

# DEVELOPMENT OF COMBINED ULTIMATE HYBRID RESPIROMETER-TITRATE METER TO ESTIMATE KINETIC PARAMETERS OF ACTIVATED SLUDGE

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*Received: Mar 20, 2009; Revised: Jun 30, 2009; Accepted: Sept 14, 2009*

## Abstract

The aim of this paper is to present a new development and an application of the ultimate hybrid respirometer-titrate meter for wastewater characterization and kinetic parameters estimation of microorganisms in activated sludge. Currently, respirometry and titrimetry are considered high performance tools with accuracy and frequency measurement. A new implementation of ultimate hybrid respirometer combined with titrate meter was developed. The new set-up of instrument was developed based on the previous knowledge and needed only sensor electrodes. The user interface programming to system control and monitor was developed based on LabVIEW 8.2 (student edition) software packages equipped with NI DAQ USB-6210 (A/D 16 bit). Finally, a laboratory implementation was tested with readily biodegradable substrate, such as sodium acetate and ammonium chloride, to estimate the essential kinetic parameters of heterotrophic as yield, maximum specific growth rate, decay rate, saturation constant for substrate and initial ammonium concentration in activated sludge samples. The parameter estimation results from using respirometric measurements data were close to the default values in Activated Sludge Model No. 1 (ASM1) and other reported values. The ammonium concentration calculate by using base information were much correlated with the amount of ammonium applied to the activated sludge sample. This demonstrated that the new instrument set-up was successfully applied to estimate kinetic parameters of activated sludge and measurement of ammonium concentration in activated sludge samples.

**Keywords:** Activated sludge, kinetic, oxygen uptake rate, respirometry, titrimetry

## Introduction

Respirometry is the measurement and interpretation of the respiration rate of activated sludge under a well-defined experimental condition, and is defined as oxygen per unit of volume and time that is consumed by the microorganisms in activated sludge (Oxygen uptake rate, OUR). It is a frequently and widely used tool for characterization of wastewater and

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activated sludge kinetic parameters. The theory of a hybrid respirometric measurement was proposed by Vanrolleghem and Spanjers (1998). In the hybrid respirometer, the principles of a flowing gas-static liquid (LFS) and static gas-flowing liquid (LSF) respirometer are combined (L is measurement in liquid phase). Table 1 compares the four principles in terms of these properties. Several details on types and measurement principles of respirometer, can be found in Spanjers *et al.* (1998). Further information in the development and evaluation of the hybrid respirometer can be found in Petersen (2000).

Besides respirometry, titration experiments can also yield information on the kinetic parameters of activated sludge in biological nitrogen removal processes. Indeed, the pH value of a biological responds to microbial reactions. The nitrification process ( $\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + \text{H}_2\text{O} + 2\text{H}^+$ ) causes pH to decrease because of two moles of proton production (Gernaey *et al.*, 1997). Ammonium concentration that interprets from proton information produced due to nitrification reaction was highly correlated with amount of ammonium applied to activated sludge sample (Massone *et al.*, 1996). The titrimetric measurement is to keep constant pH in activated sludge which results from biological reactions to monitor acid or base consumption rate.

The reliability, versatility and precision of titrimetric principle were successfully applied

to estimate kinetic parameters of activated sludge (Ramadori *et al.*, 1980; Massone *et al.*, 1996; Gernaey *et al.*, 1998). The titrimetric principle was applied to measurement under anoxic condition for estimation kinetic parameters of denitrifying bacteria and evaluation performance of denitrification process (Onnis *et al.*, 2001; Artiga *et al.*, 2005). This principle was also applied to the measurement under anaerobic condition for evaluation methanogenic activity (Rozzi *et al.*, 2001).

The results obtained from titrimetric experiments show reliability of this method in estimating kinetic parameters of activated sludge samples. Also, the advantages of each measurement principle are combined to improve performance of instrument with higher accuracy and frequency measurement (Gernaey *et al.*, 2001; Gernaey *et al.*, 2002a, 2002b). The instrument was applied to estimate kinetic parameters for Activated Sludge Model No. 1 (ASM1) calibration, monitoring and control of biological nutrient removal processes (Guisasola *et al.*, 2007; Sin and Vanrolleghem, 2007).

The aim of this paper is to develop and realize the combined ultimate hybrid respirometer-titrate meter to support the advancement in wastewater research with demonstrating the data interpretation from respirometric-titrimetric experiments. In addition, the main goal of this implementation is to construct the instrument which needs only the sensor electrodes to

**Table 1. Advantages and disadvantages of different respirometric principles ( $K_{L,a}$  is oxygen mass transfer coefficient)**

Respirometer type	Advantages	Disadvantages
Static gas-static liquid (LSS)	Easy to operate	Danger for oxygen limit Low OUR measurement frequency
Flowing gas-static liquid (LFS)	High OUR measurement frequency	$K_{L,a}$ estimation needed
Static gas-flowing liquid (LSF)	No $K_{L,a}$ needed	Low OUR measurement frequency
Hybrid respirometer	No $K_{L,a}$ needed High OUR measurement frequency	Two dissolved oxygen electrode

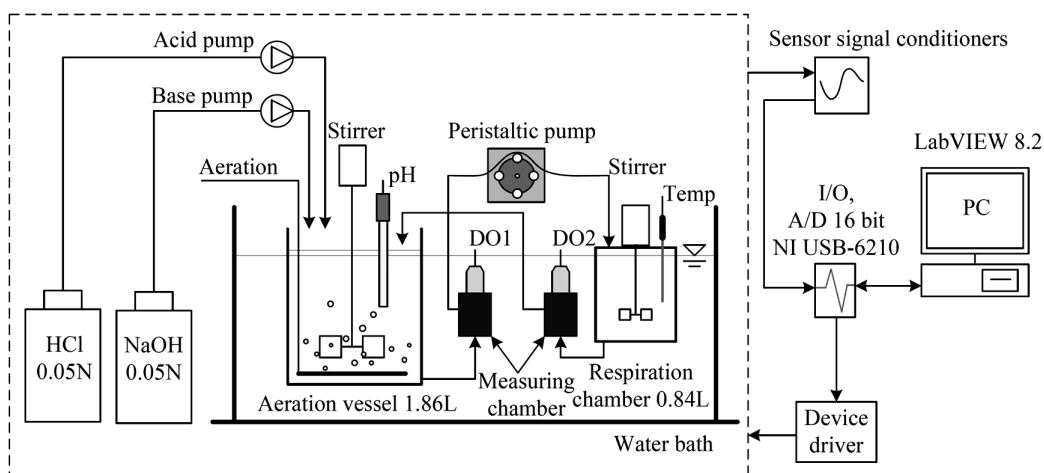
connect to the system.

## Materials and Methods

A new implementation set-up is combined ultimate hybrid respirometer-titrate meter (Vanrolleghem and Spanjers, 1998; Petersen, 2000). An overview of the instrument is shown in Figure 1. The set-up consists of an aeration vessel ( $V = 1.86$  L) and a respiration chamber ( $V = 0.84$  L). The latter is made strictly airtight, while the aeration vessel was open. For the aeration, fine-bubble aeration was provided in the aeration vessel by using a simple air stone diffuser commercially available for fish tanks. Atmospheric air, dried and compressed to 4 bar pressure, was used as air supply. Stirrers with adjustable speed mixed the content of both vessels. A peristaltic pump (six-roller) with adjustable speed was used to continuous pump the activated sludge to both vessels. The average dosage of acid and base pump (diaphragm) was  $0.031 \pm 0.001$  and  $0.032 \pm 0.001$  mL/pulse, respectively. Aeration vessel, respiration chamber, transferring tube, measuring chamber and dissolved oxygen (DO) electrodes were submerged in the water bath to reduce the difference in temperature between aeration vessel and respiration chamber.

A pH electrode (HI-1230B, Hanna Instruments) was placed at aeration vessel and two polarographic DO electrodes (YSI-200, YSI Incorporated) were placed in measuring chambers before and after respiration chamber. Signals from two DO, pH and temperature electrodes were amplified by sensor signal conditioners to the range of amplitude ( $\pm 10$  VDC). All electrode signals were noise filtered with 10 Hertz low passed analog filter circuits before being converted to digital signals by using NI-6210 (USB-DAQ, A/D 16 bit, I/O, National Instruments). The user interface for data processing and control was developed, based on LabVIEW 8.2 software package (Student edition, National Instruments). The data acquisition frequency of the sensors was set to 3 seconds. In the implementation set-up, calibration of DO electrodes was done in two steps while aerated distilled water was pumped through the set-up. First, the two DO electrodes in the measuring chamber were calibrated with DO saturation in distilled water, and then calibrated with zero DO. Calibration of pH electrode was done by using standard pH buffer solutions (4.0, 7.0, and 10.0 pH).

For the experimental work, activated sludge was sampling from the MLE pilot scale wastewater treatment plant (Environmental



**Figure 1. Overview of the combined ultimate hybrid respirometric-titrimetric set-up**

Engineering Lab, Suranaree University of Technology, Nakhon Ratchasima, Thailand), which performs nitrification, denitrification and chemical oxygen demand (COD) removal. It was fed with municipal synthetic wastewater with average COD, TKN (Total kjedahl nitrogen) and TP (Total phosphorus)  $238.99 \pm 13.15$ ,  $32.47 \pm 2.98$ , and  $4.40 \pm 0.59$  mg/L, respectively. The hydraulic retention time (HRT), solid retention time (SRT), temperature and pH were maintained at 6 h 5 days,  $28 \pm 0.10^\circ\text{C}$  and  $7.5 \pm 0.03$  respectively.

Activated sludge was aerated overnight until it reached the endogenous respiration phase and then washed 2 - 3 times with the distilled water prior to transferring to the set-up (Artiga *et al.*, 2005). At the start of the experiment, the set-up was filled with 2.74 L activated sludge. The activated sludge was aerated until the endogenous respiration phase was reached. During the experiments, small substrate pulses (e.g. 10 mL) of sodium acetate (13 g COD/L) and ammonium chloride (1 g  $\text{NH}_4^+$ -N/L) stock solutions were dosed to the activated sludge. All experiments, instrument calibration and activated sludge sample preparation were performed at  $28 \pm 0.10^\circ\text{C}$ . The pH was controlled within a narrow set-band  $7.5 \pm 0.03$ , by dosing with 0.05N HCl and 0.05N NaOH, as described in detail by Gerney *et al.* (2001).

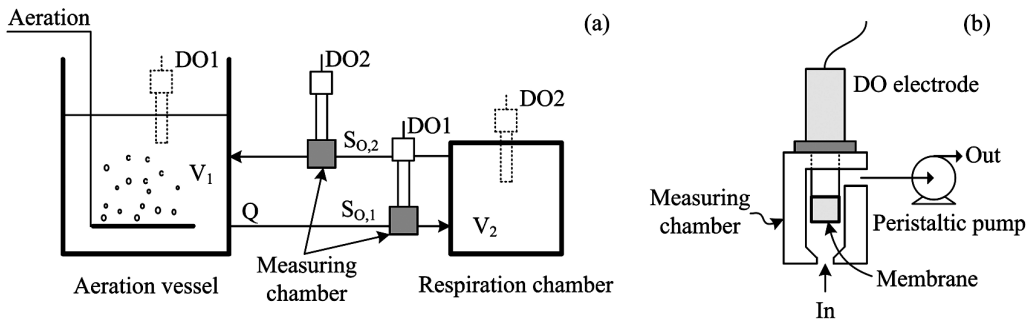
## Data Interpretation

Data derived from each experiment were interpreted using both a spreadsheet program (Excel) and a model-based data interpretation method based on ASM1.

## Experimental Results and Discussion

### Structure of Combined Ultimate Hybrid Respirometer-titrate Meter

The schematic diagram of the ultimate hybrid respirometer-titrate meter is presented in Figure 2(a). In this set-up, the DO electrode in the aeration vessel was moved to the other connecting line between the respiration chamber and the aeration vessel. In this way, the DO concentration of liquid flowing into and out of the respiration chamber,  $S_{O,1}$  and  $S_{O,2}$ , were measured by two DO electrodes, DO1 and DO2, respectively. At the same time, OUR can be calculated by derived through a mass balanced of oxygen over the respiration chamber with a similar procedure as the static gas-flowing liquid respirometer (Equation (1)) (Vanrolleghem and Spanjers, 1998). Measuring chamber can be operated in four different patterns (Lu *et al.*, 2006). So the up-flow with negative pressure (more stabled measurement than each other patterns) (Figure 2(b)) was selected as the



**Figure 2. Schematic diagram of ultimate (full line) and simple (dotted line) hybrid respirometer (a) and DO measuring chamber (b)**

regular operation pattern for DO measuring chamber.

$$\text{OUR} = -\frac{dS_{\text{O}_2}}{dt} + \frac{Q}{V_2} (S_{\text{O}_1} - S_{\text{O}_2}) \quad (1)$$

where OUR is the oxygen uptake rate (mg/L.min),  $Q$  is the flow rate (L/min) and  $V_2$  is the respiration chamber volume (L).

In the new set-up, DO electrode did not place in aeration vessel and respiration chamber. The advantages are reduced hydrodynamic noise and oscillated readings of DO electrodes in both vessels. A possible reason is because of pressure acting on the surface membrane of DO electrode (Petersen, 2000). A working volume of measuring chamber was about 4 mL, 0.5% of respiration chamber volume. Hydraulic retention time of measuring chamber at operating condition,  $Q = 0.25$  L/min was not more than 1.0 sec. So the oxygen uptake rate in measuring chamber could be ignored (Lu *et al.*, 2006). In the new implementation, an accurate and constant temperature water bath was designed to accommodate the whole system (aeration vessel, measuring chamber with DO electrodes, respiration chamber and almost all transfer tube) to achieve accurate and reliable DO measurement.

Batch experiment where the rapid change of oxygen uptake rate, the time constant of the electrode ( $\tau_{\text{electrode}}$ ) is then no longer negligible. To deal with this, the DO electrode dynamics should be taken into account before Equation (1) is used for calculation of oxygen uptake rate. Using the general accepted first order model describing the dynamics of DO electrode (Equation (2) – (3)) (Vanrolleghem and Spanjers, 1998). The DO electrode time constant 8 seconds was used in this experiment (YSI Incorporated).

$$\frac{dS_{\text{O}}^{\text{electrode}}}{dt} = -\left( S_{\text{O}} - \frac{S_{\text{O}}^{\text{electrode}}}{\tau_{\text{electrode}}} \right) \quad (2)$$

$$S_{\text{O}} = S_{\text{O}}^{\text{electrode}} + \tau_{\text{electrode}} \frac{dS_{\text{O}}^{\text{electrode}}}{dt} \quad (3)$$

The software implementation with combined ultimate hybrid respirometer was developed based on the LabVIEW 8.2 package (Student edition, National Instruments). The main content was noise filtering, sensors signal processing, devices controlling and user interface. All parameters needed to be set up by the user such as temperature and pH set-point, respiration chamber volume, internal flow rate, acid and base dosing volume, electrode time constant, signal filter and measuring frequency can input into this panel (Figure 3(a)). Meanwhile, the value of DO, oxygen uptake rate, pH, acid or base accumulations and temperature can be real-time display on panel as shown in Figure 3(b), which provides system monitoring. By this way, the user can find possible fault detection and take effective action in time.

The new implementation worked consistent, except peristaltic pump, activated sludge transfer pump between aeration vessel and respiration chamber. A two-roller peristaltic pump is the main cause of high pressure oscillating in the activated sludge transferring tube. This problem can be solved by changing transferring pump to a six-roller peristaltic pump and increasing the speed of the pump. The test with distilled water (Figure 4(a)) showed the oscillating of pressure in the activated sludge transferring tube less affect DO measurement and oxygen uptake rate calculation. After temperature reached the set-point value ( $28 \pm 0.10^\circ\text{C}$ ), DO was stable throughout the experiment period. Standard deviation (0.003 mg/L.min) was much narrowband. The test with activated sludge sample (Figure 4(b)) shows DO profile and the calculation of oxygen uptake rate with the same concentration of two pulses of substrate were applied.

### Activated Sludge Kinetic Parameters Estimation

Modeling efforts of activated sludge

processes took off in the early 1970s with the pioneering work of the Marais group from the University of Cape Town. The industry standard ASM1 was developed by the IAWPRC task group on modeling activated sludge processes (Henze *et al.*, 2000). Since then, understanding of activated sludge processes increased which resulted in the development of ASM2 and ASM3. Activated sludge models can be applied for the following purposes; design of new plants, analysis of existing plants, real-time control of plant, and serving as a guidance for researcher to achieve more efficient experimental designs (Henze *et al.*, 2000).

Respirometry, the measurement of oxygen uptake rate of biomass has been a well-established methodology for the calibration

of activated sludge models (Kappeler and Gujer, 1992; Spanjers and Vanrolleghem, 1995; Vanrolleghem *et al.*, 1999). The stoichiometric parameter (Yield,  $Y$ ), kinetic parameters (Maximum specific growth rate ( $\mu_{max}$ ), decay rate ( $b$ ) and saturation constant for substrate ( $K$ )) are the major group parameters to be calibrated. In the mathematical model, the process rates and the stoichiometric relationship between the processes and compounds are formulated mathematically by the matrix method (Table 2) for dynamic model presentation.

An accurate assessment of the heterotrophic bacteria yield coefficient,  $Y_H$ , is the most important, not only because this parameter influences sludge production and oxygen used but also because it has an impact on the value of

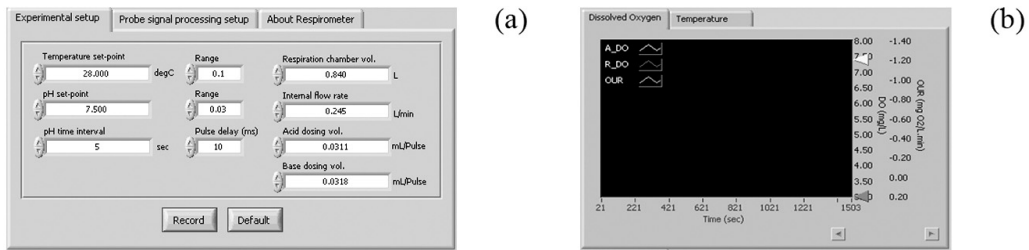


Figure 3. Software user interface (a) and real-time display panel (b)

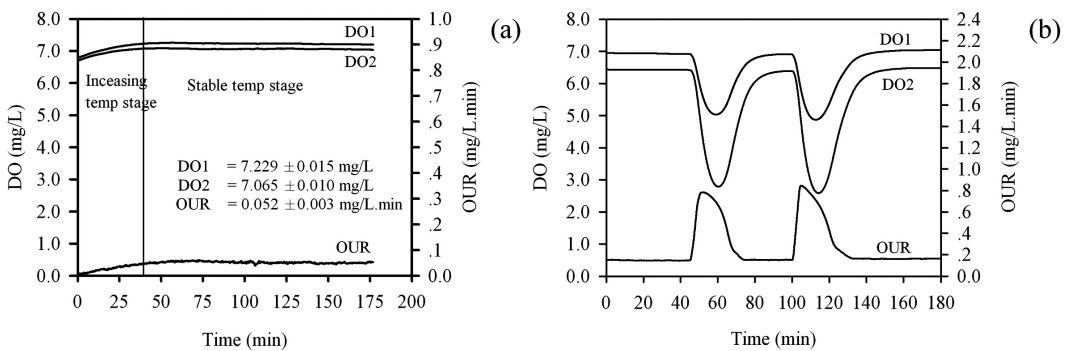


Figure 4. The experiment with distilled water (a) and activated sludge sample (b)

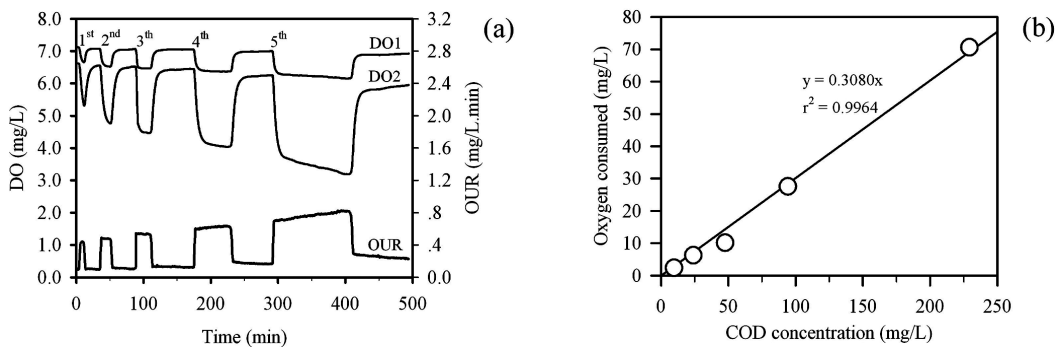
other parameters whose determination requires a value of  $Y_H$  (Vanrolleghem *et al.*, 1999). A linear relationship between the substrate ( $S_S$ ) added and oxygen consumed (OC) allows to calculate biomass yield via  $OC = (1 - Y_H)S_S$  by using the slope of the curve. The respirogram obtained in this experiment is shown in Figure 5(a).

The heterotrophic bacteria yield coefficient was evaluated through a respirometric batch experiment with 5 different pulses of a completely biodegradable organic substrate added to endogenous activated sludge sample. Sodium acetate range from 9.6 – 229.3 mg COD/L was used in this experiment with an average of mixed liquor volatile suspended solid (MLVSS) of 1,000 mg/L. A linear increase of OC was

observed when increasing amount of sodium acetate was added to the respirometer (Figure 5(b)). The maximum average yield for this data series is  $0.69 \pm 0.01$  mg COD/mg COD which is much close to the default value in ASM1 (0.67) (Henze *et al.*, 2000). Muller *et al.* (2004) reported the yield on acetate 0.69 (in the range of 0.66 - 0.71) mg COD/mg COD. Similar results were reported by Dricks *et al.* (1999), a value of yield on acetate was 0.71 mg COD/mg COD. Furthermore, the difference observed also implies that in the determination of the readily biodegradable COD (rbCOD) fraction by respirometric methods, the yield coefficient should not be based on a standard value from literature but should be experimentally determined from the activated sludge used in the experiment.

**Table 2. The matrix notation for heterotrophic bacteria ( $X_{BH}$ ) growth and decay in ASM1**

Component	$i$	1	2	3	4	Process rate, $\rho_j$
$j$	Process	$S_S$	$X_{BH}$	$S_O$	$S_{NH}$	
1	Aerobic growth of heterotrophs	$-\frac{1}{Y_H}$	1	$-\frac{1-Y_H}{Y_H}$	$-i_{XB}$	$\mu_{maxH} \frac{S_S}{K_S + S_S} \frac{S_O}{K_{OH} + S_O} X_{BH}$
2	Decay of heterotrophs		-1	1		$b_H X_{BH}$



**Figure 5. Yield determination respirogram (a) and linear relationship between oxygen consumed with substrate added (b)**

Saturation constant for substrate,  $K_S$ , is determined following the method described by Cech *et al.* (1984). This method is based on Monod equation (Equation (4) - (5)) and analysis of the oxygen uptake rate at different substrate concentration. Exogenous oxygen uptake rate ( $OUR_{ex}$ ) was measured as the difference between endogenous oxygen uptake rate ( $OUR_{end}$ ) and the total oxygen uptake rate registered in every experiment.

$$\mu_H = \frac{Y_H}{(1-Y_H)} \frac{OUR_{ex}}{X_{BH}} \quad (4)$$

$$\mu_H = \mu_{maxH} \frac{S_S}{(K_S + S_S)} \quad (5)$$

The exogenous oxygen uptake rate was divided by the maximum value of exogenous oxygen uptake rate registered in the whole experiment to calculate the relative activity ( $\mu/\mu_{maxH}$ ), depending on substrate concentration. The resulted of respirometric experimental concurrent with data fitted by simulation is shown in Figure 6. The saturation constant for substrate ( $K_S$ ) obtained is  $19.1 \pm 2.7$  mg/L, which is close to the default value in ASM1 (20 mg/L) (Henze *et al.*, 2000). The observed  $K_S$  value was closed to 20.4 mg/L (at 20°C) that was reported

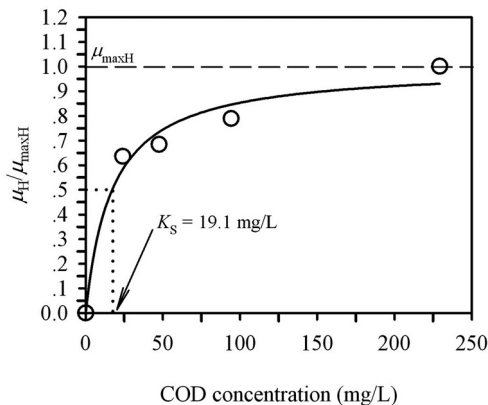


Figure 6. Assessment of saturation constant for substrate

by Playa-roja *et al.* (2002). The different in value of  $K_S$  is 15 mg/L (at 30°C) that was reported by Dosta (2007).

The decay coefficient for heterotrophic bacteria with lineal death,  $b_H$ , was determined following the method proposed by Spanjers and Vanrolleghem (1995). For this experiment, activated sludge was aerated without substrate applied. The method was based on measuring the oxygen uptake rate obtained after addition of an optimal mixture of sodium acetate (Figure 7(a)). This was repeated 3 times over a period of 9 days. If yield and maximum growth rate are constant, the value of the parameter combinations will only depend on the active biomass. The decay coefficient is calculated from the slope of a plot of  $\ln OUR_{max}$  versus time (Figure 7(b)).

As shown in Figure 7(b),  $\ln OUR_{max}$  versus time shows a straight line with the decay

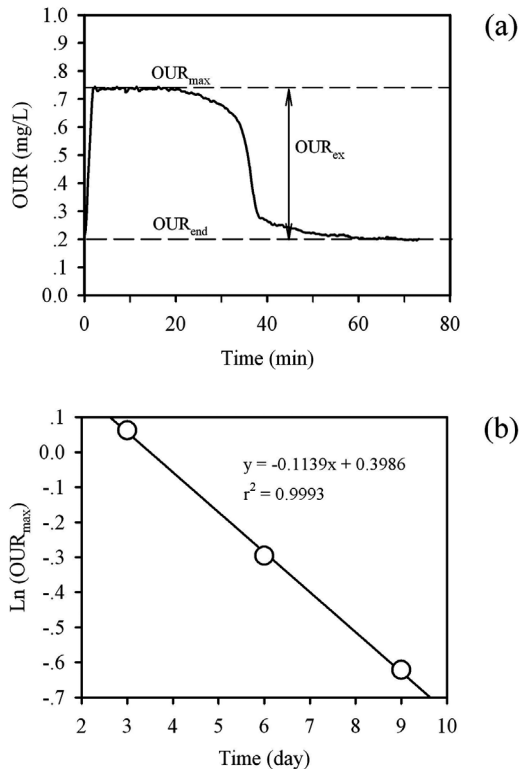


Figure 7. The respirogram obtained from a decay experiment (a) and a plot of  $\ln OUR_{max}$  versus time (b)



coefficient for heterotrophic bacteria (lineal-death),  $b_H^{\text{Lineal-death}}$ , as slope. The value determined was  $0.1 \text{ day}^{-1}$ , where obtained coefficient differs from the one in the ASM1. The death-regeneration concept, adopted in the ASM1, implies that the classical methods for determination of the decay of biomass can not be applied directly. The parameter of lineal-death concept has to be translated to the parameter of death-regeneration concept by applying Equation (6) (Orhon and Artan, 1994), where  $f_p$  is the particulate inert fraction of biomass (0.08). The decay rate was,  $b_H^{\text{Death-regeneration}}$ , obtained  $0.31 \pm 0.02 \text{ day}^{-1}$ . This decay rate value very close to  $0.28 \text{ day}^{-1}$  (at  $20^\circ\text{C}$ ) was reported by Manser *et al.* (2006). This decay rate is lower than the default value in ASM1 ( $0.62 \text{ day}^{-1}$ ), but still comparable to the range of decay rate values ( $0.05 - 1.6 \text{ day}^{-1}$ ) in the literature (Jeppsson, 1996), and  $b_H^{\text{Lineal-death}}$  is about 5% of maximum specific growth rate value (Kappeler and Gujer, 1992).

$$b_H^{\text{Death-regeneration}} = \frac{b_H^{\text{Lineal-death}}}{(1 - Y_H(1 - f_p))} \quad (6)$$

The maximum specific growth rate of heterotrophic bacteria,  $\mu_{\text{maxH}}$ , under aerobic conditions, is concomitant with the saturation

constant for substrate,  $K_S$ , an essential kinetic parameter to characterize the COD removal capacity and the biomass production of the activated sludge under study. This parameter was assessed with the procedure proposed by Kappeler and Gujer (1992) based on adding high readily biodegradable substrate to low concentration of activated sludge with none limiting of DO concentration. Sodium acetate 1,402 mg COD/L was used in this experiment with an average of MLVSS 720 mg/L. The respirogram obtained in this experiment is shown in Figure 8(a). A linear increase of oxygen uptake rate was observed when sodium acetate was added to the respirometer. The possibility of assessing  $(\mu_{\text{maxH}} - b_H)$  is the slope of the plot of LnOUR versus time (Figure 8(b)). The  $\mu_{\text{maxH}}$  for this experimental data is  $2.2 \pm 0.3 \text{ day}^{-1}$ . This  $\mu_{\text{maxH}}$  value close to  $1.68 \text{ day}^{-1}$  (at  $25^\circ\text{C}$ ) was reported by Guisasola (2005). This  $\mu_{\text{maxH}}$  is much lower than the default value in ASM1 ( $6 \text{ day}^{-1}$ ), but still comparable to the range of  $\mu_{\text{maxH}}$  values ( $0.6 - 13.2 \text{ day}^{-1}$ ) in the literature (Jeppsson, 1996). The results of all experiments are shown in Table 3.

Titrimetry, the indirect measurement of the pH effects resulting from the biomass metabolic activities, has recently reached a level of precision which makes it useful for quantifying the kinetic parameters of activated sludge

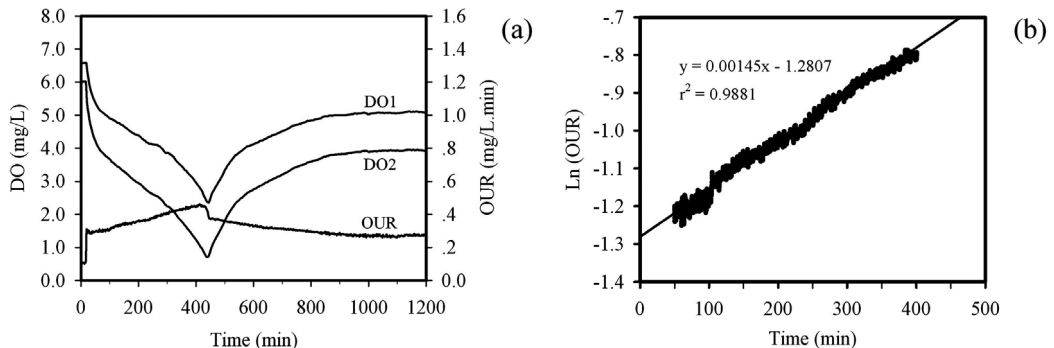


Figure 8. Maximum specific growth rate determination respirogram (a) and plot of LnOUR versus time (b)

processes (Sin and Vanrolleghem, 2007), Titrimetric measurement principle is based on adding small amount of a titration solution to keep constant pH at a fixed set-point value during the reaction.

In this study, the simultaneously respirometric and titrimetric measurements were tested with activated sludge samples to determine oxygen uptake rate (data not shown) and amount of NaOH (0.05N) to compensate for the proton production by autotrophic bacteria in nitrification process. An initial ammonium concentration can be calculated based on the difference between B2 and B1, according to Equation (7)

$$S_{\text{NH}} = \frac{(B2 - B1)N \cdot 7}{V_{\text{vessel}}} \quad (7)$$

where  $S_{\text{NH}}$  is an initial  $\text{NH}_4^+$ -N concentration in the vessel of titration unit (mg N/L), B1 is the volume of base needed to increase the pH of sludge sample to the pH set-point (mL), B2 is sum of B1 and volume of base necessary to compensate for the protons produced during nitrification (mL),  $N$  is base normality (mequiv/mL), 7 is the conversion factor (mg N/mequiv), and  $V_{\text{reactor}}$  is the volume of sludge in the vessel of the titrate meter (L) (Gernaey *et al.*, 1997).

The experiment was carried out with an average of MLVSS 2,180 mg/L at pH set-point  $7.8 \pm 0.03$ . Ammonium chloride of 1.9 and 3.7 mg  $\text{NH}_4^+$ -N/L were used in this experiment. As

shown in Figure 9(a) and 9(b), the profiles of base dosage were almost a straight line during the period of nitrification reaction. The results of titration experiments should theoretically be equal to the amount of  $\text{NH}_4^+$ -N added at the beginning of the titration experiment. The calculation of  $\text{NH}_4^+$ -N concentration of 2.0 and 3.6 mg  $\text{NH}_4^+$ -N/L were obtained. The results show that the set-up allows for determination of  $\text{NH}_4^+$ -N concentration in the activated samples using a simple and robust titration method.

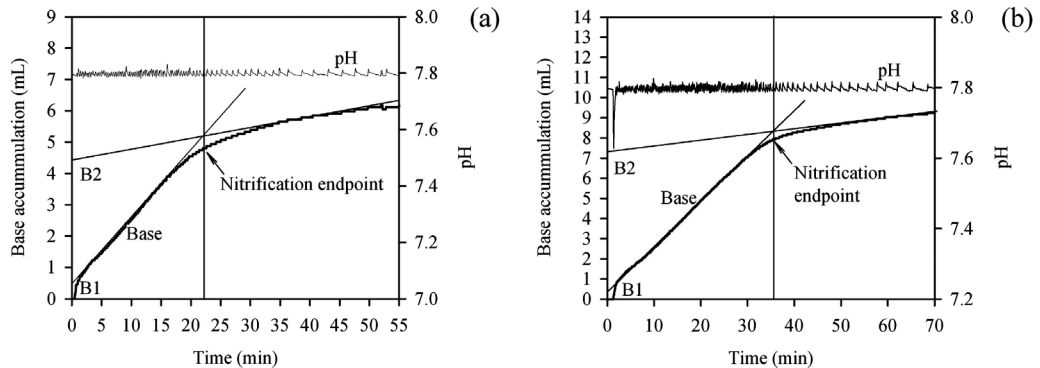
## Conclusions

The combined ultimate hybrid respirometer-titrate meter was developed and designed to be an advanced tool for wastewater engineering research based on the previous knowledge and only required sensors electrode to connect to the system. The user interface was developed based on LabVIEW 8.2 (Student edition) for controlling and experimental monitoring with real-time display. The major sources of noise for oxygen sensing and oxygen uptake rate calculation were minimized by increased number of peristaltic pump roller. The difference in temperature in each vessel was solved by means of submersed aeration vessel, dissolved oxygen measuring chamber with DO electrodes, respiration chamber and almost all transfer tubes into water bath with high accuracy and reliable temperature control.

An essential kinetic parameters of heterotrophic bacteria ( $X_{\text{BH}}$ ) are yield ( $Y_{\text{H}}$ ),

**Table 3. The kinetic parameters obtained from respirometric experiments**

Kinetic parameters of heterotrophic bacteria	Value	Unit	ASM1/ ASM3 (20°C) (Henze <i>et al.</i> , 2000)	Literature (Jeppsson, 1996)
Yield coefficient	$0.69 \pm 0.01$	mg/mg	0.67/0.85	0.38 - 0.75
Max. spec. growth rate	$2.2 \pm 0.3$	day <sup>-1</sup>	6/2	0.6 - 13.2
Saturation constant for COD	$19.1 \pm 2.7$	mg/L	20/2	5 - 30
Decay rate	$0.31 \pm 0.02$	day <sup>-1</sup>	0.62/0.2	0.05 - 1.6



**Figure 9.** Titration curves and pH profiles were obtained during titration experiment in activated sludge sample by applied 1.9 mg  $\text{NH}_4^+\text{-N/L}$  (a) and 3.7 mg  $\text{NH}_4^+\text{-N/L}$  (b)

maximum specific growth rate ( $\mu_{\max\text{H}}$ ), decay rate ( $b_{\text{H}}$ ) and saturation constant ( $K_{\text{S}}$ ) which were estimated via respirometric measurements. The results of parameter estimation using respirometric measurements data were close to the default values in ASM1 and other reported values. Even though some parameter was lower than the default value, they were still comparable within the range of values in the literature. The difference of each parameter from default value may be due to the difference of temperature, sludge sources, location, operating conditions, experimental design, and experimental time. The results of all the experiments show that the developed combined ultimate hybrid respirometer-titrator meter was successfully applied to estimate kinetic parameters of activated sludge and the measurement of ammonium concentration in activated sludge samples.

### Acknowledgments

Financial supports for this work are from Suranaree University of Technology Research Fund, Rianchai Cement Block Part. Ltd and SHELL Centennial Education Fund.

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