Simulation modeling for nitrogen removal and experimental estimation of mass fractions of microbial groups in single-sludge system

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Abstract

Nitrification–denitrification in a single-sludge nitrogen removal system (SSNRS; with a sufficient carbon source for denitrification) was performed. With an increase in the mixed liquor recycle ratio \( R_m \) from 1 to 2, the total nitrogen (TN) removal efficiency at a lower volumetric loading rate \( (\text{VLR} = 0.21 \text{ NH}_4^+ - \text{N m}^{-3} \text{ d}^{-1}) \) increased, but the TN removal efficiency at a higher VLR \( (0.35 \text{ kg NH}_4^+ - \text{N m}^{-3} \text{ d}^{-1}) \) decreased. A kinetic model that accounts for the mass fractions of Nitrosomonas, Nitrobacter, nitrate reducer and nitrite reducer \( (f_{n1}, f_{n2}, f_{dn1}, \text{ and } f_{dn2}) \) in the SSNRS and an experimental approach for the estimation of the mass fractions of nitrogen-related microbial groups are also proposed. The estimated \( f_{dn1} + f_{dn2} \) (0.65–0.83) was significantly larger than the \( f_{n1} + f_{n2} \) (0.28–0.32); the \( f_{n1} \) (0.21–0.26) was larger than the \( f_{n2} \) (0.05–0.07); and the \( f_{dn1} \) (0.32–0.45) varied slightly with the \( f_{dn2} \) (0.33–0.38). At the lower VLR, the \( f_{dn1} + f_{dn2} \) increased with increasing \( R_m \); however at the higher VLR, the \( f_{dn1} + f_{dn2} \) did not increase with increasing \( R_m \). By using the kinetic model, the calculated residual \( \text{NH}_4^+ - \text{N} \) and \( \text{NO}_2^- - \text{N} \) in the anoxic reactor and \( \text{NO}_3^- - \text{N} \) in the aerobic reactor were in fairly good agreement with the experimental data; the calculated \( \text{NO}_3^- - \text{N} \) in the anoxic reactor was over-estimated and the calculated \( \text{NH}_4^+ - \text{N} \) in the aerobic reactor was under-estimated.

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Keywords: Single-sludge system; Nitrification–denitrification; Kinetic model; Nitrogen-related microbial groups

1. Introduction

Nitrification–denitrification is to convert ammonia via nitrite to nitrate by strict aerobic nitrifiers (Nitrosomonas and Nitrobacter) and thereby to convert nitrate and nitrite to nitrogen gas by facultative aerobic denitrifiers (nitrate reducer and nitrite reducer). Many varieties of denitrifiers (e.g., Paracoccus denitrificans) have the capability to reduce nitrate and nitrite (Dodd and Bone, 1975), whereas, some groups of denitrifiers only reduce nitrate to nitrite and others reduce nitrate to nitrogen gas (McCarty et al., 1969).

To create such conditions that favor desired reactions of carbon oxidation, nitrification and denitrification, several single-sludge systems such as anaerobic/aerobic (A/O), anaerobic/anoxic/aerobic (A²/O), University of Cape Town (UCT), sequencing batch reactor (SBR), and Virginia Initiative Plant (VIP) have been developed.
### Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{k0}$</td>
<td>NH$_3$-N influent concentration (mg l$^{-1}$)</td>
</tr>
<tr>
<td>$C_{k1}$</td>
<td>NH$_3$-N concentration in anoxic reactor (mg l$^{-1}$)</td>
</tr>
<tr>
<td>$C_{k2}$</td>
<td>NH$_3$-N concentration in aerobic reactor (mg l$^{-1}$)</td>
</tr>
<tr>
<td>$C_{n1}$</td>
<td>NO$_3$-N concentration in anoxic reactor (mg l$^{-1}$)</td>
</tr>
<tr>
<td>$C_{n2}$</td>
<td>NO$_3$-N concentration in aerobic reactor (mg l$^{-1}$)</td>
</tr>
<tr>
<td>$f_{dn}$</td>
<td>mass fraction of nitrite reducer in denitrifiers (dimensionless)</td>
</tr>
<tr>
<td>$f_{dn1}$</td>
<td>mass fraction of nitrate reducer in all nitrogen-related microbial groups (dimensionless)</td>
</tr>
<tr>
<td>$f_{dn2}$</td>
<td>mass fraction of nitrite reducer in all nitrogen-related microbial groups (dimensionless)</td>
</tr>
<tr>
<td>$f_n$</td>
<td>mass fraction of <em>Nitrobacter</em> in nitrifiers (dimensionless)</td>
</tr>
<tr>
<td>$f_{n1}$</td>
<td>mass fraction of <em>Nitrosomonas</em> in all nitrogen-related microbial groups (dimensionless)</td>
</tr>
<tr>
<td>$f_{n2}$</td>
<td>mass fraction of <em>Nitrobacter</em> in all nitrogen-related microbial groups (dimensionless)</td>
</tr>
<tr>
<td>$k_{dn1}$</td>
<td>maximum specific NO$_3$-N reduction rate constant (equivalent to enrichment culture for NO$_3$-N) (d$^{-1}$)</td>
</tr>
<tr>
<td>$k'_{dn1}$</td>
<td>maximum specific NO$_3$-N reduction rate constant (mixed culture) (d$^{-1}$)</td>
</tr>
<tr>
<td>$k_{dn2}$</td>
<td>maximum specific NO$_3$-N reduction rate constant (enrichment culture for NO$_3$-N) (d$^{-1}$)</td>
</tr>
<tr>
<td>$k'_{dn2}$</td>
<td>maximum specific NO$_3$-N reduction rate constant (mixed culture) (d$^{-1}$)</td>
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<tr>
<td>$k_{n1}$</td>
<td>maximum specific NH$_4$-N oxidation rate constant (equivalent to enrichment culture for NH$_4$-N) (d$^{-1}$)</td>
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<tr>
<td>$k'_{n1}$</td>
<td>maximum specific NH$_4$-N oxidation rate constant (mixed culture) (d$^{-1}$)</td>
</tr>
<tr>
<td>$k_{n2}$</td>
<td>maximum specific NO$_2$-N oxidation rate constant (enrichment culture for NO$_2$-N) (d$^{-1}$)</td>
</tr>
<tr>
<td>$k'_{n2}$</td>
<td>maximum specific NO$_2$-N oxidation rate constant (mixed culture) (d$^{-1}$)</td>
</tr>
<tr>
<td>$K_{s,dn1}$</td>
<td>half-saturation constant for NO$_3$-N reduction (mg l$^{-1}$)</td>
</tr>
<tr>
<td>$K_{s,dn2}$</td>
<td>half-saturation constant for NO$_2$-N reduction (mg l$^{-1}$)</td>
</tr>
<tr>
<td>$K_{s,n1}$</td>
<td>half-saturation constant for NH$_4$-N oxidation (mg l$^{-1}$)</td>
</tr>
<tr>
<td>$K_{s,n2}$</td>
<td>half-saturation constant for NO$_2$-N oxidation (mg l$^{-1}$)</td>
</tr>
<tr>
<td>$Q$</td>
<td>inflow rate (l d$^{-1}$)</td>
</tr>
<tr>
<td>$Q_{m}$</td>
<td>mixed liquor recycle rate (l d$^{-1}$)</td>
</tr>
<tr>
<td>$Q_{s}$</td>
<td>sludge recycle rate (l d$^{-1}$)</td>
</tr>
<tr>
<td>$r_{dn1}$</td>
<td>first-step denitrification rate in anoxic reactor (mg l$^{-1}$ d$^{-1}$)</td>
</tr>
<tr>
<td>$r_{dn2}$</td>
<td>second-step denitrification rate in anoxic reactor (mg l$^{-1}$ d$^{-1}$)</td>
</tr>
<tr>
<td>$r_{n1}$</td>
<td>first-step nitrification rate in aerobic reactor (mg l$^{-1}$ d$^{-1}$)</td>
</tr>
<tr>
<td>$r_{n2}$</td>
<td>second-step nitrification rate in aerobic reactor (mg l$^{-1}$ d$^{-1}$)</td>
</tr>
<tr>
<td>$r_s$</td>
<td>NH$_4$-N uptake rate by microbial synthesis (mg l$^{-1}$ d$^{-1}$)</td>
</tr>
<tr>
<td>$R_m$</td>
<td>mixed liquor recycle ratio (dimensionless)</td>
</tr>
<tr>
<td>$R_s$</td>
<td>sludge recycle ratio (dimensionless)</td>
</tr>
<tr>
<td>$S_{B_{0,1}}$, $S_{B_{0,2}}$</td>
<td>NH$_4$-N or NO$_2$-N concentration in initial three consecutive samples from batch reactor (mg l$^{-1}$)</td>
</tr>
<tr>
<td>$\Delta t$</td>
<td>time interval (h)</td>
</tr>
<tr>
<td>TN</td>
<td>total nitrogen (mg l$^{-1}$)</td>
</tr>
<tr>
<td>$V_1$</td>
<td>volume of anoxic reactor (l)</td>
</tr>
<tr>
<td>$V_2$</td>
<td>volume of aerobic reactors (l)</td>
</tr>
<tr>
<td>$X$</td>
<td>average biomass concentration (mg VSS l$^{-1}$)</td>
</tr>
<tr>
<td>$X_1$</td>
<td>biomass concentration in anoxic reactor (mg VSS l$^{-1}$)</td>
</tr>
<tr>
<td>$X_2$</td>
<td>biomass concentration in aerobic reactor (mg VSS l$^{-1}$)</td>
</tr>
<tr>
<td>$X_B$</td>
<td>biomass concentration in batch reactor (mg VSS l$^{-1}$)</td>
</tr>
<tr>
<td>$X_{dn}$</td>
<td>nitrate reducer plus nitrite reducer concentrations in batch reactor (mg VSS l$^{-1}$)</td>
</tr>
<tr>
<td>$X_{dn1}$</td>
<td>nitrate reducer concentration in batch reactor (mg VSS l$^{-1}$)</td>
</tr>
<tr>
<td>$X_{dn2}$</td>
<td>nitrite reducer concentration in batch reactor (mg VSS l$^{-1}$)</td>
</tr>
<tr>
<td>$X_n$</td>
<td><em>Nitrosomonas</em> plus <em>Nitrobacter</em> concentrations in batch reactor (mg VSS l$^{-1}$)</td>
</tr>
<tr>
<td>$X_{n1}$</td>
<td><em>Nitrosomonas</em> concentration in batch reactor (mg VSS l$^{-1}$)</td>
</tr>
<tr>
<td>$X_{n2}$</td>
<td><em>Nitrobacter</em> concentration in batch reactor (mg VSS l$^{-1}$)</td>
</tr>
<tr>
<td>$\theta_c$</td>
<td>sludge age (d)</td>
</tr>
</tbody>
</table>
(Metcalf and Eddy, 2003). All the afore-mentioned systems are similar to the activated-sludge process; except SBR, the rest of the systems employ various combinations of anaerobic, anoxic, and aerobic zones, compartments or reactors. In these systems, aerobic heterotrophs, strict aerobic nitrifiers and facultative aerobic denitrifiers exist. The populations of the three microbial groups vary with different wastewater characteristics and operating conditions. They compete with one another, and eventually reach a delicate stable relationship.

Argaman and Brenner (1986a) reported that in the single-sludge nitrogen removal system (SSNRS) the yield and decay coefficients of heterotrophs were 0.29 g volatile suspended solids (VSS) g\(^{-1}\) COD and 0.02 d\(^{-1}\), respectively, while the two coefficients of autotrophs were 0.22 g VSS g\(^{-1}\) NH\(_4\)\(^+\)-N and 0.01 d\(^{-1}\), respectively. They also pointed out that microbial biomass contained in the SSNRS were heterotrophs mainly, and the predominant microorganisms were denitrifying species. In contrast, the mass fraction of autotrophs only ranged from 0.17 to 0.26; the ratio of Nitrobacter to Nitrosomonas ranged from 0.23 to 0.28 when the influent NH\(_4\)\(^+\)-N/COD ratios were maintained at 0.15–0.33.

Many mathematical models that can be used to predict residual concentrations of NH\(_4\)\(^+\) and NO\(_3\)\(^-\) in the SSNRS have been formulated (van Haandel et al., 1981; Argaman and Brenner, 1986b; Henze et al., 1987a,b; Zhao et al., 1994a,b; Argaman, 1995). An accurate prediction of all nitrogen species (NH\(_4\)\(^+\), NO\(_2\)\(^-\) and NO\(_3\)\(^-\)) in each individual reactor of the SSNRS should rely on the incorporation of mass fractions of Nitrosomonas, Nitrobacter, nitrate reducer and nitrite reducer, together with kinetic parameter values for each individual nitrogen species of concern. In addition, from the view point of engineering practice, an existing activated sludge reactor system can be rather easily upgraded to the A/O system.

Accordingly in this work, nitrification–denitrification in a laboratory-scale single-sludge anoxic aerobic activated sludge system (with sufficient carbon source for denitrification) was conducted to generate experimental data. To benefit the routine operation and management of the SSNRS, a steady-state kinetic model (with parameters as defined in Nomenclature section) that accounts for the mass fractions of nitrogen-related microbial groups (\(f_{m1}, f_{m2}, f_{dn1}\) and \(f_{dn2}\)) in the SSNRS was developed and validated by experiments. By conducting independent batch experiments, the kinetic parameter values of two-step nitrification (NH\(_4\)\(^+\) \(\rightarrow\) NO\(_2\)\(^-\) \(\rightarrow\) NO\(_3\)\(^-\)) and two-step denitrification (NO\(_2\)\(^-\) \(\rightarrow\) NO\(_3\)\(^-\) \(\rightarrow\) N\(_2\)) as well as the mass fractions of nitrogen-related microbial groups were determined. Moreover, the variations in mass fractions of nitrogen-related microbial groups with different operating conditions and simulation modeling for nitrogen removal in the single-sludge system are presented as well.

2. Model formulation

Fig. 1 illustrates the flow diagram of the single-sludge anoxic aerobic activated sludge system which is designed for nitrogen removal from wastewater. Mixed liquor and settled sludge were recycled in the single-sludge system.

The following assumptions are made for the formulation of the kinetic model:

1. Both the oxidation of NH\(_4\)\(^+\) and NO\(_3\)\(^-\) follow Monod kinetics (Williamson and McCarty, 1975; Chudoba et al., 1985).
2. Both the reduction of NO\(_3\)\(^-\) and NO\(_2\)\(^-\) follow Monod kinetics (Her and Huang, 1995).
3. All the VSS (i.e., represented by the empirical formula, C\(_3\)H\(_7\)O\(_2\)N) in the anoxic and aerobic reactors consist of viable cells.
4. Nitrogen assimilation by Nitrosomonas, Nitrobacter, nitrate reducer or nitrite reducer is 0.124 g N g\(^{-1}\) VSS, according to the organic composition of VSS (C\(_3\)H\(_7\)O\(_2\)N).

2.1. Ammonia, nitrite, and nitrate in anoxic reactor

Material-balance equations for ammonia, nitrite, and nitrate entering and leaving the anoxic reactor can be written as

\[
Q\left( {k_0 + (Q_s + Q_m)} \right)C_{s1} - (Q + Q_s + Q_m)C_{m1} = -V_1 r_{dn1} \qquad (1)
\]

\[
(Q_s + Q_m)C_{m2} - (Q + Q_s + Q_m)C_{m1} + V_1 r_{dn1} = 0 \quad (2)
\]

\[
(Q_s + Q_m)C_{n12} - (Q + Q_s + Q_m)C_{n11} - V_1 r_{dn1} = 0 \quad (3)
\]

2.2. Ammonia, nitrite, and nitrate in aerobic reactor

Material-balance equations for ammonia, nitrite, and nitrate entering and leaving the aerobic reactor can be written as
\[
(Q + Q_s + Q_m)C_{k_1} - (Q + Q_s + Q_m)C_{k_2} - V_2 r_s - V_2 r_n = 0 \quad (4)
\]

\[
(Q + Q_s + Q_m)C_{n_1} - (Q + Q_s + Q_m)C_{n_2} + V_2 r_n - V_2 r_{n_2} = 0 \quad (5)
\]

\[
(Q + Q_s + Q_m)C_{n_{al_1}} - (Q + Q_s + Q_m)C_{n_{al_2}} + V_2 r_{n_2} = 0 \quad (6)
\]

The nitrification and denitrification rates \(r_{n_1}, r_{n_2}, r_{d_{n_1}}, \text{ and } r_{d_{n_2}}\) and the ammonia uptake rate \(r_s\) in Eqs. (1)–(6) can be expressed as

\[
r_{n_1} = \frac{k_{n_1} C_{k_2} f_{n_1} X}{K_{s,n_1} + C_{k_2}} \quad (7)
\]

\[
r_{n_2} = \frac{k_{n_2} C_{k_2} f_{n_2} X}{K_{s,n_2} + C_{k_2}} \quad (8)
\]

\[
r_{d_{n_1}} = \frac{k_{d_{n_1}} C_{n_{al_1}} f_{d_{n_1}} X}{K_{s,d_{n_1}} + C_{n_{al_1}}} \quad (9)
\]

\[
r_{d_{n_2}} = \frac{k_{d_{n_2}} C_{n_{al_2}} f_{d_{n_2}} X}{K_{s,d_{n_2}} + C_{n_{al_2}}} \quad (10)
\]

\[
r_s = 0.124X/\theta_c \quad (11)
\]

where

\[
X = \frac{X_1 V_1 + X_2 V_2}{V_1 + V_2} \quad (12)
\]

2.3. Model calculation

The steady-state kinetic model includes seven operating parameters \(C_{k_{10}}, Q, Q_m, Q_s, \theta_c, V_1 \text{ and } V_2\), thirteen biological parameters \(k_{n_1}, K_{s,n_1}, k_{n_2}, K_{s,n_2}, k_{d_{n_1}}, K_{s,d_{n_1}}, k_{d_{n_2}}, K_{s,d_{n_2}}, X, f_{n_1}, f_{n_2}, f_{d_{n_1}}, \text{ and } f_{d_{n_2}}\), and six unknown parameters \(C_{k_1}, C_{k_2}, C_{n_{al_1}}, C_{n_{al_2}}, C_{n_{al_1}}\), and \(C_{n_{al_2}}\). Except \(X\) (i.e., measured in the SSNRS), the other twelve biological parameter values can be determined by conducting independent batch experiments with sludges, respectively, removed from anoxic and aerobic reactors of the SSNRS. Detailed procedures are described later in Section 3. Thus, only \(C_{k_1}, C_{k_2}, C_{n_{al_1}}, C_{n_{al_2}}, C_{n_{al_1}}, \text{ and } C_{n_{al_2}}\) have to be solved.

From simultaneous algebraic Eqs. (1) and (4), \(C_{k_1}\) and \(C_{k_2}\) can be calculated directly. The four remaining unknown parameters \(C_{n_{al_1}}, C_{n_{al_2}}, C_{n_{al_1}}, \text{ and } C_{n_{al_2}}\) can be solved by using the Newton’s iterative method, together with four simultaneous non-linear equations, Eqs. (2), (3), (5), and (6).

3. Materials and methods

3.1. Single-sludge nitrogen removal system

Two sets of identical laboratory-scale SSNRS, made of Plexiglas, were used. Each SSNRS consisted of anoxic and aerobic reactors and a settler with effective volumes of 5, 12, and 8 l, respectively. One set of stainless-steel mixing impeller (with a rotating speed of 50 rpm) was installed in the anoxic reactor. To maintain a suitable growth environment for facultative aerobic denitrifiers, the anoxic reactor was equipped with a cover (sealed on the top to prevent the intake of air) but provided with a gas vent and a sampling port. The aerobic reactor was thoroughly aerated with a diffuser to maintain the dissolved oxygen (DO) concentrations at 3–4 mg l\(^{-1}\). During the operation of the SSNRS, sludge was recycled \(R_s = 1\) from the bottom of the settler to the anoxic reactor, while mixed liquor was recycled \(R_m = 1\) and 2 from the aerobic reactor to the anoxic reactor (Fig. 1). To avoid the seasonal change of temperature, completely submersible automatic heating rods (Visi-Therm, Aquarium Systems, Italy) were installed in the SSNRS to maintain the reaction temperature at 28 ± 1 °C.

To maintain a designated sludge age \(\theta_c\) and concentration of VSS in the SSNRS, excess sludge was wasted daily from the settler. Prior to carrying out the first test run, seed sludge in the SSNRS had been acclimatized for a three-month period. To ensure that the SSNRS reached steady state, each of a total of four test runs was continuous for at least three-fold \(\theta_c\). NH\(_4\)\(^+\), NO\(_3\)\(^-\), COD in the effluent and VSS in the anoxic and aerobic reactors were monitored for each test run. Steady state was assumed to be reached after test results of the four parameters were within 5% deviation for three consecutive samples (sampling twice a week).

3.2. Feed wastewater

The ingredients of synthetic wastewater (diluted with tap water) contained methanol (267 mg l\(^{-1}\)), yeast extract (7 mg l\(^{-1}\)), KH\(_2\)PO\(_4\) (68 mg l\(^{-1}\)), K\(_2\)HPO\(_4\) (27 mg l\(^{-1}\)), NH\(_4\)Cl (460 mg l\(^{-1}\)), NaHCO\(_3\) (1330 mg l\(^{-1}\)), Ni\(^{2+}\) (0.5 mg l\(^{-1}\)), Fe\(^{3+}\) (0.5 mg l\(^{-1}\)), Co\(^{2+}\) (0.3 mg l\(^{-1}\)), Mo\(^{6+}\) (0.6 mg l\(^{-1}\)), Zn\(^{2+}\) (0.5 mg l\(^{-1}\)) and Mn\(^{2+}\) (0.5 mg l\(^{-1}\)). The concentrations of COD, alkalinity, and NH\(_4\)\(^+\)-N were 400 mg l\(^{-1}\), 790 mg l\(^{-1}\) (as CaCO\(_3\)), and 120 mg l\(^{-1}\), respectively, and the pH was 7.8–7.9.

3.3. Enrichment of nitrogen-related microbial groups

To avoid possible population shifting during the enrichment of Nitrosomonas plus Nitrobacter, Nitro- bacter alone, nitrate reducer plus nitrite reducer and nitrite reducer alone, the most influential operating conditions/parameters (chemical constituents, DO, ORP, \(\theta_c\), temperature) maintained in the four fill-and-draw reactors were close to those maintained in the SSNRS.

For the enrichment of Nitrosomonas plus Nitrobacter and Nitro-bacter alone, sludge removed from the aerobic reactor of the SSNRS was first loaded into two 5-l batch reactors, which were supplied with 1.91 g NH\(_4\)Cl, 1 g KH\(_2\)PO\(_4\), 8 g NaHCO\(_3\), and adequate amounts of trace metals and 2.49 g NaNO\(_2\), 1 g KH\(_2\)PO\(_4\), 5 g NaHCO\(_3\), and adequate amounts of trace metals, respectively, for
three times a day. The two reactors were thoroughly aerated with diffusers to maintain the DO concentrations at 3–4 mg l$^{-1}$. To keep a $\theta_c$ at about 10 d (i.e., close to the $\theta_c$ of the SSNRS), 0.5 l of mixed liquor was wasted daily prior to decanting of the supernatant. The above procedures were repeated for three months.

Similarly, for the enrichment of nitrite reducer plus nitrite reducer and nitrite reducer alone, sludge removed from the anoxic reactor of the SSNRS was first loaded into two 5-l batch reactors, which were supplied with 3.6 g KNO$_3$, 3 ml 24.3 M CH$_3$OH, 1.9 g KH$_2$PO$_4$, adequate amounts of yeast extract, and trace metals and 2.49 g NaNO$_2$, 3 ml 24.3 M CH$_3$OH, 1 g KH$_2$PO$_4$, adequate amounts of yeast extract, and trace metals, respectively, for three times a day. To maintain a suitable growth environment for facultative aerobic denitrifiers, the two reactors were equipped with a cover but provided with a gas vent and a sampling port. To keep a $\theta_c$ at about 10 d, 0.5 l of mixed liquor was wasted daily prior to decanting of the supernatant. The above procedures were repeated for three months. The above-mentioned four batch reactors were maintained at a temperature of 28 ± 1°C by installing completely submersible automatic heating rods.

### 3.4. Determination of biokinetic constants

To determine the biokinetic constants of second-step nitrification ($k_{n2}$ and $K_{s,n2}$), homogenized sludge (taken from the previous batch reactor for the enrichment of *Nitrobacter* alone) and the synthetic wastewater containing nitrite and nutrients were loaded into a 1-l batch reactor, which were immediately aerated to maintain the DO concentration at 3–4 mg l$^{-1}$. Then samples were analyzed for NO$_2^-$ remaining in the solution for every 10 min and for the final biomass concentration. Thereafter, a linear regression method (Halwachs, 1978) together with the batch data were used to determine $k_{n2}$ and $K_{s,n2}$.

To determine the biokinetic constants for first-step nitrification ($k_{n1}$ and $K_{s,n1}$), homogenized sludge (taken from the previous batch reactor for the enrichment of *Nitrosomonas plus Nitrobacter*) and the synthetic wastewater containing ammonia and nutrients were loaded into one 1-l batch reactor. Meanwhile, the same homogenized sludge and the synthetic wastewater containing nitrite and nutrients were loaded into another 1-l batch reactor. Then the initial rate method (Huang et al., 2003; Jih et al., 2003) was used to determine $k_{n1}$ and $K_{s,n1}$.

where $i$ denotes n, dn, n1, n2, dn1 and dn2; $S_{B,-1}$, $S_{B,0}$ and $S_{B,+1}$ represent the bulk concentration of NH$_3^+$-N(NO$_3^-\cdot$N) in the initial three consecutive samples; $X_B$ represents the biomass concentration in the batch reactor; and $\Delta t$ represents the time interval.

Thereafter, the batch reactor containing ammonia and nutrients, samples were analyzed for NH$_3^+$ remaining in the solution for every 10 min and for the final biomass concentration (Note: biomass of *Nitrosomonas* = (1 – f) × biomass in the batch reactor). Finally, the linear regression method was applied to determine $k_{n1}$ and $K_{s,n1}$.

Similar approach was applied to determine the biokinetic constants of first-step and second-step denitrification ($k_{dn1}$, $K_{s,dn1}$, $k_{dn2}$, and $K_{s,dn2}$) except for ammonia which was replaced with nitrate, and methanol was added as the carbon source.

### 3.5. Mass fractions of nitrogen-related microbial groups

Details of the determination of the mass fractions of *Nitrosomonas*, *Nitrobacter*, nitrate reducer, and nitrite reducer ($f_{n1}$, $f_{n2}$, $f_{dn1}$, and $f_{dn2}$) in the SSNRS are described. When the SSNRS reached steady state, sludge was removed from the aerobic reactor and loaded into two batch reactors, which were immediately aerated to maintain the DO concentration at 3–4 mg l$^{-1}$. Then, the synthetic wastewaters containing ammonia and nitrite, together with nutrients, were respectively added into the two individual batch reactors. Samples were analyzed for NH$_3^+$ and NO$_2^-\cdot$N remaining in the solution for every 10 min and for the final biomass concentration in the two individual batch reactors. Thereafter, the initial rate method (Eqs. (13) and (14)) was used to determine $f_{n1}$ and $f_{n2}$.

Similarly, sludge was removed from the anoxic reactor and loaded into two batch reactors, which were immediately purged with nitrogen gas to deprive oxygen. Then, the synthetic wastewaters containing nitrate and nitrite, together with methanol and nutrients, were respectively added into the two individual batch reactors. Samples were analyzed for NO$_3^-\cdot$N and NO$_2^-\cdot$N remaining in the solution for every 20 min and for the final biomass concentration in the two individual batch reactors. Thereafter, the initial rate method (Eqs. (13) and (14)) was used to determine $f_{dn1}$ and $f_{dn2}$.

### 3.6. Chemical analyses

Nitrate and nitrite were measured using the ion chromatography method by selecting Shim-pack IC-A3 as chromatographic column, 8.0 mM p-hydroxy benzoic acid and 3.2 mM Bis-tris as mobile phase, and CDD-6A, 3.2 μS/cm FS as conductivity detector. COD,
Table 1: Operating conditions and main results of the single-sludge nitrogen removal system

<table>
<thead>
<tr>
<th>Run</th>
<th>HRT (h)</th>
<th>NH₃-N loading (kg NH₃-N m⁻³ d⁻¹)</th>
<th>NH₃-N removal (%)</th>
<th>N removal (%)</th>
<th>COD (mg l⁻¹)</th>
<th>COD removal (%)</th>
<th>TN (mg l⁻¹)</th>
<th>TN removal (%)</th>
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a) NH₃-N influent concentration = 120 mg l⁻¹, methanol influent concentration = 400 mg COD l⁻¹, COD/NH₃-N = 3.33.

The operating conditions and main results of the SSNRS are presented in Table 1. When the influent NH₃-N and COD concentration of 120 mg l⁻¹ and 400 mg l⁻¹, hydraulic retention time (HRT) of 2.4–4.0 h for the anoxic reactor, HRT of 5.8–9.6 h for the aerobic reactor, sludge age (θₚ) of 9.4–11.0 d, sludge recycle ratio (Rₛ) of 1, and mixed liquor recycle ratios (Rₘ) of 1–2 were maintained, the COD removal efficiencies were 91–97% and, the NH₃-N removal efficiencies (95–91%) were higher than total nitrogen (TN) removal efficiencies (71–41%). A relative lower TN removal efficiency was mainly because the aerobic reactor was placed after the anoxic reactor and, thus, a part of NO₃⁻ (i.e., NO₃⁻ was the main species) generated in the aerobic reactor would discharge from the SSNRS.

When the volumetric loading rate (VLR) of the SSNRS was increased from 0.21 to 0.35 kg NH₃-N m⁻³ d⁻¹, biomass increased from 22.5–24.5 to 34.7–35.0 g VSS. Although the influent COD/NH₃-N ratio (3.3) maintained in the SSNRS was lower than the theoretical required amount of methanol for complete denitrification (i.e., COD/NO₃⁻-N ratio (3.71)), the supplied carbon source in the SSNRS should be sufficient for denitrification of the recycled NO₃⁻ (i.e., mixed liquor recycle ratios Rₘ = 1.2). Accordingly, at the lower VLR of 0.21 kg NH₃-N m⁻³ d⁻¹, the TN removal efficiency increased with increasing Rₘ because a higher Rₘ would recycle more NO₃⁻ from the aerobic reactor to the anoxic reactor for denitrification. Nonetheless, at the higher VLR of 0.35 kg NH₃-N m⁻³ d⁻¹, the TN removal efficiency decreased with increasing Rₘ because both oxygen and NO₃⁻ would recycle from the aerobic reactor to the anoxic reactor, and a higher recycled oxygen concentration imposed a competitive-inhibition effect on denitrification (Chang and Morris, 1962), especially for a shorter HRT of the anoxic reactor.

Furthermore, the calculated ΔCODexp/ΔTNexp ratios (4.1–4.3) were all larger than 2.86 (i.e., based on the transfer of one electron equivalent for dissimilative reduction of nitrate). In other words, the substrate (COD) was not only consumed in dissimilative reduction of nitrate, it was also consumed by denitrifiers for microbial synthesis. Nitrogen conversion data were also used to calculate the alkalinity change in the SSNRS and compared with the measured values. The ΔALKexp/ALKcal ratios ranged from 0.98–1.06, showing good agreement between the measured and predicted values.

4. Results and discussion

4.1. Performance of single-sludge nitrogen removal system

The operating conditions and main results of the SSNRS are presented in Table 1. When the influent NH₃-N and COD concentration of 120 mg l⁻¹ and 400 mg l⁻¹, hydraulic retention time (HRT) of 2.4–4.0 h for the anoxic reactor, HRT of 5.8–9.6 h for the aerobic reactor, sludge age (θₚ) of 9.4–11.0 d, sludge recycle ratio (Rₛ) of 1, and mixed liquor recycle ratios (Rₘ) of 1–2 were maintained, the COD removal efficiencies were 91–97% and, the NH₃-N removal efficiencies (95–91%) were higher than total nitrogen (TN) removal efficiencies (71–41%). A relative lower TN removal efficiency was mainly because the aerobic reactor was placed after the anoxic reactor and, thus, a part of NO₃⁻ (i.e., NO₃⁻ was the main species) generated in the aerobic reactor would discharge from the SSNRS.

When the volumetric loading rate (VLR) of the SSNRS was increased from 0.21 to 0.35 kg NH₃-N m⁻³ d⁻¹, biomass increased from 22.5–24.5 to 34.7–35.0 g VSS. Although the influent COD/NH₃-N ratio (3.3) maintained in the SSNRS was lower than the theoretical required amount of methanol for complete denitrification (i.e., COD/NO₃⁻-N ratio (3.71)), the supplied carbon source in the SSNRS should be sufficient for denitrification of the recycled NO₃⁻ (i.e., mixed liquor recycle ratios Rₘ = 1.2). Accordingly, at the lower VLR of 0.21 kg NH₃-N m⁻³ d⁻¹, the TN removal efficiency increased with increasing Rₘ because a higher Rₘ would recycle more NO₃⁻ from the aerobic reactor to the anoxic reactor for denitrification. Nonetheless, at the higher VLR of 0.35 kg NH₃-N m⁻³ d⁻¹, the TN removal efficiency decreased with increasing Rₘ because both oxygen and NO₃⁻ would recycle from the aerobic reactor to the anoxic reactor, and a higher recycled oxygen concentration imposed a competitive-inhibition effect on denitrification (Chang and Morris, 1962), especially for a shorter HRT of the anoxic reactor.

Furthermore, the calculated ΔCODexp/ΔTNexp ratios (4.1–4.3) were all larger than 2.86 (i.e., based on the transfer of one electron equivalent for dissimilative reduction of nitrate). In other words, the substrate (COD) was not only consumed in dissimilative reduction of nitrate, it was also consumed by denitrifiers for microbial synthesis. Nitrogen conversion data were also used to calculate the alkalinity change in the SSNRS and compared with the measured values. The ΔALKexp/ALKcal ratios ranged from 0.98–1.06, showing good agreement between the measured and predicted values.
4.2. Biokinetic constants

4.2.1. Parameters \(k_{n2}\) and \(K_{s,n2}\)

Data points shown in Fig. 2a revealed that the oxidation of NO\(_3^-\) in a batch reactor (enrichment culture; 28 °C) followed Monod kinetics. The linear regression method (Halwachs, 1978) was applied to determine the biokinetic constants \((k_{n2} \text{ and } K_{s,n2})\). The estimated \(k_{n2}\) and \(K_{s,n2}\) were 6.9 mg NO\(_3^-\) -N mg\(^{-1}\) VSS d\(^{-1}\) and 8.2 mg NO\(_3^-\) -N l\(^{-1}\), respectively. The estimated \(k_{n2}\) is close to that of Gee et al. (1990) (temperature = 23 °C; \(k_{n2} = 4.23-7.0 \text{ mg NO}_3^-\text{-N mg}^{-1}\text{VSS d}^{-1}\)), but the estimated \(k_{n2}\) and \(K_{s,n2}\) are higher than those of Chudoba et al. (1985) who used the mixed culture (temperature = 20 °C; \(k_{n2} = 0.13-0.16 \text{ mg NO}_3^-\text{-N mg}^{-1}\text{VSS d}^{-1}\); \(K_{s,n2} = 0.59-0.76 \text{ mg NO}_3^-\text{-N l}^{-1}\)). The difference in \(K_{s,n2}\) values may be because the Monod half-saturation constant \((K_s)\) depends on the substrate bulk concentration, i.e., a lower substrate bulk concentration results in a lower \(K_s\) (Chudoba et al., 1985; Charaklis and Marshall, 1990). In the present study, the NO\(_3^-\) -N bulk concentrations (<50 mg l\(^{-1}\)) were much higher than those of Chudoba et al. (<18 mg l\(^{-1}\)).

4.2.2. Parameters \(k_{n1}\) and \(K_{s,n1}\)

Except pure culture i.e., Nitrosomonas, the biokinetic constants for ammonia oxidation (NH\(_4^+ \rightarrow NO_2^-\)) are extremely difficult to be determined from mixed culture (Nitrosomonas plus Nitrobacter). Thus the initial rate method (Huang et al., 2003; Jih et al., 2003) was first used to determine the mass fraction of Nitrobacter \((f_0 = 0.22)\) in a batch reactor with enrichment culture of Nitrosomonas plus Nitrobacter. Then the same enrichment culture was loaded into another batch reactor to proceed with ammonia oxidation. Data points shown in Fig. 2b disclosed that the oxidation of NH\(_4^+\) in the batch reactor followed Monod kinetics. The estimated \(k_{n1}\) and \(K_{s,n1}\) were 1.97 mg NH\(_4^+\) -N mg\(^{-1}\) VSS d\(^{-1}\) and 0.8 mg NH\(_4^+\) -N l\(^{-1}\), respectively. The estimated \(k_{n1}\) and \(K_{s,n1}\) values are close to those of Williamson and McCarty (1975) (temperature = 20 °C; \(k_{n1} = 2.04 \text{ mg NH}_4^+\text{-N mg}^{-1}\text{VSS d}^{-1}, K_{s,n1} = 0.50 \text{ mg NH}_4^+\text{-N l}^{-1}\)) but the \(k_{n1}\) value is higher than those of Chudoba et al. (1985) who used the mixed culture (temperature = 20 °C; \(k_{n1} = 0.21-0.35 \text{ mg NH}_4^+\text{-N mg}^{-1}\text{VSS d}^{-1}, K_{s,n1} = 0.41-0.72 \text{ mg NH}_4^+\text{-N l}^{-1}\)).

4.2.3. Parameter \(k_{d,n2}\) and \(K_{s,d,n2}\)

Data points shown in Fig. 2c revealed that the reduction of NO\(_2^-\) in a batch reactor (enrichment culture; 28 °C) followed Monod kinetics. The estimated \(k_{d,n2}\) (0.74 mg NO\(_2^-\) -N mg\(^{-1}\) VSS d\(^{-1}\)) is close to that of Her and Huang (1995) (temperature = 30 °C; \(k_{d,n2} = 0.89 \text{ mg NO}_2^-\text{-N mg}^{-1}\text{VSS d}^{-1}\)), but the estimated \(K_{s,d,n2}\) (0.03 mg NO\(_2^-\) -N l\(^{-1}\)) is much lower than that of Her and Huang (temperature = 30 °C; \(K_{s,d,n2} = 10.9 \text{ mg NO}_2^-\text{-N l}^{-1}\)). In the present study, the NO\(_2^-\) -N bulk concentrations (<22 mg N l\(^{-1}\)) were much lower than those of Her and Huang (<203 mg N l\(^{-1}\)).
4.2.4. Parameter $k_{dn1}$ and $K_{s,dn1}$

To estimate the biokinetic constant for the first-step nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), the initial rate method was first used to determine the mass fraction of nitrite reducer ($f_{dn1} = 0.48$) in a batch reactor with enrichment culture of nitrate reducer plus nitrite reducer. Then the same enrichment culture was loaded into another batch reactor to proceed with nitrate reduction. Data points shown in Fig. 2d appeared when the reduction of $\text{NO}_3^-$ in the batch reactor followed Monod kinetics. The estimated $k_{dn1}$ and $K_{s,dn1}$ were 1.03 mg $\text{NO}_3^-\text{N}$ g$^{-1}$ VSS d$^{-1}$ and 14 mg $\text{NO}_3^-\text{N}$ l$^{-1}$, respectively. The estimated $k_{dn1}$ and $K_{s,dn1}$ are close to those of Her and Huang (1995) (temperature = 30 °C; $k_{dn1} = 0.375–1.25$ mg NO$3^-\text{N}$ g$^{-1}$VSS d$^{-1}$; $K_{s,dn1} = 14.3$ mg NO$3^-\text{N}$ l$^{-1}$).

4.3. Mass fractions of nitrogen-related groups

When the SSNRS reached steady state, the mass fractions of $\text{Nitrosomonas}$, $\text{Nitrobacter}$, nitrate reducer and nitrite reducer ($f_{n1}$, $f_{n2}$, $f_{dn1}$ and $f_{dn2}$) were determined. The estimated $f_{n1}$, $f_{n2}$, $f_{dn1}$, and $f_{dn2}$ were 0.21–0.26, 0.05–0.07, 0.32–0.45, and 0.32–0.38, respectively, as shown in Table 2. The mass fraction of nitrate reducer plus nitrite reducer ($f_{dn1} + f_{dn2} = 0.65–0.84$) was significantly larger than the mass fraction of $\text{Nitrosomonas}$ plus $\text{Nitrobacter}$ ($f_{n1} + f_{n2} = 0.28–0.32$). According to Metcalf and Eddy (2003), the yield coefficients of nitrifiers and denitrifiers were 0.12 g VSS g$^{-1}$ NH$4^+\text{N}$ and 0.30 g VSS g$^{-1}$ COD (i.e., equivalent to 0.30 g VSS g$^{-1}$ COD × 2.86 g COD g$^{-1}$ NO$3^-\text{N}$ = 0.86 g VSS g$^{-1}$NO$3^-\text{N}$), respectively. In the present study, the NH$4^+\text{N}$ and TN removal efficiencies in the SSNRS were 91–95% and 49–71%, respectively, and thereby the mass fraction of denitrifiers was larger than that of nitrifiers.

The mass fraction of $\text{Nitrosomonas}$ ($f_{n1} = 0.21–0.26$) was obviously larger than the mass fraction of $\text{Nitrobacter}$ ($f_{n2} = 0.05–0.07$) (Table 2). This is because the yield coefficient of $\text{Nitrosomonas}$ (0.04–0.13 mg VSS mg$^{-1}$ N) is higher than that of $\text{Nitrobacter}$ (0.02–0.07 mg VSS mg$^{-1}$ N) (Sharma and Ahler, 1977). Our estimated $f_{n1}/f_{n2}$ ratios (0.19–0.33) were close to those (0.23–0.28) of Argaman and Brenner (1986a). In addition, the mass fraction of nitrate reducer ($f_{dn1} = 0.32–0.45$) varied slightly with the mass fraction of nitrite reducer ($f_{dn2} = 0.33–0.38$) (Table 2). A similar result was also reported by Her and Huang (1995).

At the lower VLR of 0.21 kg NH$4^+\text{N}$ m$^{-3}$ d$^{-1}$, the $f_{dn1}$ plus $f_{dn2}$ value increased with increasing $R_m$. Nonetheless, at the higher VLR of 0.35 kg NH$4^+\text{N}$ m$^{-3}$ d$^{-1}$, the value $f_{dn1}$ plus $f_{dn2}$ did not increase with increasing $R_m$. This was consistent with the performance data in the present study. That is, at the higher VLR of 0.35 kg NH$4^+\text{N}$ m$^{-3}$ d$^{-1}$, the TN removal efficiencies declined with increasing $R_m$ (Table 1).

4.4. Specific nitrification and denitrification rates

By multiplying ($f_{n1} + f_{n2}$) and ($f_{dn1} + f_{dn2}$) to biomass measured in the aerobic and anoxic reactors, together with the performance data shown in Table 1, the specific nitrification and denitrification rates could be calculated (Table 2). The calculated specific nitrification and denitrification rates were 0.57–0.73 mg NH$4^+\text{N}$ mg$^{-1}$ VSS d$^{-1}$ and 0.34–0.44 mg NO$3^-\text{N}$ mg$^{-1}$ VSS d$^{-1}$, respectively. The calculated specific nitrification rates were close to those (0.41–1.33 mg NH$4^+\text{N}$ mg$^{-1}$ VSS d$^{-1}$) of Hanaki et al. (1990). However, the calculated specific nitrification rates were quite lower than the specific denitrification rate, 1.54 mg NH$4^+\text{N}$ mg$^{-1}$ VSS d$^{-1}$ (Fig. 2b if both $X_{n1}$ and $X_{n2}$ are considered). This reflects that

### Table 2

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<th>$f_{dn2}$ (–)</th>
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### Table 3

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population shifting during the enrichment of *Nitrosonomas* plus *Nitrobacter* and *Nitrobacter* alone might occur in the batch reactors. Moreover, the calculated specific denitrification rates were close to the specific denitrification rate, 0.49 mg NO$_3^-$-N mg$^{-1}$ VSS d$^{-1}$ (Fig. 2d if both $X_{dn1}$ and $X_{dn2}$ are considered). However, the calculated specific denitrification rates were relatively higher than those (0.13–0.35 mg NO$_3^-$-N mg$^{-1}$ VSS d$^{-1}$) of Engberg and Schroeder (1975) because the denitrifying loadings of as high as 0.42–1.36 kg NO$_3^-$-N mg$^{-1}$ VSS d$^{-1}$ (i.e., calculated by using the data shown in Tables 1 and 2) occurred in the anoxic reactor in the present study.

### 4.5. Model simulation and validation

Biological parameter values (i.e., determined in the present study) used in model simulation are presented in Table 3. The major difference between the ASM No. 1 (Henze et al., 1987b) and the present kinetic model was that one-step nitrification and one-step denitrification in the single-sludge (A/O) system were assumed in the ASM No. 1 (i.e., the residual concentration of NO$_3^-$ in each respective reactor could not be simulated).

By incorporating $f_{n1}$, $f_{n2}$, $f_{dn1}$, and $f_{dn2}$ together with other biological parameter values (Table 3) into the kinetic model, the residual concentrations of NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N in the anoxic and aerobic reactors could be calculated (Table 4). The calculated residual concentrations of NH$_4^+$-N and NO$_2^-$-N in the anoxic reactor and NO$_3^-$-N in the aerobic reactor were in fairly good agreement with the experimental results. However, the calculated NO$_3^-$-N in the anoxic reactor was over-estimated, while the calculated NH$_4^+$-N in the aerobic reactor was under-estimated. These systematic errors may be attributed to the under-estimation of $k_{dn1}$ and the over-estimation of $k_{dn2}$. As described earlier, microbial population shifting (from the SSNRS to the batch reactors) might occur, causing these errors. Moreover, the calculated NH$_4^+$-N and TN removal efficiencies of the SSNRS were +8.8% and ± 20% deviated from the experimental results, respectively (Table 4).

### 5. Conclusions

When the SSNRS (with sufficient carbon source i.e., methanol for denitrification) was maintained at the mixed liquor recycle ratios ($R_m$) of 1–2, the COD removal efficiencies were 91–97% and, the NH$_4^+$-N removal efficiencies (95–91%) were higher than TN removal efficiencies (71–41%). At the lower VLR of 0.21 NH$_4^+$-N m$^{-3}$ d$^{-1}$, the TN removal efficiency increased with increasing $R_m$. However, at the higher VLR of 0.35 kg NH$_4^+$-N m$^{-3}$ d$^{-1}$, the TN removal efficiency
decreased with increasing $R_m$ because a higher recycled oxygen concentration imposed a competitive-inhibition effect on denitrification.

By conducting independent batch experiments with sludges removed from each individual reactor of the SSNRs, the estimated mass fraction of nitrate reducer plus nitrite reducer ($f_{dn1} + f_{dn2} = 0.65–0.83$) was significantly larger than the mass fraction of *Nitrosomonas* plus *Nitrobacter* ($f_{n1} + f_{n2} = 0.28–0.32$); the $f_{n1}$ (0.21–0.26) was larger than the $f_{n2}$ (0.05–0.07); and the $f_{dn1}$ (0.32–0.45) varied slightly with the $f_{dn2}$ (0.33–0.38). At the lower VLR, the $f_{dn1}$ plus $f_{dn2}$ increased with increasing $R_m$, but at the higher VLR, the $f_{dn1}$ plus $f_{dn2}$ did not increase with increasing $R_m$.

By incorporating the mass fractions of nitrogen-related microbial groups together with other biological parameter values into the kinetic model, the calculated residual concentrations of NH$_4^+$-N and NO$_2^-$-N in the anoxic reactor and NO$_3^-$-N and NO$_2^-$-N in the aerobic reactor were in fairly good agreement with the experimental results. The over-estimation of NO$_2^-$-N in the anoxic reactor and the under-estimation of NH$_4^+$-N in the aerobic reactor may be attributed to the under-estimation of $k_{dn1}$ and the over-estimation of $k_{n1}$. This reflects that microbial population shifting (from the SSNRs to the batch reactors) might occur.

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**References**


