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Tuning of activated sludge in winter based on respirogram profiles under standard and site temperatures

Zhihua Li[1,*], *Zhenyu Hang*[1], *Qian Zhang*[1], *Shuangshuang Zhang*[1], *Tianyu Zhang*[2], *Hanqing Yu*[3,*]

1. *School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, Xi'an 710055, China*
2. *Department of Mathematical Sciences, Montana State University, Bozeman, MT 59717-2400, USA*
3. *CAS Key Laboratory of Urban Pollutant Conversion, Department of Chemistry, University of Science & Technology of China, Hefei 230026, China*

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ABSTRACT

Respirograms of activated sludge OUR^{T}_{x} and OUR^{20}_{x} were measured under site (*T*) and standard (20°C) temperatures, respectively, and the predicted standard temperature respirogram $OUR^{20}_{x,cal}$ was also calculated using the Arrhenius equation. These respirogram profiles reveal more information than effluent quality. A decrease of OUR^{20}_{x} is a critical alarm signal for the loss of pollutant removal capacity, and a sudden increase of the predicted value $OUR^{20}_{x,cal}$ is an alarm signal for the unrecoverable deterioration of biomass. The sign of OUR^{20}_{x}–$OUR^{20}_{x,cal}$ can be used for selection of tuning strategies. For example, a negative value of OUR^{20}_{x}–$OUR^{20}_{x,cal}$ indicates that doubling biomass is difficult, thus strategies such as extending the reaction time with limited available biomass is preferred. The findings in this study elucidated the respiration profile of activated sludge under changes of temperature and can be effectively used for the stable operation of Wastewater Treatment Plants under cold temperatures and seasonal variations.

Introduction

Temperature is one of the important factors influencing the activity and microbial community structure of activated sludge (Dong et al., 2016; Ju et al., 2014; Zhang et al., 2019) and has been extensively investigated, especially for nitrification systems (Chen et al., 2018; Delatolla et al., 2009; Hoang et al., 2014a; Mannucci et al., 2014; Sun et al., 2015; Young et al., 2017; Zhang et al., 2014). Approximately 20% of the nitrification rate at 20°C could be retained at 1°C when a proper temperature decrease step was used (Hoang et al., 2014a; Young et al., 2017). The effect of temperature on the kinetic properties of activated sludge can be well-described using the Arrhenius equation (Hwang and Oleszkiewicz, 2007; Mannucci et al., 2014).

$$\mu_T = \mu_{20} \cdot \theta^{(T-20)} \quad (1)$$

where θ is the temperature correction factor; μ_T (day^{-1}) and μ_{20} (day^{-1}) are the specific growth rates at the site temperature (*T*°C) and 20°C, respectively.

The temperature correction factor θ, taking nitrification as an example, has been extensively investigated under various conditions (Hoang et al., 2014a; Hwang and Oleszkiewicz, 2007; Mannucci et al., 2014; Young et al., 2017;

* *Corresponding authors. E-mails:* lizhihua@xauat.edu.cn, (Zhihua Li), hqyu@ustc.edu.cn. (Hanqing Yu).

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Table 1 – Critical temperatures and temperature coefficient.

Temperature variation	Operational conditions	Temperature range (°C)	Temperature correction θ (R^2)		Reactor type	Critical temperature (°C)	Reference
	Exposed time (day)		R1	R2			
Acclimated to 1°C	35–103	1–20	1.14 (0.86)	1.14 (0.84)	MBBR	5	Hoang et al. (2014a)
	103–126		1.10 (0.68)	1.10 (0.63)			
	132–162		1.11 (0.82)	1.11 (0.41)			
Acclimated to 4°C	7	4–8	1.06 (0.83)	1.06 (0.84)	BAF BioStyr (R1), MBBR (R2)	4	Delatolla et al. (2010)
Acclimated to 4°C	4		1.03	1.05	BAF BioStyr	4	Delatolla et al. (2009)
	115		1.11	1.22			
Acclimated to low temperatures		7.2–28.3	1.11 (0.99)	1.11 (0.99)	MBBR		Zhang et al. (2014)
		2.8–28.3	1.11 (0.98)	1.11 (0.98)			
Sudden temperature change		4–20	1.04	1.05			
Gradual change (>2°C/hr)	39–101	7.4–12	1.09 (0.89)		MBBR	5	Young et al. (2017)
Acclimated to 1°C	152–191	1–5	1.09				
Gradual change (<2°C/hr)		10–20	1.10		MBR	10	Mannucci et al. (2014)
Gradual change (4°C/hr)		10–20	1.60				
Sudden change		10–20	1.12		SBR		Hwang and Oleszkiewicz (2007)
Gradual change (2°C/day)		10–20	1.07				
Acclimated to low temperatures		5–20	1.08		SBR		Brdjanovic et al. (1997)
		5–30	1.06				
Gradual change (2°C/day)		8–20	1.04 (0.72)		SBR	8	Current study
		5–8	1.16 (0.89)				

Zhang et al., 2014). It varies over a wide range from 1.03 to 1.60 depending on temperature change rate, temperature range and duration of cold-shocking (Table 1). It is reasonable to compensate for the decreased rate μ_T under a low temperature with the enrichment of biomass; therefore, methods such as extending the sludge retention time (SRT) and bio-augmentation are frequently employed (Head and Oleszkiewicz, 2004; Hoang et al., 2014b; Pei et al., 2014; Wu et al., 2008). Marginal effects exist for extending SRT; i.e., after reaching a certain level, increasing SRT does not lead to further increase in the nitrification capability, and may even worsen the bioactivity when recovering from a low temperature to a high temperature (Head and Oleszkiewicz, 2004). Bio-augmentation is effective for compensating the deficiency of SRT; however, the effects depend more on hydraulic retention time (HRT) than SRT, and the enhancement of nitrification will disappear quickly once the bio-augmentation is ceased (Head and Oleszkiewicz, 2004; Pei et al., 2015), which makes such a method expensive during winter operation. Therefore, taking proper tuning measures under low temperature remains a challenge.

Cold-shocking is also encountered during seasonal variations (Hwang and Oleszkiewicz, 2007; Ju et al., 2014), e.g., from autumn to winter, resulting in fluctuations in effluent concentrations, and the temperature correction factor θ increases significantly when the temperature decreases to a critical temperature (Table 1). The significance or implications of the variation of θ is still under discussion. Also, the measured activity of nitrification has been found to remain unchanged or become even higher in the initial period of decreasing temperature (Hoang et al., 2014b), which does not follow the Arrhenius equation. The mechanism for such behavior remains unclear and invites further investigation.

The respirogram is frequently employed for the description of the activity of sludge, for the evaluation of the robustness of activated sludge systems and for estimation of the recovery potential (Huang et al., 1985; Li et al., 2018a), thus it naturally serves as a basis for the selection of tuning strategies under cold temperature shock. Therefore, this study evaluated different behaviors of cold-adapted sludge by investigating its respirogram profile under a cold temperature shocking condition, and several indexes have been proposed for the management of activated sludge in coping with temperature fluctuations.

1. Materials and methods

1.1. Reactor, wastewater and seed sludge

The reactor was a laboratory-scale sequencing batch reactor (SBR) with a diameter of 200 mm, height of 250 mm and working volume of 7 L. The duration of each cycle was 8 hr, including 0.2 hr feeding reaction, 6 hr aerobic reaction with simultaneous bubble aeration and mechanical stirring, 1.6 hr of settling and 0.2 hr of effluent discharge period. A programmable logic controller (PLC) was used to control the sequence of operations, and the mixing of suspended mixed liquor was

Table 2 – Operational temperature of the experiment.

Condition	Domestication period	Steady-state period	Temperature-decreasing period								
Period	S1								S2	S3	S4
	P1		P2		P3					P4	P5
Temperature (°C)	20 ± 0.1	20 ± 0.1	18 ± 0.2	16 ± 0.3	14 ± 0.2	12 ± 0.2	10 ± 0.1	8	10 ± 0.1	8 ± 0.2	5 ± 0.1
Time (day)	1–11	12–15	16–19	20–22	23–25	26–29	30–34	35	36–41	42–47	48–54

carried out using a mechanical mixer. The solid retention time (SRT) of the SBR was maintained at 20 days by controlling sludge wastage. The temperature of the bioreactor was adjusted by a condenser. The initial DO concentration was controlled at 0.5–1.0 mg/L by controlling the air flow rate.

Synthetic wastewater was used for the SBR which used sodium acetate as carbon source, ammonium chloride as nitrogen source, and potassium dihydrogen phosphate as phosphorus source. The specific composition was as follows (per liter): COD 0.65 g, $-NH_4^+$-N 0.08 g, TP 0.008 g and trace elements 0.4 mL. The trace elements consisted of (per liter): H_3BO_3 0.15 g, $CuSO_4{\cdot}5H_2O$ 0.03 g, KI 0.18 g, $MnCl_2{\cdot}4H_2O$ 0.12 g, $Na_2MoO_4{\cdot}2H_2O$ 0.06 g, $ZnSO_4{\cdot}7H_2O$ 0.12 g, $CoCl_2{\cdot}6H_2O$ 0.15 g, $FeSO_4{\cdot}7H_2O$ 1.54 g and EDTA 12.74 g. The pH was maintained at 7.0–7.5 by addition of 0.1 mol/L HCl, as the degradation of sodium acetate results in increase of pH. Seeding sludge, approximately 3000 mg/L, was collected from the aerobic tank of the A/A/O process of a municipal wastewater treatment plant (Xi'an, China).

1.2. Control of temperature

At the beginning of the experiment, acclimation and culture of sludge were carried out with the operating temperature of the SBR reactor at 20°C. This was continued for 4 days after the steady state was reached at 20°C, then a gradual decrease of temperature was carried out at a step of 2°C each time when the temperature was higher than 8°C, and then the temperature was maintained at a minimum temperature of 5°C (Table 2). All of the experimental temperatures were adjusted by a cryogenic bath (BILON-W-503B, Xi'an Bilon Experimental Equipment Co., China) able to control the temperature at a specific value precisely and automatically.

1.3. Determination of respirogram space

Respirogram space measurement, which includes several coordinates representing different activities (oxygen uptake rates, OUR) under different substrate conditions, has been described in previous research (Li et al., 2018b). The respiration rates were measured offline using an automatic respirogram apparatus (WBM400, Xi'an Lvbiao Water Environmental Technology Co., China) composed of a 1.2 L glass container, an aerator (compressor and fine bubble aerator stone), a DO probe (VisiFerm DO 120, Hamilton, Switzerland), a pH probe (Polilyte Plus Arc 120, Hamilton, Switzerland) and a programmable logic controller (PLC). Online monitoring of pH and temperature variations in the built-in vessel of the apparatus was performed, meanwhile the pH was controlled at 7.5–8.5 by adding HCl or NaOH, and the temperature was maintained at the target values of site (T°C) and standard (20°C) temperatures using the same bath (BILON-W-503B, Xi'an Bilon Experimental Equipment Co., China). The detailed experimental protocols for the respirogram determination are listed in Table 3.

Reprograms measured under site (T°C) and standard (20°C) temperatures were denoted as OUR^{T}_{x} and OUR^{20}_{x}, respectively, where the subscript *x* stands for different coordinates of respirogram space, i.e., OUR^{20}_{n} means the nitrogen stimulated respiration rate with a temperature of 20°C. The recovery index (RI) was defined as the ratio of OUR_e to OUR_{enc} (also expressed as OUR_t), describing the recovery potential of the activated sludge (Li et al., 2018a).

Assuming that the yield coefficient Y_H is constant, the relationship between the measured value OUR^{T}_{x} at site temperature and predicted value $OUR^{20}_{x,cal}$ at standard temperature can be expressed as follows according to Eq. (2):

$$OUR^{T}_{x} = OUR^{20}_{x,cal} \cdot \theta^{(T-20)} \quad (2)$$

When $OUR^{20}_{x,cal}$ is replaced by the measured value OUR^{20}_{x} at the standard temperature, the temperature correction factor θ can be calculated using least squares regression analysis.

Table 3 – Experimental procedures for the respirogram determination.

Respirogram	Experimental protocol
OUR_e	1. 0.3 L activated sludge and 0.9 L tap water were pumped into a built-in Plexiglas sleeve vessel of the equipment with a water bath; 2. The sludge was washed three times using PBS buffer solution and aerated for 2 hours; 3. Aeration was provided until the oxygen concentration reached 7 mg/L, and then stopped while mixing was carried out; 4. OUR_e was measured with the decline of oxygen concentration during the mixing without aeration.
OUR_{en}	1. Adding NH_4Cl to make the NH_4^+-N concentration in reactor at 50 mg/L after the measurement of OUR_e; 2. Measurement of OUR_{en}, which consisted of the endogenous OUR_e and the nitrogen-stimulated OUR_n.
OUR_{enc} (or OUR_t)	1. Adding NaAc to make the COD concentration in reactor at 300 mg/L after the measurement of OUR_{en}; 2. Measurement of OUR_{enc}, which could be considered as the sum of OUR_e, OUR_n and the carbon source-stimulated OUR_c.

Similarly, Hoang et al. (2014a) has proposed that the ammonia removal rate for MBBR systems can be expressed in terms of θ as follows:

$$R_T = R_{cal}^{20} \cdot \theta^{(T-20)} \quad (3)$$

Where R_T (g NH_4^+-N/(m^2·day)) and $R^{20}{}_{cal}$ (g NH_4^+-N/(m^2·day)) are the carriers' surface area ammonia removal rates at T and 20 °C, respectively.

1.4. Measurement of effluent and sludge characteristics

Chemical oxygen demand (COD), ammonium (NH_4^+-N), total nitrogen (TN), total phosphate (TP), mixed liquor suspended solids (MLSS) and sludge volume index (SVI) were measured according to the Standard Methods (AFWA, 2005). pH and DO were measured using a HQ40d portable DO meter (HACH Co., USA).

2. Results and discussion

2.1. Overall performance during the temperature-decreasing period

Generally, the removal rate of pollutants, in terms of COD, TP, TN and NH_4^+–N, decreased with the decrease of temperature (Fig. 1). At the same time, the value of MLSS also decreased with the decrease of temperature, except for some fluctuation during the domestication period, and the value of SVI increased with the decrease of temperature. When the temperature decreased to 8°C on day 35, the effluent concentrations of COD, TP, TN and NH_4^+–N significantly increased (Fig. 1 Period S1). However, after the temperature recovered to 10°C during days 36–41 (Fig. 1 Period S2), the effluent concentrations of COD, TP, TN, NH_4^+–N and SVI recovered almost immediately, and the same was found for

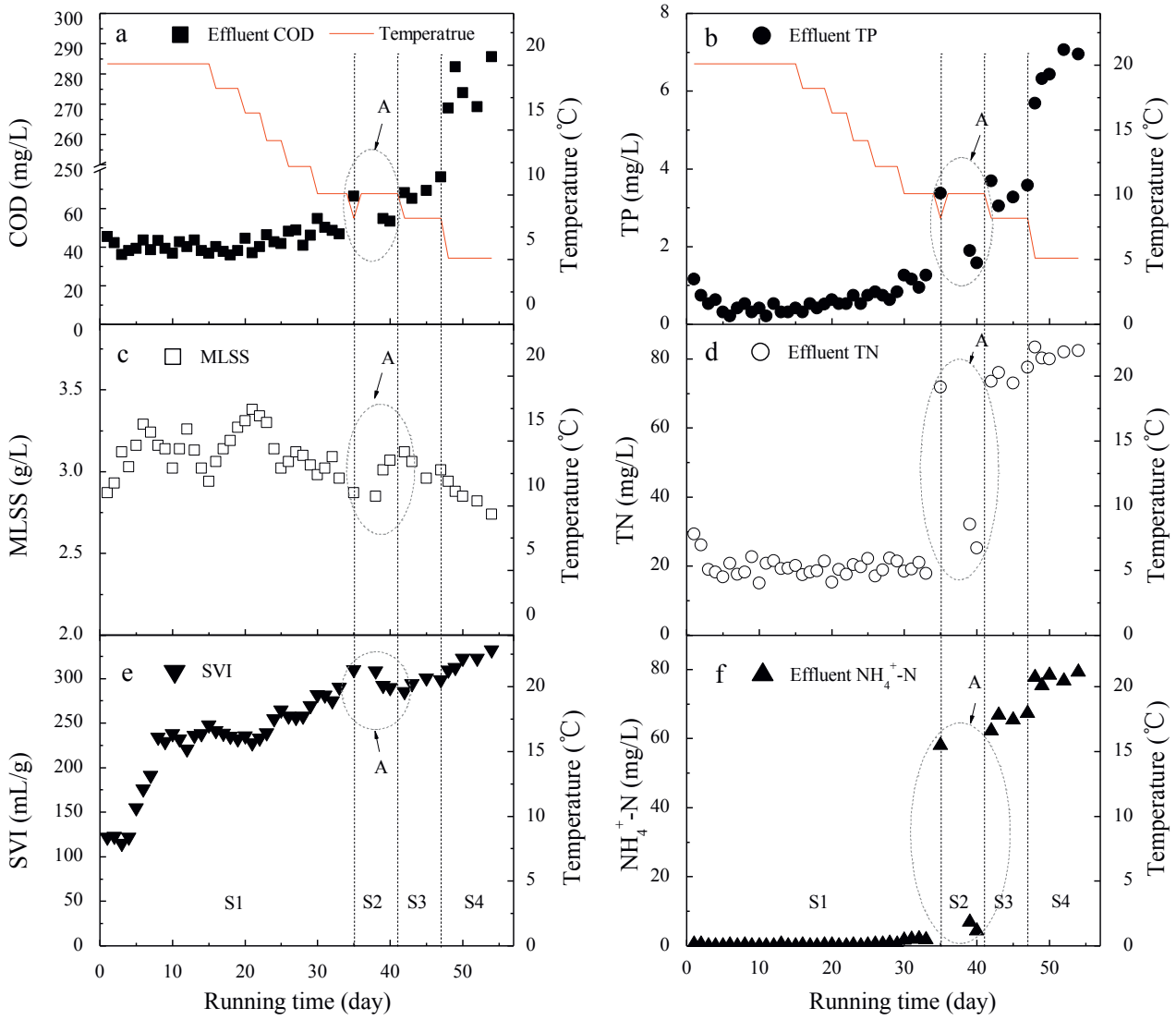

Fig. 1 – Overall performance of the activated sludge system. Variations in effluent of COD (a), TP (b), TN (d), NH_4^+-N (f) and the value of MLSS (c), SVI (e) at different temperature levels.

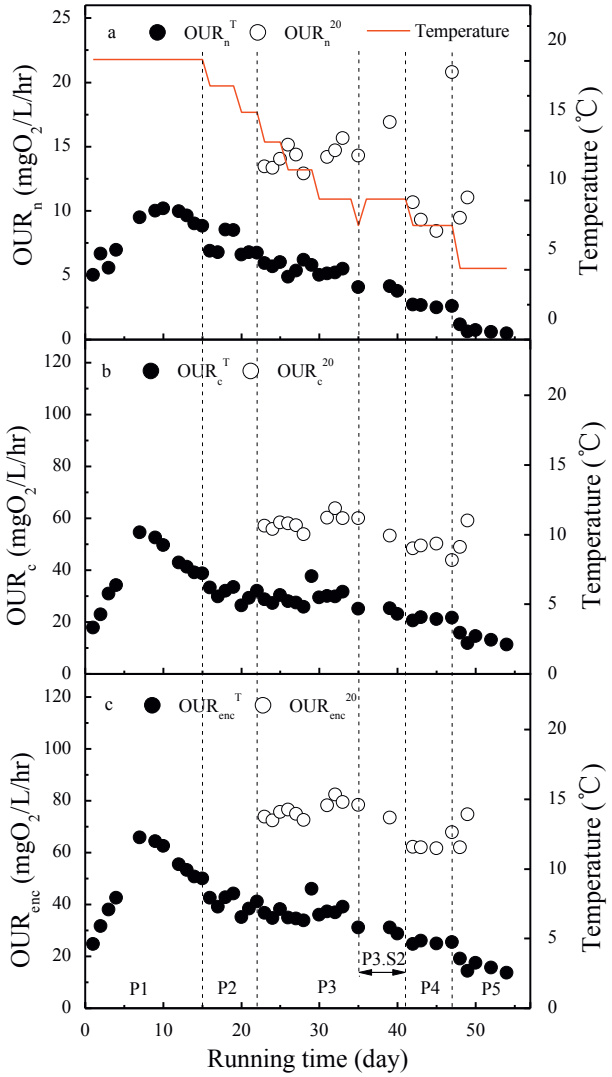

Fig. 2 – Respirogram space of standard (OUR^{20}_x) and site (OUR^{T}_x) temperature. Nitrogen stimulated respiration rate OUR^{20}_n and OUR^{T}_n (a), Carbon source stimulated respiration rate OUR^{20}_c and OUR^{T}_c (b) and total respiration rate OUR^{20}_{enc} and OUR^{T}_{enc} (c).

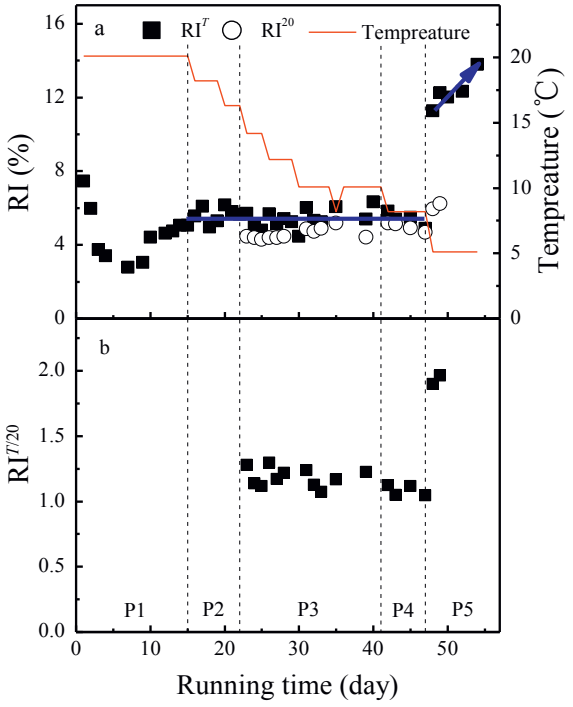

Fig. 3 – Recovery potential index RI under site and standard temperature. Variations of RI^{T}, RI^{20} (a) and $RI^{T/20}$ (b), relative to $\frac{RI^{T}}{RI^{20}}$ (%).

MLSS (Fig. 1 Region A). As the temperature decreased to 8°C again from day 42 to 47 (Fig. 1 Period S3), significant deterioration in effluent was observed again. After the temperature further decreased to 5°C from day 48 (Fig. 1 Period S4), another significant increase of effluent concentration was observed for COD, TP, TN and NH_4^+–N.

2.2. Fluctuation of respirogram during the cold-temperature adaptation

The respirogram coordinates generally decreased with the decrease of temperature and could be divided into five periods (Fig. 2), slightly different from the effluent concentration profiles (Fig. 1). Period P1 (day 0–15) could be considered as the acclimation of seed sludge from the full-scale plant condition to the lab condition, therefore fluctuation of the standard respirogram OUR^{20}_x (measured at 20°C) can be observed. The standard and site RIs in the respirogram space first decreased and then increased to 5.4% during the first period P1 (Fig. 3). During period P2 (day 15–22) when temperature decreased from 20 to 16°C, most characteristics of the sludge were stable, including the standard respirogram, SVI, and the effluent, except that the MLSS was increasing, and the respirogram OUR^{20}_x was not measured during this period. When temperature decreased to 14°C on day 23, all measured values of the standard respirogram OUR^{20}_x were at a high level until day 41 (Fig. 2 P3). During this period when temperature decreased from 14 to 8°C and then recovered to 10°C, both OUR^{T}_x and OUR^{20}_x remained approximately unchanged. When the temperature decreased from 10 to 8°C (P4, day 41–47), the measured value OUR^{20}_n decreased significantly (Fig. 2a). When temperature further decreased from 8 to 5°C (P5), significant decreases of OUR^{T}_x were observed. The RIs remained 5.4% during periods P2–P4, and during the last period P5 sludge recovery index with a temperature of T°C (RI^{T}, %) increased significantly (Fig. 3), indicating that the temperature shocking was more serious in the later period (Li et al., 2018a).

Comparing the variations of the effluent and respirogram shows that the respirogram was more sensitive to the change of temperature, as the period S1 in Fig. 1 corresponds to periods P1, P2 and early stage of period P3 in Fig. 2. The later part of period P3 in Fig. 2 was denoted as period P3.S2. Although the effluent recovered after the temperature increased to 10°C (Fig. 1 Period S2), the respiration

characteristics were different from before, since a decrease in the standard measured values of carbon source stimulated respiration rate at 20°C (OUR^{20}_c, $mgO_2/L/hr$) and total respiration rate at 20°C (OUR^{20}_{enc}, $mgO_2/L/hr$) in the respirogram took place in this period (Fig. 2b and c). The ratio $RI^{T/20}$ of sludge recovery index at T°C (RI^T, %) to sludge recovery index at 20°C (RI^{20}, %) remained 1.16 ± 0.08 during periods P2–P4 and increased to 1.93 ± 0.05 in period P5 (Fig. 3b), suggesting that the site temperature respirogram was affected more in period P5 than the other periods.

2.3. Standard respirogram for identification of temperature-related factors

Generally, the activated sludge system was robust with respect to temperature variations. A significant hold-off of effluent deterioration was observed during the period of decreasing temperature (Fig. 1 Periods S1–S3) despite the decrease in the respiration rate (Fig. 2 P1–P4). One reason is that the hydraulic retention time (HRT) of the operation was sufficiently long to buffer the temporal decline in efficiency of pollutant removal. Another reason is that the system would automatically tune its survival strategy. For example, sludge may grow more slowly and decay more slowly (Friedrich et al., 2015) under unfavorable growth conditions, which was confirmed in this study by the increase in the standard temperature respirogram under low temperatures (Fig. 2). Therefore, feedback control based on effluent quality is not reliable, as the long latency conceals the deterioration of the sludge. In contrast, the respirogram space could well distinguish different periods when the temperature was decreasing during the entire operation (Fig. 2). At the very beginning of period P3, OUR^{20}_x exhibited a sudden increase, but the effluent variation was negligible (Figs. 1 and 2). Such increase in the measured respirogram values also resulted in the deterioration of sludge, as shown by the decrease of MLSS and the increase of SVI (Fig. 1c and e). It is worth noting that OUR^{20}_x remained higher in periods P3 and P4 than in period P2 or P1, despite the decrease of temperature. Therefore, when the temperature temporally increased from a lower site temperature to the standard temperature, the maximum removal capacity of biomass in the reactor could soon recover; in other words, the temperature was the suppressing factor for the sludge activity. At the end of period P3 (day 41), the system exhibited significant decreases of OUR^{20}_x especially OUR^{20}_n (Fig. 2), and the effluent deteriorated from then on (Fig. 1). The decrease of OUR^{20}_x in period P4 suggested that a loss of potential capacity of nitrification had occurred when the temperature decreased from 10 to 8°C, and such deterioration was unrecoverable by a temporary temperature increase (from the site temperature *T* to the standard temperature 20°C). This was later evidenced by the increased RI value from the beginning of period P5 (Fig. 3), as it has been reported that an increased RI for a certain duration suggests that the system is unrecoverable (Li et al., 2018a). On the other hand, recoverable cases were also found in this study. For example, when temperature temporarily increased from 8 to 10°C on day 38 (Fig. 1 Period S2 and Fig. 2 Period P3.S2), the effluent improved despite the decreased OUR^T_x, suggesting that the system was recoverable at that time (Figs. 1 and 2), and at this point the change of RI was negligible (Fig. 3). However, compared to the RI index, OUR^{20}_x values were more effective for characterizing the potential removal capacity of activated sludge systems, especially for the nitrification process. Namely, the decrease of OUR^{20}_x is a critical alarm signal for the loss of pollutant removal capacity.

The deterioration of sludge can be caused by factors that are temperature-related or non-temperature-related, which can be determined by whether OUR^{20}_x stays stable. If OUR^{20}_x does not decrease, the system is recoverable, and the effluent can be temporarily improved by actions that can speed up the removal rate, such as increasing the oxygen concentration to increase OUR^T_x (Capodici et al., 2016; Guo et al., 2013), or extending the HRT with unchanged OUR^{20}_x. In contrast, if OUR^{20}_x decreases, stopping the loss of biomass potential removal capacity should be top priority, and possible actions include introducing attached growth systems (Hoang et al., 2014a; Young et al., 2017), extending the SRT if the MLSS in the reactor is acceptable (Yu et al., 2018), or even bio-augmentation (Head and Oleszkiewicz, 2005; Zhang et al., 2012). In fact, the effect of prolonging SRT at low temperature is very limited when the SRT is over the critical SRT that nitrifiers need (Guo et al., 2013). This study also found that the OUR^{20}_x stayed at a high value for a long time (Fig. 2 P3–P4). However, in many plants, at least in China, many operators have made the decision to extend SRT in winter without any supporting data. The improperly prolonged SRT not only worsens the operation of the settling tank, where viscous bulking generally occurs during winter, but also results in oxygen deficiency due to the large amount of biomass (Guo et al., 2013). Therefore, the measured value of OUR^{20}_x provides useful information for selection of the proper method to cope with the deterioration of effluent.

2.4. Prediction of respirogram using Arrhenius equation and its implications

A temperature was chosen as the critical temperature, the measured data were divided into two parts, above and below this temperature, respectively, and the two data sets were fitted. Then the temperature with the highest R^2 value was chosen as the critical temperature by trying different temperatures. The critical temperature for the Arrhenius equation temperature correction factor θ was calculated as 8°C in this study. The values of θ obtained through least squares fitting for OUR_n, OUR_c and OUR_{enc} were 1.08, 1.04 and 1.04 for temperatures above 8°C, and 1.51, 1.16 and 1.16 for temperatures below 8°C, respectively (Table 4), which was within the range of reported values (Table 1). The $OUR^{20}_{x,cal}$ in Eq. (2) under low temperatures was much higher than that under high temperatures, especially for nitrifiers (Table 4). Using the fitted value of θ, the predicted $OUR^{20}_{x,cal}$ was calculated for theoretical analysis, although it was not applicable under lower temperatures because the temperature 20°C was out of the range of lower temperatures (the maximum value was the critical temperature). During the periods P3–P4, the measured values of OUR^{20}_x were higher than the predicted values of $OUR^{20}_{x,cal}$. In contrast, during period P5 and the early stage of period P2 the calculated values were higher (Fig. 4). It is reasonable that the measured values were lower than the

Table 4 – Calculated value of temperature correction factor θ.

Temperature range (°C)	Sample count.	Bacteria type	θ	OUR^{20} (mg/L/hr)	R^2
8–20	n = 24	Nitrifiers	1.08 ± 0.01	8.91 ± 0.34	0.84
		Heterotrophic	1.04 ± 0.01	37.28 ± 1.34	0.62
		Total	1.05 ± 0.01	48.93 ± 1.60	0.72
5–8	n = 6	Nitrifiers	1.51 ± 0.07	348.56 ± 195.09	0.96
		Heterotrophic	1.16 ± 0.02	127.60 ± 32.74	0.89
		Total	1.16 ± 0.02	144.49 ± 36.95	0.89

predicted values, as some of the bacteria either could not recover in a short time or simply died when the temperature changed so rapidly. It should be noted that the predicted values $OUR^{20}_{x,cal}$ increased suddenly at the beginning of period P5 when the temperature dropped below the critical value of 8°C, suggesting that a lower fraction of biomass could recover if temperature increased back to normal, which also occurred in the early stage of period P2 when the temperature started to decrease. A smaller fraction of biomass was recoverable under the critical temperature, possibly due to its larger fraction of live biomass, as it has been reported that the live fraction at temperatures of 20 and 1°C was approximately 71% and 83%, respectively (Young et al., 2017). The increase of measured OUR^{20}_{x} at lower temperature lacks intuitive explanation, though it is a common phenomenon also reported by other researchers (Hoang et al., 2014a; Hoang et al., 2014b). For example, an approximately 20% increase of activity was observed when the temperature decreased from 20 to 1 °C (Hoang et al., 2014b). Such increase may be ascribed to the fact that more of the live fraction of biomass was stimulated from the dormancy status under a lower temperature (Young et al., 2017). It seems that the increased live fraction of biomass (the pink shadowed area in Fig. 4) survived in the system until the temperature decreased to the critical temperature. Similar results of increased live fraction were also found using the reported data with a similar calculation by Eq. (3). A significant increase of R^{20}_{cal} always occurred in the initial period when the temperature started to decrease (Fig. 5). Deterioration caused by sudden or frequent increase or decrease of temperature, or by long-time duration under a low temperature, resulted in an increase of R^{20}_{cal} (Fig. 5). As $OUR^{20}_{x,cal}$ changed in the same way as R^{20}_{cal}, an increase in the predicted values of respirogram $OUR^{20}_{x,cal}$ suggests an increase in the live fraction of biomass; however, a sudden increase is an alarm signal for the unrecoverable deterioration of biomass.

It is worth elucidating the importance of an increased live fraction of biomass. It has been reported that when the net growth rate increases, OUR increases and then reaches a plateau (Huang et al., 1985). Given a system with constant biomass, the theoretical OUR_{max} can be defined as the maximum specific oxygen uptake rate when all biomass is totally viable (the maximum net growth rate that can be achieved), which rarely occurs in real sludge systems but can be inferred from the relationship between OUR and SRT given an SRT value of zero. The considerably higher $OUR^{20}_{x,cal}$ suggested that the live fraction of biomass was higher under such low temperatures, as has also been reported elsewhere (Hoang et al., 2014a). When the system behaves like a system with SRT = 0, its biomass could hardly double; namely, a large amount of biomass is in a status of viable but non-culturable (VBNC). This finding explains why bio-augmentation in cold temperature must be continually

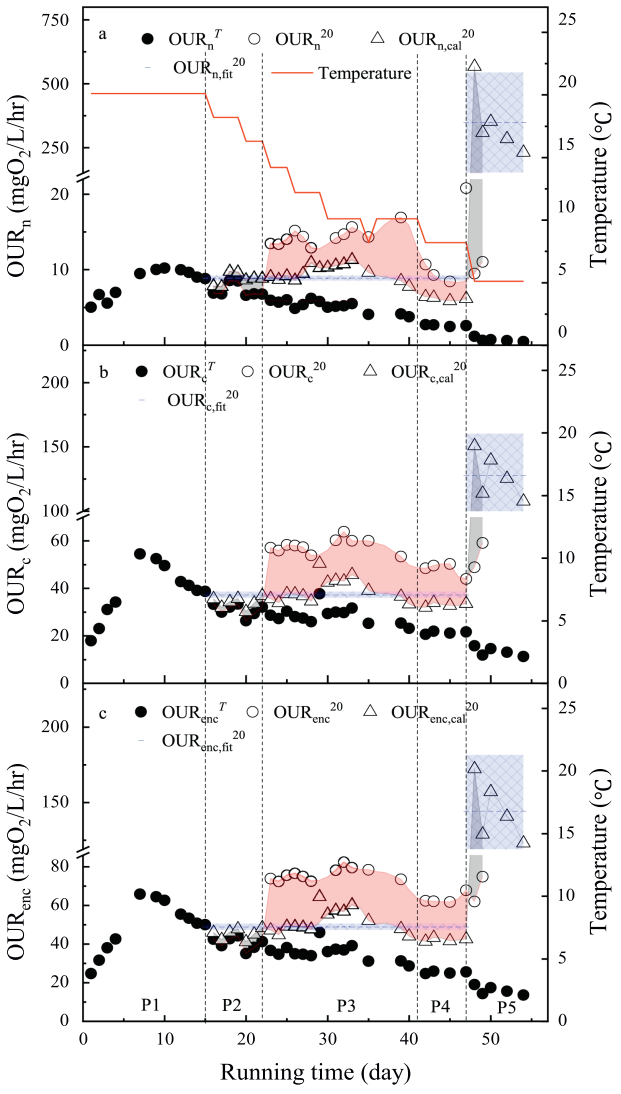

Fig. 4 – Calculated and measured standard temperature respiration rates. The blue dotted lines present the value of OUR^{20}_{x} by fitting the measured values OUR^{T}_{x} using Eq. (2). Nitrogen stimulated respiration rate OUR^{20}_{n} and $OUR^{20}_{n,cal}$ (a), carbon source stimulated respiration rate OUR^{20}_{c} and $OUR^{20}_{c,cal}$ (b) and total respiration rate OUR^{20}_{enc} and $OUR^{20}_{enc,cal}$ (c).

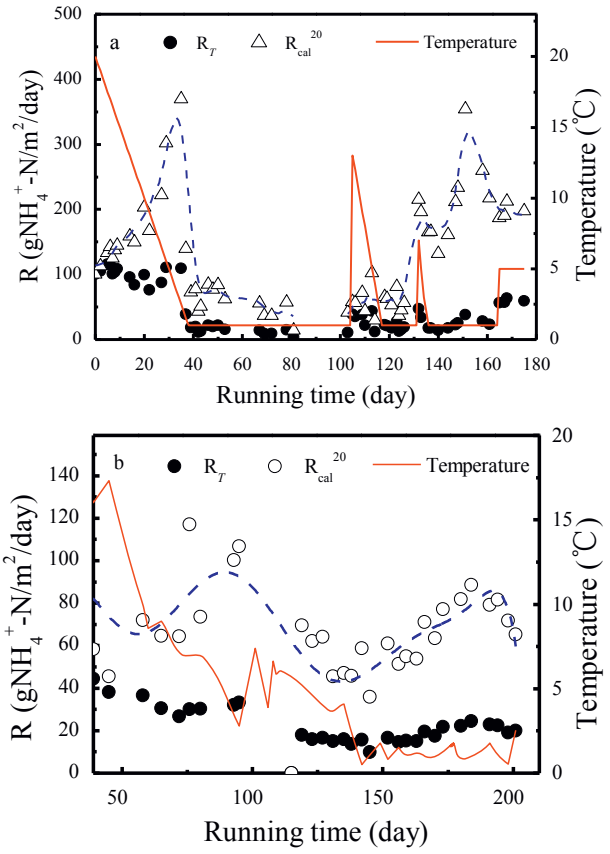

Fig. 5 – Prediction of standard removal rate using reported data, (a) from (Hoang et al., 2014b), (b) from (Young et al., 2017) . The dashed lines present the trend of the calculated value.

applied with a sufficient HRT, otherwise, the enhancement effect disappears quickly (Head and Oleszkiewicz, 2005; Wanner et al., 2005). As such, dealing with the temperature decrease by extending the SRT is not recommended (Guo et al., 2013). Increased SRT results in increased surface loading of settling tanks; however, even in a membrane bioreactor (MBR) where biomass loss does not easily occur, low-temperature operation is still a challenge. Instead, methods such as prolonging the HRT should be preferred when the predicted $OUR^{20}_{x,cal}$ value increases.

All the analysis above suggests that the sign of OUR^{20}_{x}–$OUR^{20}_{x,cal}$ can be effectively used for selection of tuning strategies. A negative value of OUR^{20}_{x}–$OUR^{20}_{x,cal}$, due to either decrease of OUR^{20}_{x} or sudden increase of $OUR^{20}_{x,cal}$, could be employed as an alarm signal for the unrecoverable deterioration of biomass, and it indicates non-temperature-related inhibition, suggesting that doubling of the biomass is difficult and thus strategies such as extending reaction time (HRT) are preferred. In contrast, a positive value of OUR^{20}_{x}–$OUR^{20}_{x,cal}$ indicates that a higher live fraction of biomass was stimulated under a low temperature, and it is a temperature-related inhibition, thus enrichment of biomass by actions such as increasing oxygen concentration or MLSS is effective in improving the performance of activated sludge systems.

3. Conclusions

In summary, effluent concentrations of COD, NH_4^+–N, TN and TP are less sensitive than the respirogram with respect to the variation of temperature. Therefore, it is necessary to measure the respirogram at site and standard temperatures simultaneously. The standard measured values OUR^{20}_{x} are effective for characterizing the potential removal capacity of an activated sludge system. An increase in the predicted values of respirogram $OUR^{20}_{x,cal}$ suggests an increase in the live fraction of biomass. The sign of OUR^{20}_{x} - $OUR^{20}_{x,cal}$ was proposed as a criterion for the selection of tuning strategies. These findings are potentially valuable for stable operation of WWTPs.

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