



## Novel modeling concept for evaluating the effects of cadmium and copper on heterotrophic growth and lysis rates in activated sludge process

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### ARTICLE INFO

#### Article history:

Received 14 June 2007

Received in revised form

23 September 2008

Accepted 6 November 2008

Available online 14 November 2008

#### Keywords:

Heterotrophic bacteria

Heavy metal

Inhibitory effect

Growth rate constant

Lysis rate constant

### ABSTRACT

A new modeling concept to evaluate the effects of cadmium and copper on heterotrophic growth rate constant ( $\mu_H$ ) and lysis rate constant ( $b_H$ ) in activated sludge was introduced. The oxygen uptake rate (OUR) was employed to measure the constants. The results indicated that the  $\mu_H$  value decreased from 4.52 to 3.26 d<sup>-1</sup> or by 28% when 0.7 mg L<sup>-1</sup> of cadmium was added. Contrarily the  $b_H$  value increased from 0.31 to 0.35 d<sup>-1</sup> or by 11%. When adding 0.7 mg L<sup>-1</sup> of copper, the  $\mu_H$  value decreased to 2.80 d<sup>-1</sup> or by 38%. The  $b_H$  value increased to 0.42 d<sup>-1</sup> or by 35%. After regression, the inhibitory effect was in a good agreement with non-competitive inhibition kinetic. The inhibition coefficient values for cadmium and copper were 1.82 and 1.21 mg L<sup>-1</sup>, respectively. The relation between the  $b_H$  values and heavy metal concentrations agreed with exponential type well. The heavy metal would enhance  $b_H$  value. Using these data, a new kinetic model was established and used to simulate the degree of inhibition. It was evident that not only the inhibitory effect on  $\mu_H$  but also that the enhancement effect on  $b_H$  should be considered when heavy metal presented.

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### 1. Introduction

The activated sludge process (ASP) has long been used for domestic as well as industrial wastewater treatment. Because the influent quality and quantity from industries is complex, some problems would be encountered as ASP is adopted, especially when heavy metals presented. If heavy metals exceed the tolerance of microorganisms, failure of ASP occurs.

Many microbial species involve in ASP to remove carbon, nitrogen and phosphorus simultaneously. In order to understand bacterial conversion in ASP, several mathematical models have been proposed such as Activated Sludge Model (ASM) [1], TaiWan Extension Activated sludge model no. 1 (TWEA1) [2] and applied in our previous works [3–6]. In the concept of these mathematical model, growth of microorganisms and consumption of substrate are predominated by two major rates, i.e. growth rate and lysis rate. Although the toxicity of heavy metals to ASP has been studied in numerous works, the main concerns are related to the effect of heavy metal on growth rate constant in most works [7–12]. Contrarily, no research has been done on the effect of heavy metals on lysis

rate constant. It is obvious that heavy metals will result in inhibition of growth rate constants, while how they affect the lysis rate is still unknown and will be an interesting issue.

Simple procedures to evaluate growth rate constant and lysis rate constant in sludge based on respirometry are available presently [2,13–15]. The measurement of oxygen uptake rate (OUR) reveals characteristic results for the active biomass due to the stoichiometric relation between biomass growth and dissolved oxygen (DO) consumed as electron acceptor. Oxygen is utilized not only by heterotrophic bacteria ( $X_H$ ) but also by ammonia oxidizing bacteria ( $X_{AOB}$ ) and nitrite oxidizing bacteria ( $X_{NOB}$ ) in ASP. Literatures show that nitrifying bacteria are more sensitive to many heavy metals than heterotrophic microorganisms. So the heterotrophic growth rate constant ( $\mu_H$ ) and heterotrophic lysis rate constant ( $b_H$ ) are considered in this study to evaluate the effects of heavy metals and to establish the kinetic model. In ASM, only the kinetic for treating domestic sewage is considered. If the effects of heavy metals on  $\mu_H$  and  $b_H$  can be obtained, these models can be extensively applied in wastewater containing heavy metals such as industrial influent.

So the objectives of this study are listed as follows. (1) To determine the growth and lysis rate constants of  $X_H$  when different cadmium and copper concentrations were added. (2) To derive novel inhibitory kinetic models which included non-competitive

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### Nomenclature

$b_H$	lysis rate constant for $X_H$ at any heavy metal concentration ( $d^{-1}$ )
$b_H'$	traditional decay coefficient for $X_H$ at any heavy metal concentration ( $d^{-1}$ )
$b_{H,0}$	lysis rate constant for $X_H$ without heavy metal ( $d^{-1}$ )
$E^*$	enhancement term (dimensionless)
$f_p$	fraction of biomass leading to particulate products (dimensionless)
$I$	concentration of heavy metal ( $mg L^{-1}$ )
$I^*$	inhibition term (dimensionless)
$K_i$	inhibition coefficient ( $mg L^{-1}$ )
$OUR_H$	oxygen uptake rate ( $mg O_2 L^{-1} h^{-1}$ or $mg O_2 L^{-1} min^{-1}$ )
$S_{O_2}$	concentration of oxygen ( $mg L^{-1}$ )
$S_S$	concentration of substrate ( $mg L^{-1}$ )
$X_H$	concentration of heterotrophic biomass ( $mg L^{-1}$ )
$Y_H$	yield coefficient of heterotrophic biomass ( $mg COD mg COD^{-1}$ )
<i>Greek symbols</i>	
$\alpha$	enhancement coefficient in exponential form (dimensionless)
$\beta$	coefficient of exponential regression (dimensionless)
$\gamma$	coefficient of linear regression (dimensionless)
$\delta$	coefficient of exponential regression (dimensionless)
$\mu_H$	growth rate constant for $X_H$ at any heavy metal concentration ( $d^{-1}$ )
$\mu_{H,0}$	growth rate constant for $X_H$ without heavy metal ( $d^{-1}$ )

kinetic, linear and exponential regression models for  $X_H$  using the values of growth and lysis rate constants. (3) To simulate the effects of cadmium and copper on heterotrophic OUR. If these objectives can be achieved, the inhibition term can be substituted into ASM or TWEA1 models. In addition, the inhibition effects of cadmium and copper on  $X_H$  in ASP process can be predicted.

## 2. Materials and methods

In this study, cadmium sulfate ( $CdSO_4$ ) and copper sulfate ( $CuSO_4$ ) were used to prepare reagent. Therefore, different concentrations of cadmium and copper including 0.0, 0.1, 0.3, 0.5 and  $0.7 mg L^{-1}$  were added into the OUR chamber, then the  $\mu_H$  and  $b_H$  values at different cadmium and copper concentrations could be calculated. Fig. 1 depicts the complete procedures. When pH, dissolved oxygen and the alkalinity vary, metal speciation reveal different in toxic effects due to different speciation including oxides, hydroxides or carbonates. Due to the fact that the quality of industrial wastewater is complex, metal speciation was ignored in previous studies [8–10].

The pilot scale plant of ASP was installed and continuously operated in a laboratory with the temperature controlled at  $20^\circ C$ . The influent flowrate, ratios of return sludge and sludge retention times were  $67 mL min^{-1}$ , 0.5 and 15 days, respectively. The mixed liquor suspended solids varied between 2000 and  $3000 mg L^{-1}$ . The DO and pH were controlled in the ranges of 1.8–2.0 and 7.0–7.2, respectively in the aeration tank.

### 2.1. Calculating $\mu_H$

Several researchers have discussed the oxygen consumption of  $X_H$  with neither substrate nor oxygen limitation [1,2, 15]. Their results were based on the kinetics without inhibition. Here, we propose a new modeling concept to describe oxygen consumption when inhibitor presents. Thus, when inhibitor presents, oxygen consumed by  $X_H$  with neither substrate nor oxygen limitation is:

$$\begin{aligned} \frac{dS_{O_2}}{dt} &= OUR_H(t) = \left[ \left( \frac{1 - Y_H}{Y_H} \right) \mu_H X_H(t_0) \right] - b_H X_H(t_0) \\ &= \left[ \left( \frac{1 - Y_H}{Y_H} \right) \mu_{H,0} I^* X_H(t_0) \right] - b_{H,0} E^* X_H(t_0) \end{aligned} \quad (1)$$

Since literatures reported that heavy metals will result in inhibition of growth rate constants, the inhibition term,  $I^*$ , is adopted in the growth rate term in Eq. (1). Although it is unknown how the heavy metals affect the lysis rate, it seems to be reasonable to assume that heavy metals will enhance the lysis rate. Therefore, the enhancement term,  $E^*$ , is adopted in the lysis rate term as shown in Eq. (1). The growth for  $X_H$  with neither substrate nor oxygen limitation when a certain amount of inhibitor presents can be written as follow:

$$\frac{dX_H}{dt} = (\mu_{H,0} I^* - b_{H,0} E^*) X_H(t) \quad (2)$$

Integration of Eq. (2) leads to:

$$X_H(t) = X_H(t_0) e^{(\mu_{H,0} I^* - b_{H,0} E^*) t} \quad (3)$$

Eq. (3) can be introduced into Eq. (1). Then the oxygen respiration is known at any time without limitations:

$$OUR_H(t) = \left[ \left( \frac{1 - Y_H}{Y_H} \right) \mu_{H,0} I^* - b_{H,0} E^* \right] X_H(t_0) e^{(\mu_{H,0} I^* - b_{H,0} E^*) t} \quad (4)$$

When Eq. (4) is differentiated with respect to time  $t$ , the resulting equation is:

$$\begin{aligned} \frac{dOUR_H(t)}{dt} &= (\mu_{H,0} I^* - b_{H,0} E^*) \left[ \left( \frac{1 - Y_H}{Y_H} \right) \mu_{H,0} I^* - b_{H,0} E^* \right] \\ &\times X_H(t_0) e^{(\mu_{H,0} I^* - b_{H,0} E^*) t} \end{aligned} \quad (5)$$

The term of  $\left[ \left( \frac{1 - Y_H}{Y_H} \right) \mu_{H,0} I^* - b_{H,0} E^* \right] X_H(t_0) e^{(\mu_{H,0} I^* - b_{H,0} E^*) t}$  equals  $OUR_H(t)$  again, so Eq. (5) can be rearranged as:

$$\frac{dOUR_H(t)}{dt} = (\mu_{H,0} I^* - b_{H,0} E^*) OUR_H(t) \quad (6)$$

Rearranging Eq. (6), the following expression can be obtained:

$$\frac{1}{OUR_H(t)} \frac{dOUR_H(t)}{dt} = (\mu_{H,0} I^* - b_{H,0} E^*) \quad (7)$$

Integrating Eq. (7), the resulting equation is:

$$\ln[OUR_H(t)] = (\mu_{H,0} I^* - b_{H,0} E^*) t + C \quad (8)$$

When time equals 0, Eq. (8) becomes:

$$\ln[OUR_H(t_0)] = C \quad (9)$$

Substituting Eq. (9) into Eq. (8), the resulting equation is:

$$\ln[OUR_H(t)] = (\mu_{H,0} I^* - b_{H,0} E^*) t + \ln[OUR_H(t_0)] \quad (10)$$

This equation represents a straight line with  $(\mu_{H,0} I^* - b_{H,0} E^*)$  as slope in a diagram of natural logarithm of OUR vs. time, i.e. Fig. 1 (b). If substituting the  $b_H E^*$  value obtained from next experiment,  $\mu_{H,0} I^*$  can be calculated:

$$\mu_{H,0} I^* = \text{slope} + b_{H,0} E^* \quad \text{or} \quad \mu_H = \text{slope} + b_H \quad (11)$$

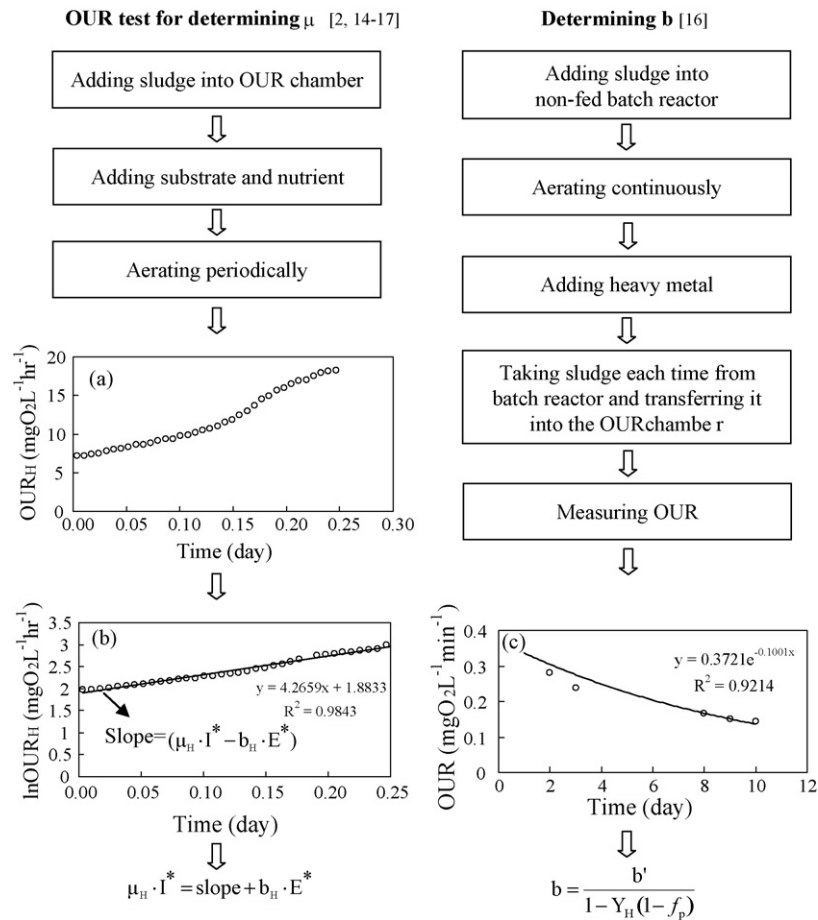


Fig. 1. Procedures for determining parameters.

## 2.2. Calculating $b_H$

The  $b_H$  value of the heterotrophic biomass was determined by the following steps. First, a fixed amount of sludge was placed into a non-fed aerated batch reactor for ten days. Second, a certain amount of sludge was taken from the above batch reactor each time and transferred into the OUR chamber to measure the OUR. By plotting the OUR vs. time, the traditional decay coefficient,  $b_H'$  could be estimated by exponential curve fitting, i.e. Fig. 1 (c) [1,16]. The  $b_H$  value can be calculated from:

$$b_H = b_{H,0}E^* = \frac{b_H'}{1 - Y_H(1 - f_p)} \quad (12)$$

The  $Y_H$  value was determined according to our previous work [17], but the default value of 0.08 for  $f_p$  (fraction of biomass leading to particulate products) was chosen according to Henze et al. [1] in this study.

## 2.3. Establishing inhibitory kinetic equation

Several types of inhibition kinetic equations have been proposed [8–10]. Beg et al. [8] pointed out that the degree of inhibition is unaffected by the concentration of substrate when using non-competitive inhibitory equation. Further, the inhibitor does not prevent the enzyme-inhibitor complex from reacting with the substrate. Beg et al. [8] chose non-competitive inhibitory equation to simulate the inhibition effect of chromium (VI) in multi-substrate carbon oxidation and nitrification process in an upflow packed bed biofilm reactor. Additionally, most inhibition terms in ASM2d or TWEA1 were the non-competitive inhibitory kinetics, so the non-

competitive inhibitory equation was also chosen to express the inhibitory behavior of  $X_H$  in this study. It is described as:

$$\mu_H = \mu_{H,0}I^* \frac{S_S}{K_S + S_S} = \mu_{H,0} \frac{1}{1 + I/K_I} \frac{S_S}{K_S + S_S} \quad (13)$$

When  $S_S$  was very high, the term of  $\frac{S_S}{K_S + S_S}$  was close to 1. Subsequently Eq. (13) becomes:

$$\mu_H = \mu_{H,0}I^* = \mu_{H,0} \frac{1}{1 + I/K_I} \quad (14)$$

Eq. (14) can be linearized as:

$$I = K_I \left( \frac{\mu_{H,0}}{\mu_H} - 1 \right) \quad (15)$$

If the  $\mu_H$  values at different heavy metal concentrations can be determined, the value of inhibition coefficient ( $K_I$ ) can be calculated by plotting the diagram of heavy metal concentrations ( $I$ ) (including 0, 0.1, 0.3, 0.5 and 0.7 mg L<sup>-1</sup>) vs.  $\left( \frac{\mu_{H,0}}{\mu_H} - 1 \right)$ .

## 2.4. OUR measurement

The measuring system consisted of four airtight, circular chambers, with same height and volume, four magnetic stirrers for stirring and an aeration stone in each chamber [2]. DO was monitored by four oxygen meters of high stability connected to a data acquisition system. Since this OUR measurer was airtight, the actual respiration rate of the tested biomass at any time during the batch-test did not depend on oxygen input. Therefore the DO concentration represented the actual OUR. A certain amount of sludge sample was added into OUR chambers. Distilled water containing

organic carbon source and nutrient including ethanol,  $\text{NH}_4\text{Cl}$  and  $\text{KH}_2\text{PO}_4$  were added resulting in total volume of 800 mL in chamber and activated sludge to COD ratio of 1/20. The pH value was maintained at 7 during batch test. The measuring system was periodically aerated, then the difference between the measured OUR and baseline oxygen respiration was calculated and compared. In order to evaluate the kinetic parameters of  $X_H$  when adding different concentrations of cadmium and copper, OUR of  $X_H$  ( $\text{OUR}_H$ ) should be measured. The determination of  $\text{OUR}_H$  were based on the subsequent addition of allylthiourea (ATU) and sodium azide ( $\text{NaN}_3$ ) [13–15,18–19], selective inhibitors of  $X_{\text{AOB}}$  and  $X_{\text{NOB}}$ , to the activated sludge sample. Ginestet et al. [19] indicated that ATU solution selectively inhibited ammonia oxidizers at a concentration of 86  $\mu\text{M}$  without affecting activities of other bacterial species. They also indicated that at a concentration of 24  $\mu\text{M}$   $\text{NaN}_3$  completely inhibited nitrite oxidation without affecting activities of other bacterial species. Thus, the same concentrations of ATU and  $\text{NaN}_3$  were added into chambers selectively inhibit ammonia and nitrite oxidizers, respectively in this study. All analytical methods used in this study were according to Standard Method [20].

### 3. Results and discussion

#### 3.1. OUR diagram and calculation

An example was used to explain the calculation procedure of OUR diagram as shown in Fig. 1. According to the results of  $\text{OUR}_H$  batch test in Fig. 1 (a), the values of  $(\mu_{H,0}I^* - b_{H,0}E^*)$  could be determined from the slope of linear regression line in Fig. 1 (b). In Fig. 1 (b), the value of  $(\mu_{H,0}I^* - b_{H,0}E^*)$  equaled 4.27.

Additionally, by plotting the OUR vs. time, the  $b_H$  value could be estimated based on the exponent term and equaled 0.10 as shown in Fig. 1 (c). Substituting the value of 0.10 into Eq. (12) with the  $Y_H$  value of 0.72 obtained from experiment, the value of  $b_H$  could be determined and its value was 0.30. Subsequently, the value of  $\mu_{H,0}I^*$  could be also determined based on Eq. (11) and equaled 4.56.

#### 3.2. Growth and lysis rate constants at different heavy metal concentrations

According to previous description, the values of  $b_H$ ,  $b_{H,0}E^*$  ( $=b_H$ ),  $(\mu_{H,0}I^* - b_{H,0}E^*)$  (slope in the diagram of natural logarithm of OUR vs. time) and  $\mu_{H,0}I^*$  ( $=\mu_H$ ) were calculated. The average values of  $b_{H,0}E^*$  and  $\mu_{H,0}I^*$  when adding different concentrations of  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  are summarized in Table 1.

In ASM1, ASM2 and ASM2d, the default values for  $\mu_{H,0}$  were 6.00  $\text{d}^{-1}$ , respectively. In ASM1, ASM2 and ASM2d, the default values for  $b_{H,0}$  were 0.62, 0.40 and 0.40  $\text{d}^{-1}$ , respectively [1]. Without addition of heavy metal ( $I^* = 1$ ,  $E^* = 1$ ), the average  $\mu_{H,0}$  and  $b_{H,0}$  values were 4.52 and 0.31  $\text{d}^{-1}$ , respectively at 20 centigrade in this study. These two values were lower than the default values in ASM [1].

Once the heavy metal was added into batch reactor, the inhibitory effect occurred. The  $\mu_{H,0}I^*$  value decreased to 3.26  $\text{d}^{-1}$

or by 28% when 0.7  $\text{mg L}^{-1}$  of cadmium was added. Contrarily the  $b_H$  value increased to 0.35  $\text{d}^{-1}$  or by 11%. When adding 0.7  $\text{mg L}^{-1}$  of copper, the  $\mu_{H,0}I^*$  value decreased to 2.80  $\text{d}^{-1}$  or by 38%. Contrarily the  $b_H$  value increased to 0.42  $\text{d}^{-1}$  or by 35%. Obviously, the inhibitory effect of copper was higher than that of cadmium. These were in good agreement with the results reported in the literature [7]. Beyenal et al. [21] also reported the combined effects of  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  on activated sludge process. According to their results, the  $\mu_H$  values without heavy metal inhibition were between 2.40  $\text{d}^{-1}$  and 6.00  $\text{d}^{-1}$ . When combined effects presented, the  $\mu_H$  values would decrease by 80% at same substrate concentration.

#### 3.3. Inhibitory kinetic equation

According to Eq. (13) and (14), the inhibitory kinetic equation for different heavy metals could be derived. The  $K_I$  value in Eq. (13) and (14) could be determined by plotting the diagram of  $I$  (0, 0.1, 0.3, 0.5 and 0.7  $\text{mg L}^{-1}$ ) vs.  $(\frac{\mu_{H,0}}{\mu_H} - 1)$  as shown in Fig. 2.

From Fig. 2, the  $R$ -squared values for regression line are 0.97 and 0.90, respectively. The high values of  $R$ -squared indicated that the inhibitory effect of these two heavy metals were in a good agreement with non-competitive inhibition kinetic. It indicated that the degree of inhibition was unaffected by the concentration of substrate. Further, the cadmium and copper did not prevent the enzyme-inhibitor complex from reacting with the substrate. The  $K_I$  values (slopes of two linear regression equations) for cadmium and copper were 1.82 and 1.21  $\text{mg L}^{-1}$ , respectively. The  $K_I$  value for cadmium was higher than that of copper. It revealed that the inhibitory effect of copper was higher than that of cadmium once again.

By plotting the  $b_{H,0}E^*$  vs. heavy metal concentrations, the relation between  $b_{H,0}E^*$  and heavy metal concentrations could be determined by exponential curve fitting as shown in Fig. 3. The  $R$ -squared values for exponential regression curve are 0.93 and 0.70, respectively. It represented that the relation between the  $b_H$  values and heavy metal concentrations were in a good agreement with exponential type as follows.

$$b_H = 0.31e^{0.137I} \quad (\text{for cadmium}) \quad (16a)$$

$$b_H = 0.31e^{0.474I} \quad (\text{for copper}) \quad (16b)$$

Here, we defined a new term, enhancement coefficient ( $\alpha$ ), to describe the enhancement effects of heavy metals on  $b_H$  values. The enhancement coefficients for cadmium and copper were 0.137 and 0.474, respectively as shown in Eq. (16a) and (16b). It revealed that the lysis rate when adding copper would be higher than that when adding cadmium.

For comparison, linear and exponential types of models were derived by regression as shown in Fig. 4 and Fig. 5. From Fig. 4 (a), the  $R$ -squared values of regression lines at different cadmium concentration for growth and lysis constants are 0.97 and 0.92, respectively. Their slopes are  $-1.8683$  ( $\beta$  in Eq. (17b)) and 0.0448 ( $\gamma$  in Eq. (17b)), respectively. Fig. 4 (b) shows the linear regression of copper. The  $R$ -squared values for growth and lysis constants are 0.98 and 0.74, respectively. Their slopes are  $-2.3692$  ( $\beta$  in Eq. (17b)) and 0.1689

**Table 1**  
The calculation of kinetic coefficients when adding different heavy metal concentrations.

	Concentration of $I$ items				
	0.0 ( $\text{mg L}^{-1}$ )	0.1 ( $\text{mg L}^{-1}$ )	0.3 ( $\text{mg L}^{-1}$ )	0.5 ( $\text{mg L}^{-1}$ )	0.7 ( $\text{mg L}^{-1}$ )
<b>Cadmium</b>					
$b_H E^*$ ( $=b$ ) ( $\text{d}^{-1}$ )	0.31 $\pm$ 0.03	0.32 $\pm$ 0.03	0.32 $\pm$ 0.03	0.33 $\pm$ 0.03	0.35 $\pm$ 0.03
$\mu_H I^*$ ( $=\mu$ ) ( $\text{d}^{-1}$ )	4.52 $\pm$ 0.31	4.46 $\pm$ 0.32	3.85 $\pm$ 0.29	3.56 $\pm$ 0.27	3.26 $\pm$ 0.25
<b>Copper</b>					
$b_H E^*$ ( $=b$ ) ( $\text{d}^{-1}$ )	0.31 $\pm$ 0.03	0.37 $\pm$ 0.04	0.38 $\pm$ 0.04	0.40 $\pm$ 0.04	0.42 $\pm$ 0.04
$\mu_H I^*$ ( $=\mu$ ) ( $\text{d}^{-1}$ )	4.52 $\pm$ 0.31	4.45 $\pm$ 0.34	3.80 $\pm$ 0.33	3.40 $\pm$ 0.28	2.80 $\pm$ 0.26

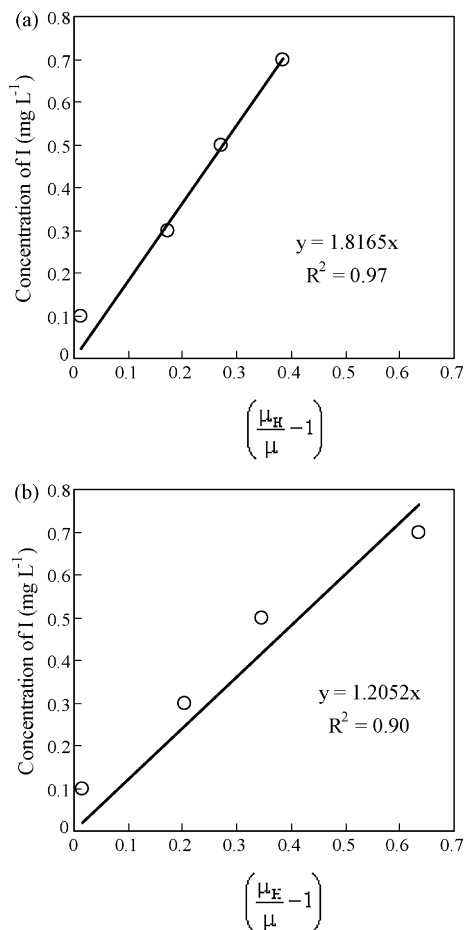


Fig. 2. Determining  $K_I$  values by linear regression. (a) Cadmium, (b) copper.

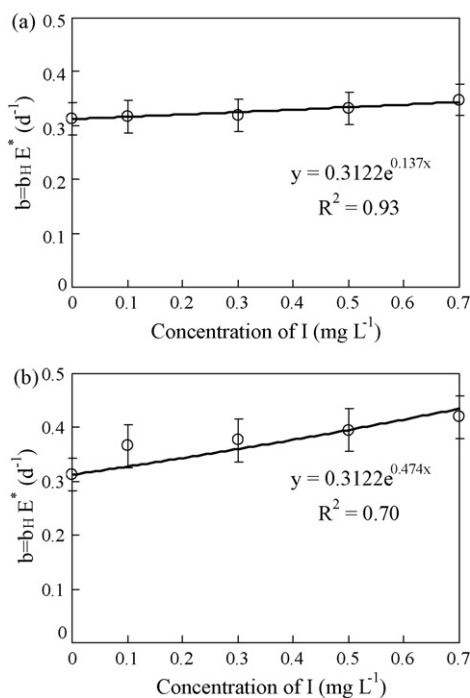


Fig. 3. The relation between the  $b_H$  values and heavy metal concentrations. (a) Cadmium. (b) Copper. (Error bars donate S.D.).

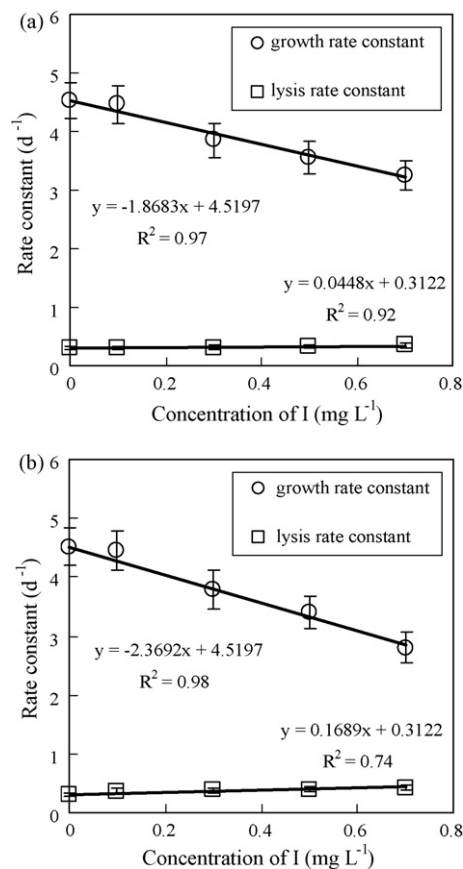


Fig. 4. Linear regression models. (a) Cadmium. (b) Copper. (Error bars donate S.D.).

( $\gamma$  in Eq. (17b)), respectively. It showed that the inhibitory effect of copper was higher than that of cadmium. Fig. 5 depicts the exponential regression of cadmium and copper. The  $R$ -squared values of growth constants for cadmium and copper are 0.98 and 0.97, respectively. The coefficients ( $\delta$  in Eq. (17c)) of their exponents are  $-0.4735$  (Fig. 5 (a)) and  $-0.6336$  (Fig. 5 (b)), respectively. In water solution with different pH, dissolved oxygen and the alkalinity, metal speciation appear as oxides, hydroxides or carbonates and different speciation is significantly different in toxic effects. At the give pH and other conditions in the system,  $\text{Cd}(\text{OH})_2$  ( $s$ ) was supposed to be the predominant precipitation. This effect reduced the available  $\text{Cd}^{2+}$  ( $aq$ ) in system. It was one reason why the inhibitory effect of copper was higher than that of cadmium.

### 3.4. Simulation of degree of inhibition

The conception of degree of inhibition was employed by several researchers to evaluate the inhibitory effect [7,11–12]. If the inhibitory effect obtained from previous section was taken into account, the OUR (Eq. (1)) could be rearranged as:

$$\text{OUR}_H = \left[ \left( \frac{1 - Y_H}{Y_H} \right) \mu_{H,0} \frac{1}{1 + I/K_I} - b_{H,0} e^{\alpha I} \right] X_H \quad (17a)$$

$$\text{OUR}_H = \left[ \left( \frac{1 - Y_H}{Y_H} \right) (\mu_{H,0} - \beta I) - (b_{H,0} + \gamma I) \right] X_H \quad (17b)$$

$$\text{OUR}_H = \left[ \left( \frac{1 - Y_H}{Y_H} \right) \mu_{H,0} e^{\delta I} - b_{H,0} e^{\alpha I} \right] X_H \quad (17c)$$

Since  $\text{OUR}_H$  represented the activity of heterotrophs, the degree of inhibition in this study could be defined as:

$$\text{Degree of inhibition} = \left( 1 - \frac{\text{OUR}_{\text{with inhibition}}}{\text{OUR}_{\text{without inhibition}}} \right) 100\% \quad (18)$$

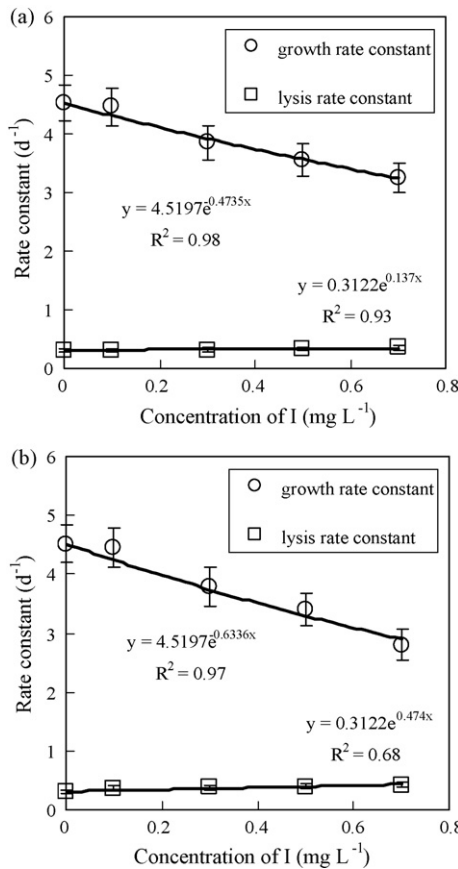


Fig. 5. Exponential regression models. (a) Cadmium. (b) Copper. (Error bars donate S.D.).

Fig. 6 shows the simulated results of degree of inhibition. In Fig. 6 (a), the degree of inhibition for cadmium (line with black point) increased smoothly and reached at 100% when 4 mg L<sup>-1</sup> was added. This simulation trend agreed with the results from Madoni et al. [7] well. In order to compare the effect of b<sub>H</sub>, the degree of inhibition when b<sub>H</sub> did not vary with cadmium concentrations was also calculated. In Fig. 6 (a), the degree of inhibition was lower when the inhibitory effect of cadmium on b<sub>H</sub> was ignored (dashed line) at the

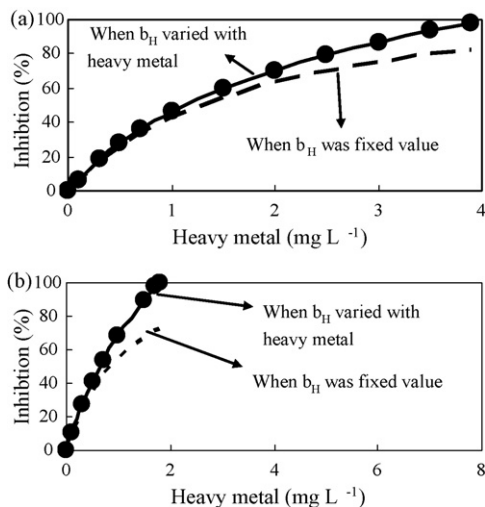


Fig. 6. The simulated degree of inhibition using noncompetitive kinetic. (a) Cadmium. (b) Copper.

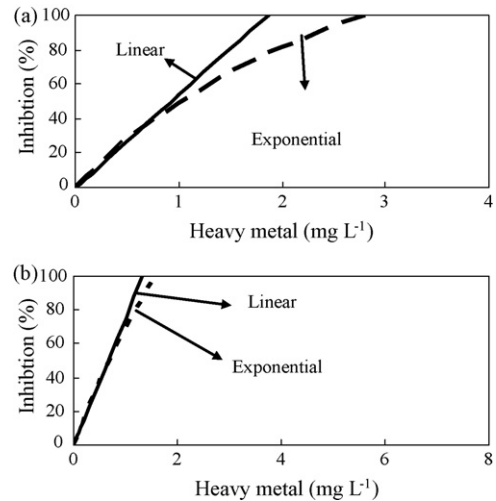


Fig. 7. The simulated degree of inhibition using linear and exponential models. (a) Cadmium. (b) Copper.

concentration of 4 mg L<sup>-1</sup>. Its value was only 84% and decreased by 16%.

Contrarily, the degree of inhibition for copper (line with black point) increased steeply and reached at 100% when 1.8 mg L<sup>-1</sup> of copper was added as shown in Fig. 6 (b). This simulation trend agreed with the results from Madoni et al. [7] well, too. If ignoring the effect of copper on b<sub>H</sub>, i.e., when b<sub>H</sub> did not vary with cadmium concentrations, the degree of inhibition was lower (dashed line). At the concentration of 1.8 mg L<sup>-1</sup>, the degree of inhibition was only 73% and decreased by 27%. It indicated that not only the inhibitory effect on μ<sub>H</sub> but also that on b<sub>H</sub> should be considered when heavy metal presented.

Fig. 7 (a) and 7 (b) depict the simulated results using linear and exponential regression. From Fig. 7 (a), the inhibition degrees of cadmium would reach 100% at 1.87 mg L<sup>-1</sup> when using linear regression model. It would reach 100% at 2.81 mg L<sup>-1</sup> when using exponential regression model. When adding copper, the inhibition degrees of linear regression model coincided with that of exponential regression model and they would reach 100% at 1.56 mg L<sup>-1</sup>. When using linear and exponential regression models, the degrees of inhibition were lower than those of non-competitive kinetics.

It was recommended that the inhibition of other heavy metals on heterotrophs and autotrophs should be evaluated in the future study.

#### 4. Conclusions

In this study, the effects of cadmium and copper on heterotrophic growth and lysis rate constants in activated sludge were evaluated. The results obtained in this study can be summarized as follows.

The μ<sub>H</sub> value decreased from 4.52 to 3.26 d<sup>-1</sup> or by 28% when 0.7 mg L<sup>-1</sup> of cadmium was added. Contrarily the b<sub>H</sub> value increased from 0.31 to 0.35 d<sup>-1</sup> or by 11%. When adding 0.7 mg L<sup>-1</sup> of copper, the μ<sub>H</sub> value decreased to 2.80 d<sup>-1</sup> or by 38%. Contrarily the b<sub>H</sub> value increased to 0.42 d<sup>-1</sup> or by 35%. After regression, the inhibitory effect of these two heavy metals was in a good agreement with non-competitive inhibition kinetic. The K<sub>I</sub> values for cadmium and copper were 1.82 and 1.21 mg L<sup>-1</sup>, respectively. The relation between the b<sub>H</sub> values and heavy metal concentrations agreed with exponential type well. It revealed that heavy metal would enhance the b<sub>H</sub> value. Using these data, a new kinetic model was established and used to simulate the degree of inhibition. If ignoring the effect of cadmium and copper on b<sub>H</sub>, the degree of inhibition was lower.

Thus, it was evident that not only the inhibitory effect on  $\mu_H$  but also that on  $b_H$  should be considered when heavy metal presented.

### Acknowledgements

The authors are grateful to the National Science Council of R.O.C. for financial support under the grant number NSC 95-2221-E-324-018.

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