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Modeling urea biodegradation in activated sludge using combined respirometric–titrimetric measurements

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ABSTRACT

Urea biodegradation kinetics determination has been performed in the literature using a two-step nitrification model that was calibrated using on-line respirometric measurements. However, the model neglected the initial hydrolysis step that converts urea to carbon dioxide and ammonia nitrogen, and assumed constant carbon dioxide transfer rate (CTR), though it is inherently a non-linear process which has an impact on the titrimetric modeling. Hence, in this paper, it is aimed to propose a complete two-step nitrification model for urea biodegradation paying attention to urea degradation pathway along with due consideration given for non-linear CTR process occurring in activated sludge system. Experiments were performed in a simple batch reactor equipped with respirometric and titrimetric set-up. Three different initial urea concentrations were added to the reactor for investigating the process kinetics. Proposed model was successfully calibrated with respirometric, titrimetric and combined respirometric–titrimetric measurements; and the estimated parameters were compared for model evaluation. Furthermore, the proposed model was validated with off-line ammonium, nitrite and nitrate measurements. The study revealed that urea was hydrolyzed at a faster rate in liquid phase. The maximum growth rates of the *Nitrosomonas* species and the *Nitrobacter* species were found to be 0.065–0.1 d^{-1} and 0.006–0.008 d^{-1} respectively.

Keywords: Oxygen uptake rate (OUR); Urea biodegradation; Model calibration; Parameter estimation

1. Introduction

Urea is a common organic nitrogen compound that exists particularly in industrial wastewater. High level of urea concentration causes elevated nitrogen concentrations in the wastewater effluent which in-turn results in adverse impacts on animals, birds, fishes and plants growth [1]. Consequently, an in-depth understanding on urea removal dynamics using activated sludge is very important to understand the removal mechanism that can aid optimization of the performance of wastewater treatment plants. On-line monitoring system has been recommended to obtain details of the bio-kinetic information of substrate biodegradation process. Respirometric and titrimetric measurement techniques, thereby, have become popular in recent years since these methods are capable of producing high frequency on-line data required for the investigation of substrate removal mechanisms in a bio-culture. Many researchers applied respirometric measurements [2–6] and titrimetry measurements [7–10] during activated sludge model calibration. Moreover, researchers emphasized the application of combined respirometric-titrimetric measurements for precise model parameter estimation [10–13].

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Gernaey et al. [14] employed two-step nitrification model for the interpretation of urea biodegradation in activated sludge where on-line respirometric measurements were used for model calibration. They assumed a constant carbon dioxide transfer rate (CTR) for modeling. According to Pratt et al. [9,15], a constant CTR may be applicable only when the system is controlled with a low CO_2 transfer coefficient at a pH higher than 8. Consequently, Sin and Vanrolleghem [10] considered a non-linear CTR in the liquid phase and proposed a titrimetric model for acetate biodegradation. However, there is no reference in the literature depicting the titrimetric model for nitrification that pays due attention to the dynamic CTR process taking place in the liquid phase in an activated sludge system. In addition, Gernaey et al. [14] determined the urea nitrification kinetics without including the hydrolysis process in the model structure that does not reflect reality (see sub-section 2.1 below).

Hence, in this paper, a nitrification model was proposed considering urea hydrolysis process and the physical-chemical interactions of CO_2 in the liquid medium to enable a model-based interpretation of both the respirometric and titrimetric behavior in an activated sludge system. The proposed model was calibrated for three different initial urea concentrations. In addition, three different calibration approaches: using the respirometric measurements alone, the titrimetric measurements alone and combined respirometric-titrimetric measurements were performed to estimate the parameters more precisely and to validate the proposed model.

2. Materials and methods

2.1. Model theory

The major steps during the biodegradation process include ammonification, ammonium oxidation, nitrite oxidation, endogenous respiration, aqueous CO_2 equilibrium and CO_2 stripping (see Table 1 for process matrix). The following sub-sections describe the basic theory corresponding to the proposed titrimetric model development.

2.1.1. Ammonification

Ammonification represents the hydrolysis of urea (NH_2CONH_2) to ammonium (NH_4^+) in the presence of the enzyme *urease* in the environment. Eq. (1) shows the conversion of urea to ammonium where proton (H^+) is consumed and bicarbonate (HCO_3^-) is released in the environment [16]. Sometimes, the proton consumption is expressed in terms of hydroxyl ion (OH^-) production in the system [17], which, in turn, represents the same conversion process.

$$NH_2CONH_2 + H^+ + 2H_2O \rightarrow 2NH_4^+ + HCO_3^- \quad (1)$$

Based on the chemical conversion as shown in the above equation, the proton production during hydrolysis can be estimated using the model matrix (Table 1). The kinetic expression used by Spanjers and Vanrolleghem [18] for ammonification (as hydrolysis) was applied in the proposed model based on the assumption that ammonification is not dependent on biomass concentration.

2.1.2. Ammonium oxidation (Nitrification step 1)

Ammonium is oxidized to nitrite by *Nitrosomonas* species during the first nitrification step by releasing proton in the liquid medium. Eq. (2) represents the biochemical conversion of ammonium to nitrite assuming CO_2 as the carbon source required for biosynthesis of autotrophic microorganisms [7]. The equation is expressed in molar unit basis where elemental conservation and a balance of the degrees of reduction were used to determine the stoichiometric coefficient.

$$\begin{aligned}&\left(\frac{1}{Y_{SA1}}+c\right)NH_4(\text{mmol-N})+\frac{1}{2}\left(\frac{3}{Y_{SA1}}-\frac{\gamma_X}{2}\right)O_2(\text{mmol})\\&+CO_2(\text{mmol-C})\rightarrow 1CH_aO_bN_c(\text{C-mmol})\\&+\frac{1}{Y_{SA1}}NO_2(\text{mmol-N})+(...)H_2O+\left(\frac{2}{Y_{SA1}}+c\right)H^+(\text{mmol})\end{aligned} \quad (2)$$

In the above equation, $CH_aO_bN_c$ represents the elemental composition of biomass and γ_X represents the degree of reduction of the biomass which is calculated as $4 + a - 2b - 3c$. Y_{SA1} is the autotrophic biomass yield of the first nitrification step (molar unit basis). Eq. (3) is the stoichiometric expression that can be derived by converting the units from mol-N to g N and C-mol to g COD and dividing both sides of Eq. (2) with "$8\gamma_X$" where 8 gCOD is assumed as equivalent for each mol electron [19].

$$\begin{aligned}&\left(\frac{1+Y_{A1}i_{NBM}}{Y_{A1}}\right)(\text{mgN})NH_4+\left(\frac{3.43-Y_{A1}}{Y_{A1}}\right)(\text{mgCOD})\,O_2\\&+\frac{1}{8\gamma_X}(\text{mmol})CO_2\rightarrow 1\,(\text{mgCOD})\,CH_aO_bN_c\\&+\frac{1}{Y_{A1}}(\text{mgN})NO_2+\,(...)\,H_2O+\left(\frac{2+Y_{A1}i_{NBM}}{14Y_{A1}}\right)(\text{mmol})H^+\end{aligned} \quad (3)$$

Here, Y_{A1} refers to the autotrophic biomass yield of the first nitrification step (mg COD/mg N) which is equal to $8Y_{SA1}.\gamma_X/14$. The coefficient related to CO_2 and proton (H^+) production/consumption are expressed in molar units that is more relevant to titrimetric analysis. In Table 1, the parameter "p" represents the fraction of NH_4^+ in the liquid phase which is derived as $1/(1 + 10^{pH - pKNH_4})$ by Gernaey et al. [20]. A single component for biomass concentration (X_B) was used to keep the proposed model simple. A combined parameter $f_{BA}.X_B$ was used to express

the growth kinetics, where the coefficient f_{BA} represents the fraction of autotrophs in the mixed culture (Table 1).

2.1.3. Nitrite oxidation (Nitrification step 2)

Second nitrification step represents the conversion of nitrite (NO_2) to nitrate (NO_3) by *Nitrobacter* species. In a similar way as stated above, the consumption of CO_2 due to the biomass growth on nitrite can be estimated by using the following C-mol basis expression [Eq. (4)]:

$$\begin{aligned} &c\mathrm{NH_3(mmol\text{-}N)} + \frac{1}{Y_{SA2}}\mathrm{NO_2(mmol\text{-}N)} \\ &+\frac{1}{2}\left(\frac{1}{Y_{SA2}} - \frac{\gamma_X}{2}\right)\mathrm{O_2(mmol)} + \mathrm{CO_2(mmol\text{-}C)} \\ &\rightarrow 1\mathrm{CH_aO_bN_c(C\text{-}mmol)} + \frac{1}{Y_{SA2}}\mathrm{NO_3(mmol\text{-}N)} \\ &+ (...)\ \mathrm{H_2O} \end{aligned} \tag{4}$$

In Eq. (4), Y_{SA2} is the autotrophic biomass yield of the second nitrification step (molar unit basis). The same biomass composition was assumed for both the *Nitrosomonas* and *Nitrobacter* species to avoid complexity in the modeling. Eq. (5) can be derived similarly as described above where the growth yield, Y_{A2} is presented in terms of g COD/g N that is equal to $8Y_{SA2}.\gamma_X/14$. The coefficient related to ammonia uptake (i_{NBM}) is expressed as g N per g COD biomass unit basis and can be determined from the relation $14c/8\gamma_X$ [21].

$$\begin{aligned} &i_{\mathrm{NBM}}(\mathrm{mgN})\mathrm{NH_3} + \frac{1}{Y_{A2}}(\mathrm{mgN})\mathrm{NO_2} \\ &+\left(\frac{1.14 - Y_{A2}}{Y_{A2}}\right)(\mathrm{mgCOD})\mathrm{O_2} + \frac{1}{8\gamma_X}(\mathrm{mmol})\mathrm{CO_2} \\ &\rightarrow 1\ (\mathrm{mg\ COD})\ \mathrm{CH_aO_bN_c} + \frac{1}{Y_{A2}}(\mathrm{mgN})\mathrm{NO_3} \\ &+ (...)\ \mathrm{H_2O} \end{aligned} \tag{5}$$

In addition, the above equation demonstrates the stoichiometric components related to ammonia and oxygen uptake for a unit biomass growth (mg COD basis) during the second step nitrification process (see model matrix in Table 1).

2.1.4. Endogenous respiration

The biological reaction during endogenous respiration leads to CO_2 production that can be estimated using the stoichiometric expression as shown in Eq. (6).

$$\begin{aligned} &1\ (\mathrm{mgCOD})\ \mathrm{CH_aO_bN_c} + 1\ (\mathrm{mgCOD})\ \mathrm{O_2} \\ &\rightarrow \frac{1}{8\gamma_X}(\mathrm{mmol})\ \mathrm{CO_2} + (...)\ \mathrm{H_2O} + i_{\mathrm{NBM}}(\mathrm{mgN})\mathrm{NH_3} \end{aligned} \tag{6}$$

Table 1 presents the production of CO_2 for the respective oxygen uptake of $(1 - f_{XI})$ g COD (as derived by Sin and Vanrolleghem [10]).

2.1.5. Aqueous CO_2 equilibrium and CO_2 stripping

Sin and Vanrolleghem [10] used the dynamic model in their study to explain the physical–chemical interactions of CO_2 in typical biological reactors as well as the transfer of aqueous CO_2 to the gas phase for the investigation on acetate biodegradation. Similar approaches are applied in our proposed model to represent the aqueous CO_2 equilibrium and CO_2 stripping in an aerobic activated sludge system (see Table 1 for more details).

2.2. Batch study

Batch experiments were conducted using a titrimetric respirometer to investigate urea biodegradation in an activated sludge system. The set-up consists of dissolved oxygen (DO) and pH sensors along with a reactor having a capacity of 3.5 L [22]. Both pH and DO were monitored every 5 s interval and pH was controlled at a set point of 7.8 ± 0.03. Data acquisition of the analogue signals from the sensors was processed by a personal computer equipped with the *Labview* software package. Compressed air was supplied for the proper aeration in the bioreactor. The sludge was collected from Wetalla Water Reclamation Plant (operated by Toowoomba City Council), Australia. The facility maintains 1.5–2 g/L of MLSS as sludge concentration in the reactors and operates at 15 days of sludge retention time. The sludge was acclimatized with urea for five days prior to the commencement of the main experiments to allow the microorganisms to perform at their maximum capacity. Basic trace nutrient were added to ensure that bacterial growth was not limited by their absence. In addition, $NaHCO_3$ was dosed to provide sufficient inorganic carbon, to keep the metabolic function of the biomass normal. Urea with varying initial concentration (5, 10 and 20 mg N/L) was used to investigate the biodegradation mechanism under aerobic condition. All raw data related to DO and pH was processed using a spreadsheet program as prescribed by Gernaey et al. [7,14]. OUR was calculated based on the procedure explained in Gernaey et al. [14] using the experimentally determined value for the oxygen transfer coefficient (K_La). Re-aeration procedure was followed to calculate the parameter K_La [23].

2.3. Model parameter estimation

The proposed model was calibrated using three different calibration approaches: using respirometric measurements alone, titrimetric measurements alone and combined respirometric–titrimetric measurements, followed by model parameter estimation. A non-linear technique employing the algorithms in the optimisation

Table 1
Process matrix involved in the proposed model for urea biodegradation

Process	X_B (g COD)	X_N (g N)	S_{NH} (g N)	S_{NO_2} (g N)	S_{NO_3} (g N)	S_O (g O_2)	S_{HCO_3} (mol)	S_{CO_2} (mol)	S_{Hp} (mol)	Kinetics
Ammonification	—	–1	1	—	—	—	1/28	—	–(1/28)	$k_N X_N$
S_{NH} oxidation (Nitrification 1)	1	—	$-(1/Y_{A1}) - i_{NBM}$	$1/Y_{A1}$	—	$-(3.43 - Y_{A1})/Y_{A1}$	—	$-(1/8\gamma_X)$	$i_{NMB}p/14 + 1/7Y_{A1}$	$\left(1-e^{-t/\tau}\right).\mu_{max,A1}.\frac{S_{NH}}{K_{SA1}+S_{NH}}.f_{BA}.X_B$
S_{NO_2} oxidation (Nitrification 2)	1	—	$-i_{NBM}$	$-(1/Y_{A2})$	$1/Y_{A2}$	$-(1.14 - Y_{A2})/Y_{A2}$	—	$-(1/8\gamma_X)$	$i_{NMB}p/14$	$\left(1-e^{-t/\tau}\right).\mu_{max,A2}.\frac{S_{NO_2}}{K_{SA2}+S_{NO_2}}.f_{BA}.X_B$
Endogenous respiration	–1	—	$i_{NBM} - i_{NXI}f_{XI}$	—	—	$-(1 - f_{XI})$	—	$(1 - f_{XI})/8\gamma_X$	$-[(i_{NMB} - f_{XI}i_{NXI})/14]p$	$b{\cdot}X_B$
Aqueous CO_2 equilibrium	—	—	—	—	—	—	1	–1	1	$k_1 S_{CO_2} - k_1 10^{pk_1 - pH} S_{HCO_3}$
CO_2 stripping	—	—	—	—	—	—	—	1	—	$K_L a_{CO_2}\left(S^*_{CO_2} - S_{CO_2}\right)$

The proposed model is based on the assumption that ammonification is not dependent on biomass concentration [18]. The first order expression ($1–e^{-t/\tau}$) is used to explain the start-up phase in the batch experiment [3]. In this model matrix, the parameter S_{Hp} represents the proton production in the liquid medium where a negative value for this parameter indicates the proton consumption in the system.

toolbox included in MATLAB (R2007a) was used during the parameter estimation process. Minimization of the mean squared error (MSE) between the model and the experimental output was calculated as the main criterion for curve fitting. For proper model evaluation, the proposed model was calibrated using varying initial urea concentration (e.g. 5, 10 and 20 mg N/L).

The model parameters k_N, K_{SA1}, K_{SA2}, $\mu_{max,A1}$, $\mu_{max,A2}$, Y_{A1}, Y_{A2} and τ were estimated along with calculation of 95% confidence intervals. The parameter f_{BA} was assumed to be 0.3 based on the fact that the heterotrophic biomass outweighs autotrophic biomass in subtropical regions [24]. Readers are referred to "Symbols and abbreviations" for the description of model parameters.

The ASM default values for the parameters b (0.15 d^{-1}), f_{XI} (0.2) and i_{NXI} (0.02 g N/g COD X_I) were assumed here for the proposed model calibration and parameter estimation. The relationship $OUR_{end}(0) = (1 - f_{XI}).b.X_B(0)$ was employed to calculate the initial concentration of biomass, $X_B(0)$. Total inorganic carbon in the aqueous medium, $C_{T,init}$ was adjusted reasonably for different assays to fit the experimental profile with the model one. The initial concentrations of CO_2 and HCO_3 in the reactor were calculated using their relationship with $C_{T,init}$ [21]. The parameter k_1 was adjusted to 1.5 min^{-1} for better curve fitting and lies within the range (0.15–1.8 min^{-1}) noted by Stumm and Morgan [25]. During the model calibration, the value for $K_L a_{CO_2}$ was calculated as 0.0728 min^{-1} from the oxygen transfer coefficient ($K_L a$) using the relationship between their diffusivity coefficients [10,26]. The parameter pK_1 was taken as 6.39 [26]. The default values suggested by Stumm and Morgan [25] for the parameters pK_{NH_4} (9.25) and $S^*_{CO_2}$ (0.017 mmol/L) were assumed during the parameter estimation process. In addition, the degree of reduction of the biomass (γ_X) and the nitrogen content of the biomass (i_{NBM}) were calculated as 4.2 and 0.083 g N/g COD X_B respectively based on the biomass formula of $CH_{1.8}O_{0.5}N_{0.2}$ that was revised later for better curve fitting.

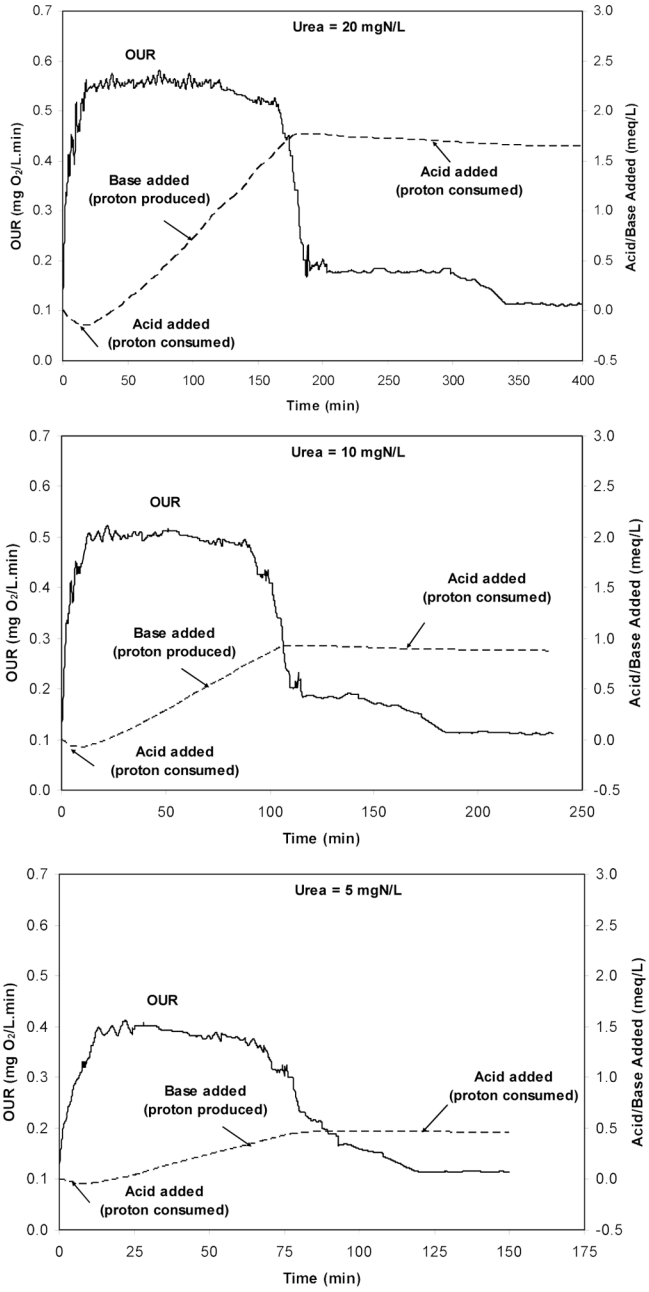

Fig. 1. OUR with titrimetric profiles for three different urea concentrations in an activated sludge system.

3. Results and discussion

3.1. Results and discussion of batch experiments

A series of batch experiments with varying initial urea concentrations of 5, 10 and 20 mg N/L was conducted to observe the influence of initial concentrations of urea on the biodegradation process. The activated sludge, which was used in this study, was fed with urea for five days prior to the commencement of the main experiments to the biomass was acclimatize with the test substrate to optimize its adoption capacity. A constant pH at 7.8 ± 0.03 was maintained during this study.

Fig. 1 represents the OUR and titrimetric profiles when three different initial urea concentrations were added to an activated system. The OUR profiles follow the same pattern in all concentration studies. The OUR increases to a maximum level due to the consumption of urea under the feast period [14]. The peak of the OUR profile is found to increase proportionally with the increase of initial substrate concentration. The OUR then drops to a level producing a "tail" in the OUR profile which finally decreases gradually to an endogenous OUR level. This kind of "tail" in the nitrification process was also noted in the literature and explained as due to nitrite accumulation in the liquid medium [27]. It was also confirmed

through off-line measurements where significant nitrite accumulated during the nitrification process (Fig. 4).

Urea biodegradation initially causes acid addition to the reactor followed by a continuous base addition under feast conditions (Fig. 1). Eq. (1) also shows that urea hydrolysis results in proton consumption in the liquid medium. The current study reveals that urea is hydrolyzed to ammonium at a very fast rate (see the sub-section 3.2 for the process rate). Hence, the substrate urea was often treated as a readily biodegradable compound like ammonium and hydrolysis was excluded in the nitrification modeling [14] to keep the model simple. However, this does not reflect the real life situation. Though the proton consumption (acid addition) during the urea biodegradation is minor compared to the proton production (base addition) in the system, both the acid and base addition were found to increase proportionally with the increase in initial urea concentration as presented in Fig. 1. After the end of the feast period, the CO_2 stripping leads the titrimetric process to drop the profile to the background proton consumption (acid addition) rate which was also observed before the addition of urea to the reactor when pH was maintained at 7.8.

3.2. Results and discussion of model calibration

The proposed model was calibrated with the experimental OUR and Hp measurements for the initial urea concentration of 20 mg N/L which is presented in Fig. 2. The model calibration graphs for the urea concentrations of 10 and 5 mg N/L are not demonstrated in this paper. The parameter estimation results are shown in Tables 2–4 where the calibration approaches: using respirometric measurements alone, titrimetric measurements alone and combined respirometric–titrimetric measurements, were applied.

This study reveals that the parameter k_N varies from 0.034 to 0.081 min^{-1} as the urea concentration decreases from 20 to 5 mg N/L. There is little reported in the literature about urea ammonification (hydrolysis) kinetics to compare with current observations. However Spanjers and Vanrolleghem [18] noted the organic nitrogen hydrolysis rate to be 0.04 min^{-1} when using raw wastewater as a test substrate. The autotrophic maximum growth rate for the first nitrification step ($\mu_{max,A1}$) is found to increase from 0.065 to 0.1 d^{-1} when the urea concentration changes from 5 to 20 mg N/L respectively. On the other hand, a very slow biomass growth rate was noticed during the second nitrification step (conversion of nitrite to nitrate) showing an average $\mu_{max,A2}$ value of 7.98×10^{-3} d^{-1} (Tables 2–4). Consequently, it results in nitrite accumulation in the liquid medium which was also confirmed by off-line NO_2-N measurement (Fig. 4). Though for the overall nitrification process ASM suggested an autotrophic maximum growth rate higher (0.8 d^{-1} in ASM1, 1.0 d^{-1} in ASM3) than the current observation, Gernaey et al. [14] observed a

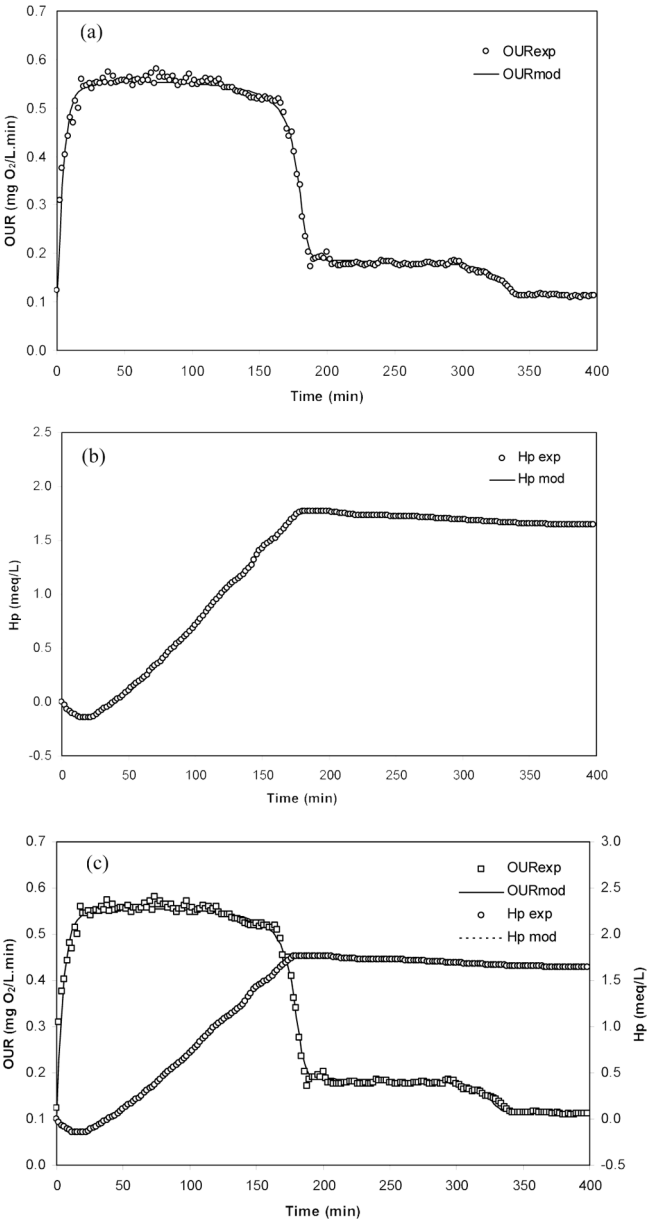

Fig. 2. Model calibration using (a) respirometric data alone (b) titrimetric data alone and (c) combined respirometric–titrimetric data (Urea = 20 mg N/L).

maximum autotrophic biomass growth rate as slow as 4.7×10^{-3} d^{-1} during the first step of ammonium nitrification process. Parameter estimation results show that the calculated combined parameter $(3.43 - Y_{A1})\,\mu_{max,A1}.f_{BA}.X_B/Y_{A1}$ lies between 0.264–0.393. It is found to be consistent with the observation of Gernaey et al. [14] who estimated the average value for the combined parameter as 0.319 for the first step of the urea nitrification process.

The estimated parameter K_{SA1} gives an average value of 0.29 mg N/L that leads to the combined parameter $(3.43 - Y_{A1}).K_{SA1}$ as 0.936. However, Gernaey et al. [14] recorded

Table 2
Parameter estimation results using respirometric data alone for three different concentration studies (confidence intervals are shown in brackets as percentages)

Parameters	Urea 20 mg N/L (Confidence interval, %)	Urea 10 mg N/L (Confidence interval, %)	Urea 5 mg N/L (Confidence interval, %)
Parameters estimated:			
$\mu_{max,A1}$ (1/min)	$6.91\times10^{-5} \pm 4.11\times10^{-8}$ (0.06)	$6.22\times10^{-5} \pm 7.14\times10^{-8}$ (0.12)	$4.5\times10^{-5} \pm 4.53\times10^{-8}$ (0.1)
$\mu_{max,A2}$ (1/min)	$5.54\times10^{-6} \pm 1.87\times10^{-9}$ (0.03)	$4.86\times10^{-6} \pm 1.4\times10^{-9}$ (0.03)	$4.3\times10^{-6} \pm 2.42\times10^{-9}$ (0.06)
k_N (1/min)	$0.036 \pm 1.37\times10^{-5}$ (0.04)	$0.061 \pm 6.27\times10^{-5}$ (0.1)	$0.081 \pm 9.9\times10^{-4}$ (1.22)
K_{SA1} (mg N/L)	$0.278 \pm 5.56\times10^{-4}$ (0.2)	$0.273 \pm 8.9\times10^{-4}$ (0.33)	$0.271 \pm 2.59\times10^{-4}$ (0.33)
K_{SA2} (mg N/L)	$0.202 \pm 1.82\times10^{-3}$ (0.9)	$0.2 \pm 2.22\times10^{-3}$ (1.1)	0.198 ± 0.011 (5.56)
Y_{A1} (mg COD X_B/mg N S_{NH})	$0.204 \pm 3.62\times10^{-5}$ (0.02)	$0.204 \pm 7.81\times10^{-5}$ (0.04)	$0.2 \pm 9.23\times10^{-4}$ (0.46)
Y_{A2} (mgCOD X_B/mg N S_{NO_2})	$0.029 \pm 6.37\times10^{-6}$ (0.02)	$0.025 \pm 4.27\times10^{-5}$ (0.17)	$0.024 \pm 8.42\times10^{-4}$ (3.51)
Parameters assumed:			
b (1/min)	0.0001042	0.0001042	0.0001042
f_{XI} (mg COD/mg COD)	0.2	0.2	0.2
Parameters calculated:			
X_B (mg COD/L)	1200	1200	1200
MSE[a]	1.25×10^{-4}	1.01×10^{-4}	9.6×10^{-5}

[a]MSE refers to the mean squared error which is calculated from sum of squared errors divided by number of observations

this combined parameter slightly higher (1.277) than the current observation when investigating urea nitrification kinetics in an activated sludge system. In this current study, the average value for the biomass yield coefficient Y_{A1} is found to be 0.2, whereas the estimated parameter Y_{A2} is found to vary from 0.023 to 0.029. Though Kim et al. [28] revealed the yield coefficients Y_{A1} and Y_{A2} as 0.33 and 0.083 respectively; Marsili-Libelli and Tabani [29] noted the combined autotrophic biomass yield ($Y_{A1} + Y_{A2}$) varied from 0.258 to 0.296. In addition, ASM prescribed the overall autotrophic biomass yield (Y_A) to be 0.24 which supports the current observation.

The titrimetry related component $C_{T,init}$ was adjusted to 1.5 mmol CO_2/L for all three urea concentration studies for the better fit of experimental profiles with the model one. For all three calibration approaches the model parameters are found to be consistent with reasonable confidence intervals (Tables 2–4) which validates the accuracy of the model calibration and parameter estimation processes. In addition, the mean squared errors (MSEs), calculated from three different initial urea concentrations and calibration approaches, are acceptable and statistically confirm the soundness of the proposed model.

3.3. Proposed model evaluation

The proposed model explains well both the experimental respirometric and titrimetric measurements as evident by the good fit of the model profiles with the experimental observations. In addition, the estimated model parameters show consistent results for all three calibration approaches (i.e. calibration with respirometric measurements alone, titrimetric measurements alone and combined respirometric titrimetric measurements). Moreover, the parameter estimation errors (calculated for 95% confidence intervals) as well as the mean squared errors (MSEs) for all three calibration approaches were reasonable and confirm the statistical soundness of the proposed model.

In the proposed model, the biomass formula was assumed to be $CH_{1.5}O_{0.2}N_{0.1}$ to achieve a good fit between the model and experimental profiles for all three calibration approaches. Pratt et al. [9] noted that the best fit between measured and simulated data was obtained with the assumption of a biomass formula of $CH_{1.87}O_{0.66}N_{0.17}$ during their model calibration study. Based on the assumed biomass formula ($CH_{1.5}O_{0.2}N_{0.1}$) the calculated degree of reduction of biomass, γ_x was fixed at 4.8 during calibra-

Table 3
Parameter estimation results using titrimetric data alone for three different concentration studies (confidence intervals are shown in brackets as percentages)

Parameters	Urea 20 mg N/L (Confidence interval, %)	Urea 10 mg N/L (Confidence interval, %)	Urea 5 mg N/L (Confidence interval, %)
Parameters estimated:			
$\mu_{max,A1}$ (1/min)	$6.91\times10^{-5} \pm 7.51\times10^{-8}$ (0.11)	$6.22\times10^{-5} \pm 8.72\times10^{-8}$ (0.14)	$4.51\times10^{-5} \pm 6.83\times10^{-8}$ (0.15)
$\mu_{max,A2}$ (1/min)	$5.52\times10^{-6} \pm 1.51\times10^{-9}$ (0.03)	$4.83\times10^{-6} \pm 2.98\times10^{-9}$ (0.06)	$4.3\times10^{-6} \pm 1.15\times10^{-9}$ (0.03)
k_N (1/min)	$0.034 \pm 2.12\times10^{-5}$ (0.06)	$0.059 \pm 1.82\times10^{-5}$ (0.03)	$0.078 \pm 1.04\times10^{-4}$ (0.13)
K_{SA1} (mg N/L)	$0.289 \pm 2.77\times10^{-4}$ (0.1)	$0.3 \pm 3.5\times10^{-4}$ (0.12)	$0.297 \pm 2.55\times10^{-3}$ (0.86)
K_{SA2} (mg N/L)	0.202 ± 0.043 (21.29)	0.2 ± 0.059 (29.5)	0.186 ± 0.092 (49.46)
Y_{A1} (mg COD X_B/mg N S_{NH})	$0.202 \pm 6.79\times10^{-5}$ (0.03)	$0.204 \pm 1.8\times10^{-5}$ (0.01)	0.198 ± 0.014 (7.07)
Y_{A2} (mg COD X_B/mg N S_{NO_2})	$0.029 \pm 7.24\times10^{-5}$ (0.25)	$0.026 \pm 4.39\times10^{-5}$ (0.17)	0.023 ± 0.013 (56.52)
Parameters assumed:			
b (1/min)	0.0001042	0.0001042	0.0001042
f_{XI} (mg COD/mg COD)	0.2	0.2	0.2
k_1[b] (1/min)	1.5	1.5	1.5
$K_La_{CO_2}$ (1/min)	0.0728	0.0728	0.0728
$C_{T,init}$[b] (mmol/L)	1.5	1.5	1.5
Parameters calculated:			
HCO_3 (mmol/L)	1.4447	1.4447	1.4447
CO_2 (mmol/L)	0.0553	0.0553	0.0553
X_B (mg COD/L)	1200	1200	1200
MSE[a]	1.09×10^{-4}	4.77×10^{-5}	4.42×10^{-5}

[a]MSE refers to the mean squared error which is calculated from sum of squared errors divided by number of observations
[b]Parameters were fixed by trials for the better fit of experimental profile with the model

tion where the i_{NBM} content was calculated as 0.036 g N/g COD X_B, though the typical value for the nitrogen content of biomass was reported between the range 7–8.6% [19]. Conversely, Sin and Vanrolleghem [10] estimated the iNBM content within the range 2.4–5.7% which supports the current observation. Gernaey et al. [8] also reported the parameter i_{NBM} as low as 3.8% during their organic carbon biodegradation study.

Gernaey et al. [14] verified the respirometric method of their proposed model by investigating the linearity between BOD_{st} values and NH_4-N concentrations added to the activated sludge. With this in mind, an attempt was made in this current study to determine the relationship between BOD_{st} and urea concentration (expressed as mg N/L). This is presented in Fig. 3. The area under the OUR profiles were considered to calculate the BOD_{st} for respective urea concentration study. The slope ($4.57 - Y_A$) of the curve is typically expected to be 4.33 g O_2/g NH_4-N [30]. From Fig. 3 the slope of the curve is found to be 4.31 g O_2/g NH_4-N which supports the literature value [30]. However, Gernaey et al. [14] noted the slope as high as 4.44 g O_2/g NH_4-N for urea nitrification.

In addition to on-line respirometric and titrimetric methods, the proposed model was validated using off-line NH_4-N, NO_2-N and NO_3-N measurements during the urea biodegradation study (Fig. 4).

The model-simulated profiles present reasonably the off-line experimental observations confirming the precision of the proposed model. In addition, the nitrite accumulation is noted significant during urea nitrification. It was supported by the parameter estimation results where the maximum growth rate of *Nitrobacter* species ($\mu_{max,A2}$) was observed to be significantly slower compared to that of *Nitrosomonas* species ($\mu_{max,A1}$).

Table 4
Parameter estimation results using combined respirometric–titrimetric data for three different concentration studies (confidence intervals are shown in brackets as percentages)

Parameters	Urea 20 mg N/L (Confidence interval, %)	Urea 10 mg N/L (Confidence interval, %)	Urea 5 mg N/L (Confidence interval, %)
Parameters estimated:			
$\mu_{max,A1}$ (1/min)	$6.91\times10^{-5} \pm 7.7\times10^{-8}$ (0.11)	$6.22\times10^{-5} \pm 1.07\times10^{-8}$ (0.02)	$4.5\times10^{-5} \pm 2.45\times10^{-8}$ (0.05)
$\mu_{max,A2}$ (1/min)	$5.57\times10^{-6} \pm 2.25\times10^{-9}$ (0.04)	$4.86\times10^{-6} \pm 2.7\times10^{-9}$ (0.06)	$4.3\times10^{-6} \pm 9.96\times10^{-9}$ (0.23)
k_N (1/min)	$0.034 \pm 1.15\times10^{-5}$ (0.03)	$0.059 \pm 1.95\times10^{-5}$ (0.03)	$0.08 \pm 8.93\times10^{-4}$ (1.11)
K_{SA1} (mg N/L)	$0.287 \pm 8.88\times10^{-4}$ (0.31)	$0.289 \pm 9.52\times10^{-4}$ (0.33)	$0.28 \pm 2.42\times10^{-4}$ (0.09)
K_{SA2} (mg N/L)	0.202 ± 0.003 (1.49)	0.2 ± 0.005 (2.5)	0.2 ± 0.047 (23.5)
Y_{A1} (mg COD X_B/mg N S_{NH})	$0.203 \pm 7.23\times10^{-5}$ (0.04)	$0.204 \pm 1.22\times10^{-4}$ (0.06)	$0.2 \pm 4.9\times10^{-3}$ (2.45)
Y_{A2} (mg COD X_B/mg N S_{NO_2})	$0.029 \pm 7.64\times10^{-5}$ (0.26)	$0.025 \pm 8.21\times10^{-5}$ (0.33)	$0.024 \pm 3.61\times10^{-3}$ (15.04)
Parameters assumed:			
b (1/min)	0.0001042	0.0001042	0.0001042
f_{XI} (mg COD/mg COD)	0.2	0.2	0.2
k_1[b] (1/min)	1.5	1.5	1.5
$K_L a_{CO_2}$ (1/min)	0.0728	0.0728	0.0728
$C_{T,init}$[b] (mmol/L)	1.5	1.5	1.5
Parameters calculated:			
HCO_3 (mmol/L)	1.4447	1.4447	1.4447
CO_2 (mmol/L)	0.0553	0.0553	0.0553
X_B (mgCOD/L)	1200	1200	1200
MSE[a]	1.33×10^{-4}	6.13×10^{-5}	5.66×10^{-5}

[a]MSE refers to the mean squared error which is calculated from sum of squared errors divided by number of observations
[b]Parameters were fixed by trials for the better fit of experimental profile with the model

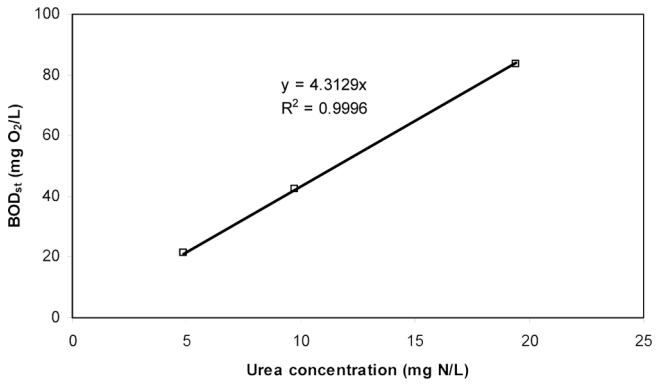

Fig. 3. BOD_{st} as a function of the initial urea concentration (expressed as mg N/L).

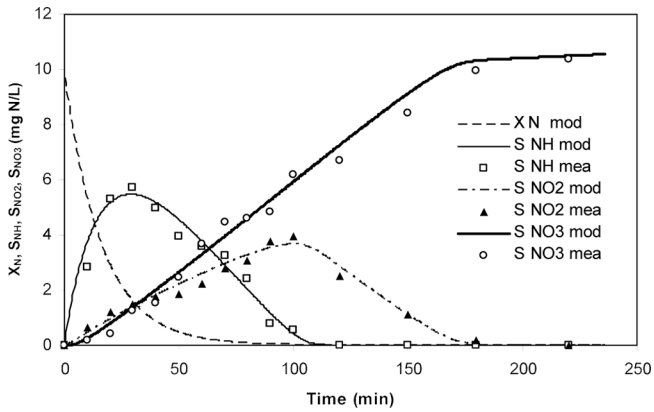

Fig. 4. Model validation using off-line ammonium, nitrite and nitrate measurements during urea (10 mg N/L) biodegradation.

4. Conclusions

A nitrification model was proposed to interpret urea biodegradation process by paying due attention to urea biodegradation pathway and dynamic CO_2 transfer in the liquid medium. The proposed model was justified for different initial urea concentrations. The model was found to explain well the experimental respirometric and titrimetric measurements of urea biodegradation process. In addition, the estimated model parameters were found to be consistent for all three calibration approaches thereby validating the proposed model. Moreover, the estimated model parameters compared favorably with values recorded in the literature. The model was validated with off-line NH_4-N, NO_2-N and NO_3-N measurements for urea nitrification process which confirms the precision of the proposed model.

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Symbols and abbreviations

ASM — Activated sludge model
b — Endogenous decay coefficient of biomass, d^{-1}
BOD_{st} — Short-term biochemical oxygen demand
$CH_aO_bN_c$ — Elemental composition of biomass, C-mol
$C_{T,init}$ — Total inorganic carbon in the aqueous medium, mmol CO_2/L
CTR — CO_2 transfer rate, mmol CO_2/L.d
DO — Dissolved oxygen
f_{BA} — Fraction of autotrophic biomass in the mixed culture, mg COD/mg COD
f_{XI} — Inert fraction of biomass, mg COD/mg COD
H_p — Proton concentration in liquid phase, meq/L
i_{NXI} — Nitrogen content of the inert fraction of biomass, g N/g COD X_I
i_{NBM} — Nitrogen content of biomass, g N/g COD X_B
k_1 — Forward reaction rate for aqueous CO_2 equilibrium, d^{-1}
k_N — Ammonification (hydrolysis) rate, d^{-1}
K_La — Oxygen mass transfer coefficient, d^{-1}
K_La_{CO2} — CO_2 mass transfer coefficient, d^{-1}
K_{SA1} — Substrate affinity constant for the first nitrification step, mg N/L
K_{SA2} — Substrate affinity constant for the second nitrification step, mg N/L
MSE — Mean squared error
OUR — Oxygen uptake rate, mg O_2/L.d
OUR_{end} — Endogenous oxygen uptake rate, mg O_2/L.d
pK_1 — Negative logarithm of the first acidity constant in the CO_2 equilibrium
pK_{NH_4} — Negative logarithm of the equilibrium constant for NH_4^+ dissociation
S_{CO_2} — CO_2 concentration in liquid phase, mmol/L
$S^*_{CO_2}$ — CO_2 saturation concentration at 1 atm, mmol/L
S_{HCO_3} — Bicarbonate concentration in liquid phase, mmol/L
S_{NH} — Ammonium concentration, mg N/L
S_{NO_2} — Nitrite concentration, mg N/L
S_O — Dissolved oxygen concentration in liquid phase, mg/L
X_B — Biomass concentration, mg COD/L
$X_B(0)$ — Initial biomass concentration, mg COD/L
X_I — Inert particulate COD, mg COD/L
X_N — Degradable organic nitrogen, mg N/L
Y_{A1} — Autotrophic biomass yield of the first nitrification step, mg COD/mg N
Y_{A2} — Autotrophic biomass yield of the second nitrification step, mg COD/mg N

Greek

γ_X — Degree of reduction of biomass, mol electron/C-mol
$\mu_{max,A1}$ — Maximum autotrophic biomass growth rate for the first nitrification step, d^{-1}
$\mu_{max,A2}$ — Maximum autotrophic biomass growth rate for the second nitrification step, d^{-1}
τ — First order time constant, d

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