Modeling a nitrite-dependent anaerobic methane oxidation process: Parameters identification and model evaluation

Zhanfei He, Chen Cai, Sha Geng, Liping Lou, Xiangyang Xu, Ping Zheng, Baolan Hu

Department of Environmental Engineering, Zhejiang University, Hangzhou 310058, China

**Highlights**

- First time identified several key kinetic parameters for n-damo bacteria.
- Methane was not a limiting factor of n-damo process.
- The optimal nitrite concentration was 1.92 mmol L\(^{-1}\).
- A kinetic model for n-damo process was first established and evaluated.

**Abstract**

Nitrite-dependent anaerobic methane oxidation (n-damo) is a recently discovered process that is mediated by n-damo bacteria that oxidize methane with nitrite to generate nitrogen gas. In this work, a kinetic model based on Monod type kinetics and diffusion-reaction model was developed to describe the bioprocess. Some key kinetic parameters needed in the model were obtained from a series of batch activity tests and a sequencing batch reactor (SBR) operation over 100 days. The growth rate, decay rate, methane affinity constant, nitrite affinity constant and inhibition constant were 0.0277 ± 0.0022 d\(^{-1}\), 0.00216 ± 0.00010 d\(^{-1}\), 0.092 ± 0.005 mmol L\(^{-1}\), 0.91 ± 0.09 mmol L\(^{-1}\) and 4.1 ± 0.5 mmol L\(^{-1}\) for n-damo bacteria at 30°C, respectively. The results showed that the model could simulate actual performance of the SBR in the first 76 days, that methane was not a limiting factor at atmospheric pressure for its high affinity, and that the optimum nitrite concentration was 1.92 mmol L\(^{-1}\).

**1. Introduction**

Nitrite-dependent anaerobic methane oxidation (n-damo), a microbial process using nitrite as the electron acceptor to anaerobically oxidize methane, has been discovered in the last decade (Raghoebarsing et al., 2006). The stoichiometric equation is showed in Eq. (1). The process has been noticed widely (Ettwig et al., 2008; Hu et al., 2009; Shen et al., 2012), because it has several advantages over conventional wastewater treatment processes, including efficient utilization of methane (a potent greenhouse gas) and removal of nitrogenous pollutants simultaneously. Although the methane in the gas phase can be recycled and used for electric energy generation, the dissolved methane in the effluent water from a wastewater treatment plant is difficult to recover and will be released slowly into the environment contributing to the greenhouse effect (Cakir and Stenstrom, 2005). The process was suggested to treat the effluent from low-temperature anaerobic sewage treatments that contained dissolved methane and nitrogen, in which dissolved methane could be used as solo electron donor for denitrification. Kampman et al. proposed a new concept for treating anaerobic treatment effluent that consisted of a UASB-digester system, an n-damo reactor and a nitritation reactor (Kampman et al., 2012). Luesken et al. verified that n-damo bacteria and anammox bacteria could coexist experimentally, and the application of such a coculture for nitrogen removal may be feasible in the near future (Luesken et al., 2011). However, the growth of n-damo bacteria is too slow to obtain sufficient enrichment culture (Ettwig et al., 2008; Raghoebarsing et al., 2006), which has prevented research progress from being made on the n-damo process. The growth could be accelerated by breaking some "bottle necks", and several limiting factors were discussed in this work.

\[
\text{3CH}_4 + 8\text{NO}_2 + 8\text{H}^+ \rightarrow 3\text{CO}_2 + 4\text{N}_2 + 10\text{H}_2\text{O} \quad (\Delta G^\circ = -928\text{kJ mol}^{-1}\text{CH}_4) (1)
\]

Mathematical model is a useful tool to give an insight of biologic reaction system, to help design an activated sludge treatment system, and to optimize operational parameters. There have been many successful cases where mathematic models were used to...
help design and operate biologic wastewater treatment systems (Amand and Carlsson, 2012; Young et al., 2013). Despite the usefulness of the mathematic model, there is no mathematic model of the n-damo process reported to our knowledge, mainly because several key kinetic parameters have not been determined. In this work, some key kinetic parameters were identified from a series of batch activity tests and a sequencing batch reactor (SBR) operation. The main goals of this work are to develop a concise kinetic model to describe the n-damo process and to identify the key kinetic parameters of n-damo bacteria. The n-damo model is based on Monod type kinetics (Ni et al., 2009) and diffusion-reaction model (Wanner and Gujer, 1986). Moreover, the growth limiting factors of n-damo bacteria were discussed on the foundation of the results in this work.

2. Methods

2.1. Biomass and medium

The inoculum of a 1.0 L SBR was taken from a previous lab-scale SBR fed with artificial medium after 500 days. The medium contained the following components (per liter): 1.0 g KHCO₃, 0.05 g KH₂PO₄, 0.3 g CaCl₂·2H₂O, 0.2 g MgSO₄·7H₂O, 0.014 g NaNO₂, 0.5 ml of an acidic trace element solution, and 0.2 ml of an alkaline trace solution element. The acidic (100 mM HCl) trace element solution contained (per liter) 2.085 g FeSO₄·7H₂O, 0.068 g ZnSO₄·7H₂O, 0.12 g CoCl₂·6H₂O, 0.5 g MnCl₂·4H₂O, 0.32 g CuSO₄·0.995 g NiCl₂·6H₂O, and 0.014 g H₂BO₃. The alkaline (10 mM NaOH) trace element solution contained (per liter) 0.067 g SeO₂, 0.050 g Na₂WO₄·2H₂O, and 0.242 g Na₂MoO₄ (Ettwig et al., 2009). The medium was flushed by Ar–CO₂ (95:5) for 15 min and pH was control at 7.0–7.2 before feeding.

2.2. Operation of SBR

The previous lab-scale SBR (2.0 L) was operated for 500 days, and then another SBR (1.0 L) was operated continually for 100 days and shut down for 25 days. The inoculum of the new SBR was taken from the previous SBR after 500 days. The biomass used in the batch tests for parameters identification (affinity constant and inhibition constant) was taken from the previous lab-scale SBR on day 420. The growth rate of n-damo bacteria was obtained from the 100 days operation period of the new SBR and the decay rate coefficient was measured over the 25 days shutdown period. Moreover, the biomass from the new SBR was sampled on day 1, 45 and 94 to measure the n-damo activity.

The new reactor owned 1.0 L of working volume and 0.3 L of headspace, and the inoculum sludge volume was 0.3 L. The operation cycles contained 22 h of reaction, 1.8 h of settling and 0.2 h of influent inflow and effluent discharge. The exchange volume was 0.5 L (exchange ratio was 0.5), and the hydraulic retention time was 48 h. The reactor was supplied continually by methane (99.99%) at 10 ml min⁻¹ and stirred by a magnetic stirring son at 150 rpm, and the operating temperature was 30 ± 0.5 °C.

2.3. N-damo activity measurement

N-damo activity measurement was conducted in 70 ml serum bottles containing 10 ml of biomass (sediment after 1.8 h of settling), 30 ml of medium and 30 ml of gases. The biomass was washed with a nitrite-free medium and transferred immediately into serum bottles with 30 ml medium. Subsequently, the serum bottles were flushed by Ar–CO₂ (95:5) for 10 min and sealed rapidly. These bottles were divided into two groups equally, one of which was the experimental group, and the other was the control group. Every group has three replicates. Methane (99.99%) was only replaced into the experimental group to obtain certain partial pressures. The bottles were incubated in a shaking table at 30 ± 0.5 °C, and 0.5 ml sample was taken and centrifuged (5 min, 7440g) every 1.5 h from 0.5 h to 8.0 h. The concentrations of nitrite in the supernatant were determined. The n-damo activity was calculated by subtracting the nitrite conversion rate in the control group from that in the corresponding experimental group.

2.4. Analytical methods, data treatment and modeling software

The measurements of nitrite and volatile suspended solids (VSS) were performed according to standard methods (APHA, 2005), while pH was measured using a PHS-9V acidimeter (Hangzhou Huaguang Radio Factory, China) and DO was measured by a JPB-607 DO meter (Shanghai Rex Instrument Factory, China). The experimental data were combined, processed, and analyzed using MS Excel 2007, the figure of contour lines was constructed by Matlab 7.0, the other figures were plotted by Origin 8.0, and the model was simulated by Matlab 7.0.

3. Model development

3.1. Fundamental assumptions

To mathematically solve the process of biologic reaction and methane diffusion, some simplifying assumptions were made as follows: (1) the biomass was in solid dominantly and the rest in liquid could be ignored; (2) the biomass was constant in the batch experimental time (about 8 h); (3) the reaction system reached a quasi-steady-state quickly; (4) the methane transfer coefficient (Kₐ) was constant; (5) the effects of sampling were ignored; (6) the medium and sludge were mixed completely; and (7) the differences of methane solubility and mass transfer coefficient between medium and distilled water were neglected.

3.2. Biologic reaction kinetics

In this model, multiple Monod kinetic type was applied to describe the dependency of the n-damo bacteria growth on nitrite and dissolved methane concentrations. Notably, the inhibitory effect of nitrite was also described by Monod type. Hence, the conversion rates of methane and nitrite can be presented as Eqs. (2) and (3), respectively.

\[
r_{\text{CH}_4} = \frac{\mu_{\text{max}} X_{\text{DA}}}{Y_{\text{DA}}} \cdot \frac{S_{\text{CH}_4}}{K_{\text{CH}_4} + S_{\text{CH}_4}} \cdot \frac{S_{\text{NO}_2}}{K_{\text{NO}_2} + S_{\text{NO}_2}} \cdot \frac{K_{\text{NO}_2}'}{K_{\text{NO}_2}'} + S_{\text{NO}_2}
\]

\[
r_{\text{NO}_2} = \frac{8 \mu_{\text{max}} X_{\text{DA}}}{3 Y_{\text{DA}}} \cdot \frac{S_{\text{CH}_4}}{K_{\text{CH}_4} + S_{\text{CH}_4}} \cdot \frac{S_{\text{NO}_2}}{K_{\text{NO}_2} + S_{\text{NO}_2}} \cdot \frac{K_{\text{NO}_2}'}{K_{\text{NO}_2}'} + S_{\text{NO}_2}
\]

where \( r_{\text{CH}_4} \) is the conversion rate of methane, \( r_{\text{NO}_2} \) is the conversion rate of nitrite for n-damo process, \( \mu_{\text{max}} \) is the maximum growth rate of n-damo bacteria, \( Y_{\text{DA}} \) is the yield coefficient for n-damo bacteria growth on methane, \( S_{\text{CH}_4} \) is the concentration of dissolved methane, \( S_{\text{NO}_2} \) is the concentration of nitrite, \( K_{\text{CH}_4} \) is the methane affinity constant for n-damo bacteria, \( K_{\text{NO}_2} \) is the nitrite affinity constant for n-damo bacteria, \( K_{\text{NO}_2}' \) is the nitrite inhibition constant for n-damo bacteria, and \( X_{\text{DA}} \) is the active biomass of n-damo bacteria. Notably, all the parameters of n-damo in this work were used for a group of bacteria that intermediate the n-damo process.
3.3. Methane gas–liquid diffusion

The n-damo process involves in an essential gas substrate, methane, so the first step is the diffusion of methane from the headspace (gas phase) into the culture solution (liquid phase) where the biologic reaction occurs. The diffusion-reaction concept was introduced to describe the n-damo process including gas–liquid diffusion and biologic reaction. The Eq. (4) could describe the diffusion-reaction kinetics in a gas–liquid system at a quasi-steady-state.

\[ k_l a (S_{CH_4} - S_{CH_4}^*) - r_{CH_4} = 0 \]  

(4)

where \( k_l a \) is the volumetric mass transfer coefficient of methane and \( S_{CH_4}^* \) is the saturation concentration of methane in liquid phase.

The mass transfer is an important factor of heterogeneous bioreactions, and may become a rate-limiting step sometimes (Lebrero et al., 2013; Vangsgaard et al., 2012). Thus n-damo process may also be limited by methane mass transfer. The \( k_l a \) value could reflect the mass transfer property, and the effect of \( k_l a \) on n-damo activity was studied in this work.

3.4. Model analysis

Combining the above biologic kinetics and mass transfer dynamics, Eq. (5) could be obtained by inserting Eq. (2) into Eq. (4).

\[
\frac{r_{CH_4}}{r_{max}} = \left\{ 1 + 2 \left[ \left( \frac{r_{max}}{K_{CH_4}K_a} + 1 - \frac{S_{CH_4}}{K_{CH_4}} \right)^2 + 4 \frac{S_{CH_4}}{K_{CH_4}} \right] \right\}^{-1} 
\]

(5)

where \( r_{max} = \frac{\alpha_k}{Y_{CH_4}K_{NO_2}} = \frac{\alpha_k}{Y_{CH_4}K_{NO_2}}X_{DA} \) Actually, Eq. (5) is equivalent to the switching function, \( S_{CH_4}/(S_{CH_4}+K_{CH_4}) \), in Eqs. (2) and (3), and they present the same thing, the limitation of methane from different perspectives. The \( r_{max} \) in Eq. (5) is the maximal conversion rate of methane that can be obtained without methane limitation, when the biomass and nitrite concentrations are fixed. From Eq. (5), the ratio of conversion rates, \( r_{CH_4}/r_{max} \), is a function of two dimensionless groups, \( k_l a K_{CH_4}/r_{max} \) and \( S_{CH_4}/K_{CH_4} \), in which the former group is effected by mass transfer and the latter is determined by methane solubility. According to the above correlation, contour lines of the ratio of methane conversion rates are presented in Fig. 1. The conversion ratios are constant along each curve. The curves close to the abscissa axis have less intervals than the farther ones, which indicates that the \( S_{CH_4}/K_{CH_4} \) has a larger effect or higher sensitivity on the conversion ratios when the \( S_{CH_4}/K_{CH_4} \) value is lower, and the \( K_l a K_{CH_4}/r_{max} \) has the same characteristic. In short, the short figure shows the sensitivities or limitations of the two dimensionless groups (or \( S_{CH_4}^* \) and \( k_l a \)). At \( k_l a K_{CH_4}/r_{max}<1 \), the limitation of the n-damo process is linked to \( k_l a \), at \( k_l a K_{CH_4}/r_{max}>2 \), the limitation is linked to \( S_{CH_4}^* \), and \( S_{CH_4}/K_{CH_4} \) has the similar feature.

4. Results and discussion

4.1. Parameters identification

Fig. 2 shows the results of the specific activity tests with varying nitrate concentration, and the data points are fitted by a multiple Monod kinetic equation, Eq. (3). The multiple Monod type could describe the inhibitory effect of nitrite for n-damo process well, because the adjusted coefficient of determination (Adj. R-square) was up to 0.941. The nitrite affinity constant and inhibition constant of n-damo bacteria, \( K_{NO_2} \) and \( K_{NO_2}^* \), are 0.91 ± 0.09 mmol L\(^{-1}\) and 4.1 ± 0.5 mmol L\(^{-1}\), respectively, which are identified from the curve fitting.

Low nitrite concentration limited the growth of n-damo bacteria, but over high nitrite concentration would inhibit that. The optimal nitrite concentration was the highest point of the fitted curve in Fig. 2, and could be calculated by taking the square root of the product of affinity and inhibition coefficient, \( (K_{NO_2}K_{NO_2}^*)^{1/2} \), according to the multiple Monod kinetic type. The optimal value was 1.92 mmol L\(^{-1}\) and 1–3 mmol L\(^{-1}\) could be recommended as the appropriate concentration range under the experimental conditions based on the fitted curve.

The results of the specific activity tests with different solubilities of methane are shown in Fig. 3, the data points are fitted by Eq. (3) as well, and its Adj. R-square is up to 0.995. The methane affinity constant of n-damo bacteria was a crucial parameter, and it was 0.092 ± 0.005 mmol L\(^{-1}\) identified by the fitting in Fig. 3.

The n-damo activity increased but the increase rate dropped over methane concentration, which indicated that the dissolved methane did not have inhibitory effect among the experimental range and only have very little effect when its solubility was high. As long as the solubility of methane was up to 0.83 mmol L\(^{-1}\) (the methane partial pressure in headspace was 67 kPa at 30 °C), the n-damo activity could be very close (>90%) to its maximum activity.

Fig. 1. Contour lines with different ratios of methane conversion rates, \( r_{CH_4}/r_{max} \).
is the decay rate coefficient of n-damo bacteria, the initial nitrite concentration was 0.2 mmol L\(^{-1}\), and the agitation rate of the shake table was 150 rpm.

The methane affinity constant for sulfate-dependent anaerobic methane oxidation (SAMO) microbe was >1.0 mmol L\(^{-1}\) (Roel Meulepas, 2010; Zhang et al., 2010) that was significantly higher than that of n-damo bacteria identified in this study. N-damo bacteria have a strong affinity for methane, possibly because these microbe have evolved in fresh water systems (low pressure and low dissolved methane environment) in the past for a long time (Deutzmann and Schink, 2011; Kojima et al., 2012), while SAMO microbe mainly exist in ocean sediments (Antje Boetsius, 2000; Wankel et al., 2012).

The decay rate coefficient of n-damo bacteria, \(b_{\text{DA}}\), was determined by measuring the decreasing of nitrite conversion rate for n-damo twice before and after the 25 days reactor shutdown period. Both batch tests were conducted under the same experimental conditions (e.g. temperature, agitation rate, methane partial pressure, initial nitrite concentration, etc.), the parameters and substance concentrations in Eq. (3) were assumed to be constant, and \(b_{\text{DA}}\) could be calculated by Eq. (6). The value of \(b_{\text{DA}}\) was 0.00216 ± 0.00010 d\(^{-1}\), which was slightly lower than that of ANAMMox bacteria, another low growth bacteria anaerobic consuming nitrite (Ni et al., 2009; Scaglione et al., 2009).

\[
b_{\text{DA}} = \frac{\ln(r_{\text{NO}_2}/r_{\text{NO}_2}^0)}{\Delta t}
\]

where \(b_{\text{DA}}\) is the decay rate coefficient of n-damo bacteria, \(r_{\text{NO}_2}^0\) is the initial nitrite conversion rate for n-damo, \(r_{\text{NO}_2}\) is the final nitrite conversion rate for n-damo, and \(\Delta t\) is the reactor shutdown period.

The actual growth rate of n-damo bacteria, \(\mu_{\text{DA}}\), is a key kinetic parameter of n-damo bacteria enrichment and cultivation. According to mass balance principle, the growth biomass of n-damo bacteria consists of net growth biomass in reactor, wash out biomass with effluent, and decay biomass, so the actual growth rate of n-damo bacteria is the sum of net growth rate, \(\mu_{\text{Net}}\), wash out rate, \(w_{\text{DA}}\), and decay rate, \(b_{\text{DA}}\). Biomass was fetched out from the SBR on 0 and 45 day, and n-damo activity measurement was conducted to estimate the \(\mu_{\text{Net}}\) value. The \(\mu_{\text{Net}}\) value was 0.01368 ± 0.00120 d\(^{-1}\) that was obtained by measuring the increasing of nitrite conversion rate for n-damo before and after the 45 days operation period, which was similar to \(b_{\text{DA}}\) determination. Wash out rate could be estimated by multiplying volume exchange ratio by biomass withdrawn fraction (biomass concentration ratio of in effluent and in reactor). The biomass withdrawn fraction and the fraction of n-damo bacteria in VSS were assumed to be constant under stable experimental conditions. The biomass withdrawn fraction was 0.0237 ± 0.0018 obtained by measuring VSS in effluent and in reactor (0.0564 ± 0.0029 and 2.38 ± 0.06 gVSS L\(^{-1}\), respectively), so the \(w_{\text{DA}}\) value was 0.01185 ± 0.00099 d\(^{-1}\) and the \(\mu_{\text{DA}}\) value was 0.0277 ± 0.0022 d\(^{-1}\). Moreover, the doubling time of n-damo bacteria could be calculated by \(\ln(2)/\mu_{\text{DA}}\), and its value was 25.0 d that is similar to reported values (Ettwig et al., 2008; Kampman et al., 2012; Raghoebarsing et al., 2006). Finally, the kinetic coefficients of n-damo bacteria identified in this study are listed in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu_{\text{DA}})</td>
<td>Growth rate on CH(_4)</td>
<td>0.0277 ± 0.0022</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>(b_{\text{DA}})</td>
<td>Decay rate coefficient</td>
<td>0.00216 ± 0.00010</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>(K_{\text{CH}_4})</td>
<td>CH(_4) affinity constant</td>
<td>0.092 ± 0.005</td>
<td>mmol L(^{-1})</td>
</tr>
<tr>
<td>(K_{\text{NO}_2})</td>
<td>NO(_2) -N affinity constant</td>
<td>0.91 ± 0.09</td>
<td>mmol L(^{-1})</td>
</tr>
<tr>
<td>(K_{\text{NO}_3})</td>
<td>NO(_3) -N inhibition constant</td>
<td>4.1 ± 0.5</td>
<td>mmol L(^{-1})</td>
</tr>
</tbody>
</table>
4.3. The simulation of effluent nitrite concentration

The SBR reactor was operated for 100 days and the influent and effluent nitrite concentrations were determined daily. Moreover, batch tests were conducted on 1, 45, and 94 day, to measure n-damo activities. The results of batch tests showed that the nitrite conversion rate for other processes, \( r_{\text{other}} \), was stable, \( 0.0029 \pm 0.0002 \text{ mmol h}^{-1} \), and was considered to be a constant during the operation period in this model work. Besides, the dissolved methane was also regarded as a constant, for methane gas was supplied continuously and sufficiently. To simulate the effluent of the SBR, the Eq. (3) was reduced into Eq. (7).

\[
\begin{align*}
\frac{dn_{\text{NO}_2}}{dt} &= K e^{\mu_{\text{net}}} \frac{S_{\text{NO}_2}}{K_{\text{NO}_2} + S_{\text{NO}_2}} - \frac{K_{\text{NO}_2}^i}{K_{\text{NO}_2} + S_{\text{NO}_2}} \\
&= \frac{K e^{\mu_{\text{net}}} S_{\text{NO}_2} - K_{\text{NO}_2}^i}{K_{\text{NO}_2} + S_{\text{NO}_2}} \\
&= \frac{K e^{\mu_{\text{net}}} S_{\text{NO}_2}}{K_{\text{NO}_2} + S_{\text{NO}_2}} - \frac{K_{\text{NO}_2}^i}{K_{\text{NO}_2} + S_{\text{NO}_2}} \\
&= r_{\text{NO}_2} - \frac{K_{\text{NO}_2}^i}{K_{\text{NO}_2} + S_{\text{NO}_2}}
\end{align*}
\]

where, \( K = \frac{K_{\text{NO}_2}^i}{S_{\text{NO}_2}} \) is a constant under the above simplifying assumptions, \( S_{\text{NO}_2} \) is the initial n-damo biomass, \( \mu_{\text{net}} \) is the net biomass growth rate of n-damo bacteria, and \( t \) is the operating time. The \( K \) value was estimated by best fitting the first 10 days’ data. Best fitting was obtained when the objective function, \( \Sigma_{i=1}^{n} (S_{\text{experimental}} - S_{\text{model}})^2 / \Sigma_{i=1}^{n} (S_{\text{experimental}})^2 \), reached a minimum (Tang et al., 2012). The function is a relative least-squares criterion, and \( S_{\text{experimental}} \) and \( S_{\text{model}} \) are the effluent experimental and simulated nitrite concentrations of the first ten data points indicated by \( i \).

On the basis of Eq. (7) and mass balance principle, Eq. (8) (an ordinary differential equation, ODE) was derived to solve the SBR system. The effects of settling and exchanging medium on the conversion rate of nitrite were assumed to be neglected. According to Eq. (8), the simulated data of the effluent nitrite concentration were calculated numerically by Matlab 7.0 and presented in Fig. 5.

\[
V_{\text{working}} \frac{dS_{\text{NO}_2}}{dt} = (K e^{\mu_{\text{net}}} \frac{S_{\text{NO}_2}}{K_{\text{NO}_2} + S_{\text{NO}_2}} - \frac{K_{\text{NO}_2}^i}{K_{\text{NO}_2} + S_{\text{NO}_2}} + r_{\text{other}}) \text{d}t
\]

where \( V_{\text{working}} \) is the working volume and \( r_{\text{other}} \) is the nitrite conversion rate for other processes.

The comparison between the experimental and simulated results indicated that the model could predict the effluent nitrite concentrations well during 1–76 days, but failed to meet the experimental results in the last 24 days. The high experimental effluent nitrite concentrations were probably caused by some important limiting factors not considered in the model. Some previous studies indicated that the nitrite consumption rates decreased after reaching a maximum (Ettwig et al., 2008; Kampman et al., 2012), and a similar phenomenon was found in this reactor where the nitrite consumption rates no longer continued to increase in the later stage of operation. Nevertheless, the specific reasons of decreasing or stagnating were not clear and it was difficult to be included in model at present.

4.4. The discussion of limiting factors

Methane is a really easy limiting factor of n-damo process for its low solubility in water, and especially previous studies of anaerobic methane oxidation indicated that high methane pressure could increase biologic activity (Katja Nauhaus, 2002; Zhang et al., 2010). Nevertheless, it is convinced that methane is not a limiting factor of n-damo process at atmospheric pressure from this study. On the one hand, the optimal nitrite concentration is 1.92 mmol L\(^{-1}\) calculated from \( K_{\text{NO}_2} \) and \( K_{\text{NO}_2}^i \), and its corresponding dissolved methane concentration is 0.72 mmol L\(^{-1}\) according to theoretic stoichiometric ratio in Eq. (1). On the other hand, methane affinity constant is 0.092 mmol L\(^{-1}\). The solubility of methane is 1.237 mmol L\(^{-1}\) at 30 °C and 100 kPa (Haynes, 2011) that is bigger than both of the two values above, and the dissolved methane switching function, \( S_{CH_4}/(S_{CH_4} + K_{CH_4}) \), can be up to 0.93.

Now that methane was not a limiting factor and nitrite could not be a potent inhibitor in the later period, the n-damo bacteria activity decrease or stagnation may be caused by the absence of growth factors (Ettwig et al., 2008). To enrich n-damo bacteria and limit heterotrophic bacteria, there was no organic matter added into the present medium, which may limit the growth of n-damo bacteria in the later period for growth factors deficiency. A previous study had focused on the problem, and tried to add sewage treatment effluent as a source of potential growth factors, but did not draw a clear conclusion (Kampman et al., 2012). Further research should focus on the culture medium optimization.

5. Conclusions

A kinetic model for n-damo process was established and well predicted the effluent nitrite concentration of an SBR in the first 76 days. Several key kinetic parameters for n-damo bacteria were identified and listed in Table 1. The optimal nitrite concentration for n-damo bacteria was 1.92 mmol L\(^{-1}\), and the recommended
$k_0$ value was up to 10 h$^{-1}$. Methane was not a limiting factor of n-damo process at atmospheric pressure for its high affinity, and there probably were some unknown growth factors missing in the present medium.

Acknowledgement

The authors wish to thank the Natural Science Foundation (Nos. 51108408 and 41276109).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.08.001.

References

Haynes, W.M., 2011. CRC Handbook of Chemistry and Physics, 92 ed. CRC Press Taylor & Francis Group, Colorado, USA.