cold regions.
as temporary declines caused by storm water collected in combined sewerage systems on snowy days. Therefore, the anammox sludges, especially in Low–R2, have sufficient potential to develop versatile nitrogen removal processes with no heating system in mild climate regions.

From these results, it was inferred that high T-N removals can be achieved in all reactors at low temperatures and in Low–R1 at high temperatures if the SRT is kept sufficiently long for anammox bacteria (e.g. 150 d) (Figs. 2 and 3). Actually the typical SRT is 45–160 d in a full-scale granular-type anammox reactor. Immobilization techniques have been used for extending the SRT in anammox reactors using supporting media such as non-woven polyester fabric materials, polyvinyl alcohol (PVA) gel beads, and polyethylene glycol gel (PEG) carrier. Reportedly, anammox bacteria can coexist with heterotrophic bacteria even under high organic loadings when SRT is extended extremely to 700 d.

An important assumption made for this study was that the parameter values of anammox bacteria (Tables 2–4) were unchanged by the temperature variation although the values can actually affect competition among bacteria. The Low–R1 and Low–R2 sludges held diverse bacteria including uncultured anammox-like or planctomycete-like bacteria, although Candidatus Kuenenia stuttgartiensis dominated in the Mod–R sludge. Population dynamics of bacteria should be monitored and controlled in a real scale-up process of the anammox reactors. The initial bacterial composition would be conserved for a long time in cell-entrapping immobilization carriers such as PVA and PEG. Otherwise, the bacterial community in the anammox sludge would be adapted further to low temperatures.

The anammox process for wastewater treatment requires combination with nitrite-producing processes, such as nitritation by ammonia-oxidizing bacteria and nitrate reduction by heterotrophic bacteria. Reportedly, the nitritation rate was more sensitive to temperatures lower than the anammox rate at 15℃ in a single-stage nitrogen removal using anammox and partial nitritation process. Continued modeling and simulation of the temperature dependence of nitritation and denitrification and the spatial distribution of anammox bacteria, nitrifying bacteria, and heterotrophic bacteria in biofilms or granules are necessary for the design and operation of nitrogen removal reactors using the anammox process.

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