Monitoring the nitrification and identifying the endpoint of ammonium oxidation by using a novel system of titrimetry

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ABSTRACT

Based on the structure of the hybrid respirometer previously developed in our group, a novel implementation for titrimetry was developed, in which two pH electrodes were installed at the inlet and outlet of the measuring cell. The software capable of digital filtering and titration time delay correction was developed in LabVIEW. The hardware and software of the titrimeter and the respirometer were integrated to construct a novel system of respirometry–titrimetry. The system was applied to monitor a batch nitrification process. The obtained profiles of oxygen uptake rate (OUR) and hydrogen ion production rate (HPR) are consistent with each other and agree with the principle of the biological nitrification reaction. According to the OUR and HPR measurements, the oxidized ammonium concentrations were estimated accurately. Furthermore, the endpoint of ammonium oxidation was identified with much higher sensitivity by the HPR measurement. The system could be potentially used for on-line monitoring of biochemical reactions occurring in any kind of bioreactors because its measuring cell is completely independent of the bioreactor.

Key words | ammonium oxidation, hydrogen ion production rate, nitrification, oxygen uptake rate, respirometry, titrimetry

INTRODUCTION

Biological wastewater treatment is a dynamic process involving various complex biochemical reactions, which often results in oxygen consumption and/or hydrogen ion production/consumption. Due to the close relations to the kinetic processes of micro-organism growth and pollutant biodegradation, oxygen uptake and hydrogen ion production/consumption are important information sources to monitor, identify and control the biological wastewater treatment process (Thornton et al. 2010). Based on the measurement of oxygen uptake rate (OUR), Blackburne et al. (2008) used the strategy of controlled aeration duration to achieve shortcut nitrification successfully. In the last decade, this topic has been studied intensely, including much progress in developing an understanding of the stoichiometry of oxygen utilization and/or hydrogen ion production/consumption versus aerobic conversions of COD (Gernaey et al. 2002a; Pratt et al. 2004), NH₄⁺-N and NO₂⁻-N (Gernaey et al. 1998a; Guisasola et al. 2005; Jubany et al. 2005), as well as anoxic conversions of COD, NO₂⁻-N and NO₃⁻-N (Petersen et al. 2002; Mcmurray et al. 2004; Ficara & Canziani 2007). Moreover, respirometry and titrimetry have been applied to measure OUR and hydrogen ion production rate (HPR) for kinetic parameter estimation (Gernaey et al. 1998a; Carvalho et al. 2002), model calibration (Jubany et al. 2005) and process monitoring (Artiga et al. 2003; Gapes et al. 2003; Fiocchi et al. 2006, 2008) in biological wastewater treatment.

Compared with the wide application of pH measurements in wastewater treatment plants (WWTPs), HPR measurement is still limited to few laboratories and only used for research due to the lack of available measurement technology and instrumentation (Vanrolleghem & Lee 2003). The titration unit (Gernaey et al. 1998b), titration and off-gas analysis (TOGA) sensor (Pratt et al. 2003) and TITAA (Fiocchi et al. 2006) developed in the past are all pH-stat titrimeters, namely, they measure HPR indirectly by dosing acid or base into the titration cell to keep constant pH during the measuring process (Petersen et al. 2002; Gernaey et al. 2002a; Guisasola et al. 2007;...
Fiocchi et al. (2008), thus making the titrimetric approach only available to monitor batch experiments.

Lu et al. (2006) designed the hybrid respirometer according to the principle proposed by Vanrolleghem & Spanjers (1998), which has been successfully applied to wastewater characterization (Lu et al. 2010), parameter estimation and inhibition evaluation. In this study, a novel implementation of titrimetry with 2 pH electrodes installed at the inlet and outlet of the measuring cell is proposed. The new titrimetric system can be used to monitor both a continuous and a batch bioreactor. The hardware and software of the titrimeter and the respirometer were integrated to construct a novel system of respirometry–titrimetry. The system was applied to monitor the characteristics of both oxygen consumption and hydrogen ion production in a laboratory (lab)-nitrifying batch reactor.

MATERIALS AND METHODS

Principle and configuration of the titrimeter

The configuration of the hybrid respirometer (Lu et al. 2006) is shown in Figure 1. The 5 L open aeration vessel and the 1 L airtight measuring cell are connected with plastic tubes. A peristaltic pump (Baoding Longer, BT00-600M) with adjustable speed continuously pumps the activated sludge mixed liquor around the two reactors. DO concentrations of the liquid flowing into and out of the measuring cell, $S_{0,1}$ and $S_{0,2}$, are measured by the two DO probes (Mettler Toledo Inpro6800) positioned at the inlet and outlet of the measuring cell, respectively. The OUR signal is obtained through a mass balance for oxygen in the measuring cell.

On the basis of the hybrid respirometer, two pH probes (Mettler Toledo Inpro4250) are introduced and located at the inlet and outlet of the measuring cell to measure the pH values of the influent (pH1) and effluent (pH2), respectively. A computer collects the pH data through transmitters (Mettler Toledo pH2100e) and a DAQ card (National Instruments M series) and compares pH2 with pH1 every 5 seconds. Once a difference between pH1 and pH2 occurs, micro-pumps (Bio-Chem Valve, 120SP24504EE) controlled by the computer via the output signal of the DAQ card will automatically add acid or base into the mixed liquor to keep pH2 identical to pH1. In theory the dosage of base or acid reflects the hydrogen ion production or consumption of the mixed liquor of activated sludge and wastewater only if pH1 and pH2 are kept equal to each other. The basic and derived mass balance equation for hydrogen ions over the measuring cell in the titration process can be obtained as Equations (1) and (2):

$$\frac{d[H^+]}{dt} = \frac{Q_i[H^+]_{i}}{V} - \frac{Q_e[H^+]_{e}}{V} + \text{HPR} - \alpha$$

(1)

$$\frac{d[H^+]}{dt} = \text{HPR} - \alpha$$

(2)

$[H^+]_{i}$, $[H^+]_{i}$ and $[H^+]_{e}$ are the hydrogen ion concentrations in the mixed liquor in the measuring cell, in the influent and effluent of the measuring cell, respectively (mmol L$^{-1}$); $V$ is the volume of the measuring cell (L); $Q_i$ and $Q_e$ are the flow rates of the influent and effluent of the measuring cell (L min$^{-1}$), and here $Q_i = Q_e$; $\alpha$ is the chemical addition rate (mmol.(L min$^{-1}$)).

Assuming that the variation of the hydrogen ion concentrations of the mixed liquor in the measuring cell is small...
enough to be ignored during the acquisition of one HPR data point (for example, 1 min), Equation (3) can be obtained.

\[
\text{HPR} = \alpha
\]  

(3)

**Software for titrimetry**

**Data processing and dosing control**

The software for titrimetry was developed based on LabVIEW 7.1. Besides the functions for data collection and processing, results display and saving described in Lu et al. (2006), comparison of pH1 and pH2 and micro-pump control for titrant dosing are important modifications to the software for titrimetry. The pH1 is taken as the reference, and the difference between pH2 and pH1 (i.e. \(\Delta pH = pH2 - pH1\)) is taken as the control variable, which is set to a narrow interval (for example [-0.03, 0.03]). The computer would then send out a signal to start the micro-pump to add alkaline or acid solution into the measuring cell when \(\Delta pH\) exceeds the set interval. Every 1 min (adjustable), the computer displays and saves pH1, pH2 and the accumulated volume of the dosed acid or alkaline solution. From the volume of the measuring cell, and the volume and concentration of the dosed alkaline or acid solution, the hydrogen ion production amount (HPA) is calculated. The HPR is determined as the difference between HPA in the current minute and the former minute.

**Digital filtering**

In order to eliminate the random measurement noise, a 5-rank sliding average filter was employed to smooth the HPR values. The results showed that the filter could effectively remove the measurement noises and improve the data quality.

**Titration time delay correction**

The signal controlling titrant dosing derives from the \(\Delta pH\), which results in a titration dosing delay because of a residence time \(\Delta t\) of the mixed liquor in the measuring cell. When hydrogen ion production or consumption is beginning, it would take a time interval \(\Delta t\) for the mixed liquor to fill the measuring cell. In this period, there is no available \(\Delta pH\) to excite titration even though hydrogen ion production or consumption has occurred. The loss of the measured HPA due to the titration delay is estimated by multiplying the measured average HPR with \(\Delta t\), and is added to the measured HPA in order to obtain the actual HPA.

**Monitoring nitrification in a batch reactor**

The novel respirometric–titrimetric system was applied to monitor a lab-scale nitrification process in order to evaluate the performance of the system. Activated sludge collected from the aeration tank of a WWTP in Chongqing, China was washed to remove residual substrates and toxic metabolites and aerated overnight to reach endogenous respiration. The endogenous respiration sludge was seeded into the 5 L aerobic bioreactor with a concentration of 3,000 mg MLSS L\(^{-1}\). The recycle pump transferred the mixed liquor from the bioreactor to fill the measuring cell and then back to the bioreactor. The temperature of the mixed liquor was kept at 25°C by means of a hot water jacket enwrapping the bioreactor. A 0.10 mol L\(^{-1}\) HCl and a 0.1 mol L\(^{-1}\) NaOH solution were used as titrant.

Concentrated ammonium stock solution was injected into the bioreactor to result in initial \(NH_4^+\cdot N\) concentrations of 3, 4.5, 6, 7.5, 9 and 10.5 mg L\(^{-1}\). OUR and HPR increased because of the nitrification activity and then decreased to the endogenous level when the substrate was depleted. The oxidized ammonium amount during any time interval could be calculated using Equations (4) and (5).

\[
\Delta S_{NH}(t_i) = \int_{t_0}^{t_i} \frac{OUR_{ex}(t) \, dt}{4.57 - \frac{Y_A}{C}}
\]  

(4)

\[
\Delta S_{NH}(t_i) = 7 \times \int_{t_0}^{t_i} HPR(t) \, dt = 7 \times HPA(t_i)
\]  

(5)

Here, \(\Delta S_{NH}(t_i)\) is the amount of ammonium oxidized from \(t_0\) to \(t_i\) (mg L\(^{-1}\)), \(OUR_{ex}(t)\) and \(HPR(t)\) are the exogenous OUR (mg (L min\(^{-1}\)) and the HPR (mmol (L min\(^{-1}\)) at time \(t\), HPA\((t_i)\) is the HPA at time \(t_i\) (mmol L\(^{-1}\)), and \(Y_A\) is the autotrophic yield coefficient (mg COD.(mg N\(^{-1}\)).

**RESULTS AND DICUSSION**

**Characteristics of OUR and HPR in nitrification**

The results measured for one batch nitrification test are presented in Figure 2. The pH2 almost always kept pace with pH1 (see Figure 2(a)), which indicated that the control of
the chemical dosage was effective enough to implement the titrimetric principle. At the 70th minute, pH1 started to increase rapidly and chemical analysis showed that the NH$_4^+$-N of the mixed liquor was depleted at this time, which suggested that the bending point of the pH1 profile indicated the end of the ammonium oxidation. In fact, pH measurement has been used for real-time control (by terminating aeration) to achieve shortcut nitrification (Peng et al. 2004). Correspondingly, the profiles of DO, OUR and HPR also displayed obvious bending points at the 70th minute, which demonstrated that both OUR and HPR could indicate the endpoint of ammonium oxidation. Furthermore, the profiles of OUR and HPR are much more distinct for the identification of ammonium oxidation than that of pH. Meanwhile, at this point the HPA curve could be divided into two parts with different slope, which is similar to the results obtained by titrimetry with only one pH electrode (Gernaey et al. 1998b).

The OUR and HPR could reveal the dynamics of the activated sludge process. However, they depend on the quantity and activity of functional microorganisms in the activated sludge, as well as the substrate species and concentrations. When COD and NH$_4^+$-N oxidation occur simultaneously, it would be difficult to identify the endpoint of ammonium oxidation based on only OUR because the OUR from COD oxidation would cover that OUR from NH$_4^+$-N oxidation. It can be seen from Figure 2 that HPR could be more sensitive to identifying the endpoint of ammonium oxidation than OUR.

**Estimation of the oxidized NH$_4^+$-N based on OUR and HPR measurements**

A series of batch nitrification experiments were conducted with different initial NH$_4^+$-N concentrations, and the results of respirometry and titrimetry are shown in Figures 3 and 4, respectively. As the initial NH$_4^+$-N concentrations increased, the maximum OUR and HPR increased and lasted for a longer time. However, the minimum values of DO1 and DO2 became lower and lower. HPA linearly increased at first and then leveled off.

In the first four tests no NaHCO$_3$ was added into the mixed liquor. It was found that the pH of the mixed liquor in the system decreased more significantly due to the higher initial substrate concentrations (data not shown). Moreover, instead of returning to the initial pH values, the pH values at the end of each test decreased. Therefore, a small amount of NaHCO$_3$ solution was added into the mixed liquor before the 5th test (from about $t = 315$ min) and the peak values of OUR and HPR in the 5th test increased slightly. In the 6th test, more NaHCO$_3$ solution was added. The OUR peak values showed a further increase,
while the HPR peak values didn’t increase obviously but fluctuated greatly. This situation might be due to the fact that too much alkalinity and the higher pH of the mixed liquor probably affected the titrimetry because of CO₂ stripping in the bioreactor.

Based on OUR and HPR, the total amount of oxidized ammonium (ΔSNH₄) during each batch experiment was calculated and compared with the dosage. The results are shown in Figure 5. The relative errors between the calculated ΔSNH₄ based on OUR and the amount which had been dosed are between −5.73 and −10.67%, with an average of −8.08%. Similarly, the corresponding errors for the calculated results based on HPR are in the range of −8.22 and −16.93% (except the last test). The calculated results are always lower than the dosages. Gernaey et al. (1998a) reported that the NH₄⁺-N concentrations obtained by interpreting titration data were 1.32 ± 0.185 mg L⁻¹, which correlated well with the 1.33 mg L⁻¹ measured. Yuan & Bogaert (2001) showed that the nitrified nitrogen concentration calculated by titration data was 3.64 mg L⁻¹, with an error of 7.37% from the initial ammonium nitrogen concentration of 3.39 mg L⁻¹. Compared with the work of Gernaey et al. (1998a) and Yuan & Bogaert (2001), errors in this paper are a little higher.

There are good linear correlations between the dosages and the calculated results based on both OUR and HPR (Figure 5), with correlation coefficients of 0.9982 and 0.9968, respectively. This finding indicates that some kind of systematic error exists, and the trend of those errors is consistent. The errors might primarily come from the following aspects: (1) According to the operating principle of the novel system, the biological reaction dynamics of the measuring cell is employed to represent that of the bioreactor. However, the structure of the system causes a delay of the former compared with the latter, and results in a reduction of the measured OUR and HPR, and as a result the measured values could always be lower than the true values. (2) The hypothesis that the variation of the hydrogen ion concentration of the mixed liquor in the measuring cell could be ignored during the acquisition of one HPR data point could not be completely satisfied in practice. The pH decreased slowly during the nitrification of the batch reactor, so dH⁻/dt in Equation (2) was positive (even close to zero), and made the measured HPR lower than the true value. (3) The buffer systems in the activated sludge system could neutralize a part of the hydrogen ion production by nitrification, which results in a reduction of the amount that is titrated. (4) The nitrifier growth takes up CO₂ into the cells, and endogenous respiration of microbial organisms consumes oxygen and produces CO₂. The dissolution of the produced CO₂ results in hydrogen ion production as a result of carbonate acid dissociation. Therefore, the CO₂ effects of nitrifier growth and endogenous respiration of microbial organisms in the mixed liquor of the measuring cell, which were not taken into consideration here, could result in the errors of the calculated amount of oxidized ammonium based on OUR and HPR measured in the nitrification process. In addition, the usage of Yₐ without calibration could also be a source of the errors.

Advantages of the novel respirometric-titrimetric system

The advantages of the novel respirometric-titrimetric system developed in this study are as follows: (1) The configuration of the system with two DO electrodes and two pH electrodes located at the inlet and outlet of the measuring cell, makes the measuring cell completely independent of the bioreactor that is monitored. Therefore, the measuring cell can be connected to batch and continuous flow bioreactors with or without aeration to continuously monitor the biochemical reaction occurring in the bioreactors. (2) The measuring cell of 1 L is much smaller than the biological wastewater treatment tank, and it is not necessary to keep the bioreactor at a constant pH during the measurement. This would largely decrease the consumed amount of chemicals for titrimetry. (3) In the airtight measuring cell there is nearly no CO₂ stripping which would otherwise result in a change in the hydrogen ion concentration through acid/base buffering systems. The impact of CO₂ stripping on HPR is difficult to be accurately accounted for when the carbon dioxide transfer rate (CTR) is not measured unless under specific conditions, and this limits the use of pH-stat titration instruments. In order to address this problem,
Pratt et al. (2003) developed the TOGA sensor using a quadrupole mass spectrometer as an off-gas measurement technique to measure CO₂ in the bioreactor off-gas and calculate the CTR. By this means, the impact of the bicarbonate system on the HPR signal can be determined. However, the evolution of CO₂ in the off-gas could not accurately reflect that in the liquid phase, because the former is influenced by the bicarbonate system balance and mass transfer dynamics of CO₂ between gas and liquid phases. Meanwhile, it is acknowledged that construction of the TOGA sensor is very expensive and its application for online calibration of wastewater treatment systems is practically difficult. Sin & Vanrolleghem (2007) extended the Gernaey model expressing respirometric–titrimetric data resulting from carbon degradation experiments with a dynamic CO₂ model to describe the nonlinear effect of CO₂ stripping. This made the calibration of activated sludge models more complex. Pratt & Yuan (2007) assessed the impact of adjustments to the inorganic carbon pool on titrimetric data by considering a pH-stat titration of heterotrophic carbon oxidation, and used model simulations to quantify the impact of CO₂ transfer for a wide range of operating conditions. (4) Respirometry and titrimetry can be conducted either simultaneously or separately. Respirometry is suitable for aerobic processes, while titrimetry is applicable to any biochemical reactions with hydrogen ion production or consumption. And, due to the software developed in LabVIEW, the level of automation of the instrumentation is improved and its operation is simplified.

Besides monitoring the biological wastewater treatment processes, OUR and HPR were also used for calibrating activated sludge models. Petersen et al. (2001) verified the parameters of a 2-step nitrification model based on titrimetric data and OUR data, respectively. The results showed that high precision of parameter estimation could be obtained from both methods, while a fast convergence of the objective function towards a minimum was obtained for estimation on titrimetric data. Gernaey et al. (2002b) reported that the confidence interval of the parameter estimation for an aerobic carbon degradation model was improved by using both OUR and titrimetric data. Meanwhile, the cell yield coefficient (Y_H) and nitrogen content of biomass (N_BHM) could be estimated simultaneously. Sin & Vanrolleghem (2007) calibrated an aerobic carbon degradation model with a nonlinear CO₂ stripping process using respirometric–titrimetric measurements in batch experiments, which provided a good basis for validation of the aerobic activated sludge models (carbon oxidation model and nitrification model) using titrimetric data and OUR.

The respirometric and titrimetric system developed in this study can be used not only for off-line calibration in batch tests in the laboratory, but also for on-line calibration of activated sludge models for any WWTP configuration.

**CONCLUSIONS**

A novel implementation of titrimetry was developed including two pH electrodes installed at the inlet and outlet of a measuring cell. The hardware and software of the titrimeter and the hybrid respirometer were integrated to construct a novel respirometric–titrimetric system, which can conduct the measurements of DO, pH, OUR and HPR simultaneously with high frequency and level of automation. The obtained kinetic features of DO, pH, OUR and HPR are consistent with each other and agree with the principle of nitrification. Based on the OUR and HPR measurements, the amount of oxidized ammonium could be predicted accurately. HPR is more sensitive to identifying the endpoint of ammonium oxidation than OUR, especially when COD and NH₄-N oxidation occur simultaneously.

The system could be connected to batch and continuous flow bioreactors with or without aeration to continuously monitor the biochemical reaction occurring in the bioreactors, for example in-situ measurement of the activity of a biological wastewater treatment tank. Further study of its application in other unit processes of biological wastewater treatment is certainly worthwhile.

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