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ESTABLISHMENT OF THE POWER–TIME CURVE EQUATION OF BACTERIAL GROWTH IN THE LOG PHASE

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Abstract

The power-time curves of two species of bacteria, *Vibro metschnikovii*, *Vibro bollisae* were determined calorimetrically by using a 2277 bioactivity monitor. The power–time curve equation of bacterial growth in the log phase can be expressed as $\nu=A(e^{k_1t}-e^{-kt})$. A self-function recursion equation, $f_i=b_1f_{i+1}+b_2f_{i+2}$, was obtained through the perfect non-linear function $f(t)=A+Be^{-k_1t}+Ce^{-k_2t}$. A linear equation, $\nu_i/\nu_{i+1}=b_1+b_2\nu_{i+2}/\nu_{i+1}$, was obtained by using the self-function recursion equation. The rate constants of bacterial growth k_1, the time constant of the calorimeter k, the generation times G, and the pre-exponential factors A were obtained from the power–time curve equations.

Power–time curve equations of bacterial growth in the log phase are expressed for *V. metschnikovii* as $\nu = 1.05(e^{0.0228t}- e^{-0.0175t})$, and for *V. bollisae* as $\nu = 1.58(e^{0.0278t}- e^{-0.0170t})$.

Keywords: bacterial growth, microcalorimetry, power–time curve equation, self-function regression method, thermokinetics

Introduction

In thermokinetics, the amplitudes of the calorimetric signal at a given time are expected to be proportional to the heat power generated in bacterial growth [1]. Some kinds of power–time curve equations of bacterial growth have been obtained by using different kinetic models of bacterial growth [2–4]. For bacterial growth in a conduction microcalorimeter, the relationship of the heat power of a non-steady-state process with respect to time satisfies the Tian equation [5]. In the present paper, a novel power–time curve equation of bacterial growth in the log phase has been obtained according to the exponential law of bacterial growth, the Tian equation and a self-function regression method [6]. This power–time curve equation of bacterial growth has been used to study two species of bacteria, *Vibro metschnikovii* and *Vibro bollisae*, and to calculate the growth rate constants, the generation times, the time constants and the pre-exponential factors.

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Theory

Power–time curve equation of bacterial growth

According to the basic theory of thermokinetics, when bacteria grow in a conduction microcalorimeter, the heat power of a non-steady-state process is described by the Tian equation [5]:

$$P = K\nu + \Lambda\frac{d\nu}{dt} \tag{1}$$

where P is the heat production rate or the heat power, ν is the amplitude of the calorimetric signal at time t, K is the heat constant and Λ is proportional to the heat capacity of a thermokinetic system.

Let

$$k = \frac{K}{\Lambda} \tag{2}$$

where k is the time constant of the calorimeter. On inserting Eq. (2) into Eq. (1), we have

$$\frac{k}{K}P = k\nu + \frac{d\nu}{dt} \tag{3}$$

According to the literature [7], for an isothermal, isobaric and closed system, the heat production rate of bacterial growth P is proportional to the bacterial growth rate r:

$$P = Q_o r \tag{4}$$

where Q_o is the heat generated by one bacterium during the whole growth.

The kinetic equation of bacterial growth in the log phase is given by [8]

$$r = \frac{dN}{dt} = k_1 N \tag{5}$$

where N is the bacterial number at time t and k_1 is the growth rate constant. The bacterial number and culture time are in accordance with the exponential law [8]

$$N = N_o e^{k_1 t} \tag{6}$$

where N_o is the bacterial number at time t_o. From Eqs (4), (5) and (6), we obtain

$$P = N_o Q_o k_1 e^{k_1 t} \tag{7}$$

From Eqs (7) and (3), we obtain

$$\frac{k}{K}N_o Q_o k_1 e^{k_1 t} = k\nu + \frac{d\nu}{dt} \tag{8}$$

Using Laplace transformation and anti-Laplace transformation, from Eq. (8) we obtain

$$\nu = A(e^{k_1 t} - e^{-kt}) \tag{9}$$

where A is called the pre-exponential factor, whose dimension is the same as that of ν.

$$A = \frac{N_o Q_o}{K} \frac{k_1 k}{k_1 + k} \tag{10}$$

Equation (9) is the power–time curve equation of bacterial growth in the log phase. It is a perfect non-linear function.

Self-function recursion equation

According to the literature [6], for a perfect non-linear function:

$$f(t) = A + Be^{-k_1 t} + Ce^{-k_2 t} \tag{11}$$

The following recursion equation can be derived:

$$f_i = b_1 f_{i+1} + b_2 f_{i+2} \quad \text{or} \quad \frac{f_i}{f_{i+1}} = b_1 + b_2 \frac{f_{i+2}}{f_{i+1}} \tag{12}$$

where

$$f_i = A + Be^{-k_1 t_i} + Ce^{-k_2 t_i}$$

$$f_{i+1} = A + Be^{-k_1 (t_i + \Delta t)} + Ce^{-k_2 (t_i + \Delta t)}$$

$$f_{i+2} = A + Be^{-k_1 (t_i + 2\Delta t)} + Ce^{-k_2 (t_i + 2\Delta t)}$$

and b_1 and b_2 are constants.

The function of the amplitude of the calorimetric signal ν with respect to time t is called the power–time curve equation. From a power–time curve, we can take three amplitudes (ν_i, ν_{i+1}, ν_{i+2}) at fixed time intervals, $\Delta t = t_{i+2} - t_{i+1} = t_{i+1} - t_i$. From Eq. (9), we can prove that

$$\frac{\nu_{i+1} e^{-k_1 \Delta t} - \nu_i}{\nu_{i+2} e^{-k_1 \Delta t} - \nu_{i+1}} = e^{k \Delta t} \tag{13}$$

Thus, we have

$$\nu_i = (e^{-k_1 \Delta t} + e^{k \Delta t}) \nu_{i+1} - e^{-k_1 \Delta t} e^{k \Delta t} \nu_{i+2} \tag{14}$$

From Eqs (12) and (14), we obtain

$$\frac{\nu_i}{\nu_{i+1}} = b_1 + b_2\frac{\nu_{i+2}}{\nu_{i+1}} \tag{15}$$

$$b_1 = e^{-k_1\Delta t} + e^{k\Delta t} \tag{16}$$

$$b_2 = -e^{-k_1\Delta t}e^{k\Delta t} \tag{17}$$

If Δt is fixed, ν_i/ν_{i+1} and ν_{i+2}/ν_{i+1} are in a linear relation. From certain sets of data, ν_i, ν_{i+1}, ν_{i+2} (i=1, 2, 3, ...), constants b_1 and b_2 can be obtained by using linear regression analysis with Eq. (15). From Eqs (16) and (17), the constants k_1 and k can be written as

$$k_1 = -\frac{1}{\sqrt{\Delta t}}\ln\left(\frac{b_1 - \sqrt{b_1^2+4b_2}}{2}\right)$$
$$k = \frac{1}{\sqrt{t}}\ln\left(\frac{b_1 + \sqrt{b_1^2+4b_2}}{2}\right) \tag{18}$$

Bacterial generation time and pre-exponential factor

According to the literature [9], the generation time G is calculated by

$$G = \frac{\ln 2}{k_1} \tag{19}$$

The pre-exponential factor A can be obtained by using Eq. (9) with k_1, k and ν.

Materials and experimental method

Bacteria and medium

The bacteria employed were *V. metschnikovii* and *V. bollisae.* The peptone culture medium contained 1 g NaCl and 1 g peptone per 100 ml (pH=8.4–8.6). It was sterilized in high-pressure steam at 120°C for 30 min.

Instrument

A type of heat-flow microcalorimeter, the 2277 bioactivity monitor manufactured by LKB, Browwa, Sweden, was used to obtain the metabolic power–time curves of the bacteria. The voltage signal was recorded by means of an LKB-2210 recorder (1000 mV range). The baseline stability for the instrument was 0.2 μW per 24 h.

The experiment was performed in a stopped-flow manner.

Experimental method

In the calorimetric experiment, first the flow cell (0.6 ml) was thoroughly cleaned and sterilized. The procedure: sterilized distilled water, 0.1 mol l^{-1} HCl,

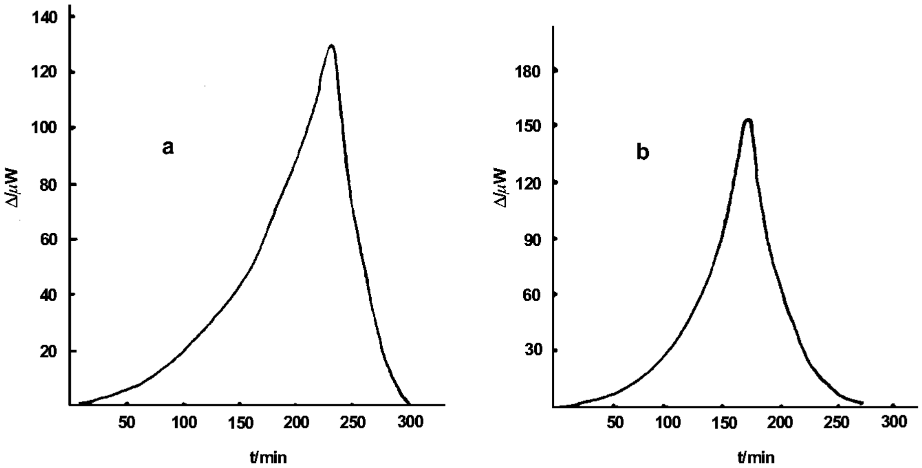

Fig. 1 Thermoanalytical curves of bacterial growth at 36°C; a – *V. metchnikovii*; b – *V. bollisae*

75% ethanol solution, and sterilized distilled water were pumped with an LKB-2132 microperpex peristaltic pump through the cell, each for 30 min, at a flow rate of 30 ml h^{-1}.

Once the system had been cleaned and sterilized, and the baseline had stabilized, the bacterial sample initially containing $1.2 \cdot 10^6$ bacteria ml^{-1} was pumped into the flow cell system and a power–time curve of continuous bacterial growth was recorded. Re-establishment of a stable baseline indicated that the process of bacterial growth had finished. The experimental temperature was fixed at 36°C.

Results and discussion

Experimental results

From the bacterial growth power–time curves, the amplitudes of the calorimetric signal *v* were obtained. According to Eq. (15), the linear equations for *V. metschnikovii* and *V. bollisae* were obtained with the data on the amplitudes v:

$$\text{for } \textit{V. metschnikovii}\text{: } \frac{v_i}{v_{i+1}} = 2.01 - 0.923\frac{v_{i+2}}{v_{i+1}} \quad (20)$$

$$\text{for } \textit{V. bollisae}\text{: } \frac{v_i}{v_{i+1}} = 2.06 - 0.920\frac{v_{i+2}}{v_{i+1}} \quad (21)$$

From Eqs (18), (19), (20), (21) and (9), the bacterial growth rate constant k_1, the time constant of the calorimeter *k*, the generation time *G*, and the pre-exponential factor *A* can be calculated. They are listed in Table 1.

Table 1 Bacterial growth at 36°C

	V. metschnikovii					*V. bollisae*				
ν_i/mV	20.0	26.5	36.0	49.5	68.8	11.0	16.5	25.0	38.0	58.0
ν_{i+1}/mV	26.5	36.0	49.5	68.8	96.0	16.5	25.0	38.0	58.0	88.5
ν_{i+2}/mV	36.0	49.5	68.8	96.0	134.2	25.0	38.0	58.0	88.5	135.5
Δt/min	15					15				
R	–0.993					–0.952				
$10^2 \cdot k_1$/min^{-1}	2.28					2.78				
$10^2 \cdot k$/min^{-1}	1.75					1.70				
G/min	30.4					24.9				
A/mV	1.05					1.58				

From these data, bacterial growth power–time curve equations in the log phase can be expressed as follows:

$$\text{for } V.\ metschnikovii\text{: } \nu = 1.05(e^{0.0228t} - e^{-0.0175t}) \quad (22)$$

$$\text{for } V.\ bollisae\text{: } \nu = 1.58(e^{0.0278t} - e^{-0.0170t}) \quad (23)$$

Discussion

It is shown from the power–time curve equations of bacterial growth in the log phase that the amplitudes consist of two parts. This is a result of using the Tian equation. According to the Tian equation (Eq. (1)), the heat production rate P is generally not proportional to the amplitude ν. Only in case $d\nu/dt=0$ P is proportional to ν. In the literature, it is general for authors to use P proportional to ν, even when $d\nu/dt$ is significantly different from zero [10]. This appears to be particularly general in reports on living cellular systems.

For the power–time curve equation of bacterial growth, if the bacterial growth time t is long enough, then $e^{k_1t} \gg e^{-kt}$. In this event, the power–time curve equation can be simplified as follows:

$$\nu = Ae^{k_1t} \quad (24)$$

$$\text{for } V.\ metschnikovii\text{: } \nu = 1.05e^{0.0228t} \quad (25)$$

$$\text{for } V.\ bollisae\text{: } \nu = 1.58e^{0.0278t} \quad (26)$$

In the literature [2, 8]:

$$P = P_0e^{k_1t} \quad (27)$$

where P_0 is constant. From Eqs (24) and (27), we have

$$P = \frac{P_o}{A}\nu = \varepsilon \tag{28}$$

where $\varepsilon=(P_0/A)$ is a constant. Therefore, P is proportional to ν. Equation (9) can be substituted by Eq. (24). If it does not satisfy $e^{k_1 t} >> e^{-kt}$, the power–time curve equation should be expressed as Eq. (9), and the heat production rate of bacterial growth P is not proportional to the amplitude ν.

As stated above, the kinetic equation in the log phase of bacterial growth, according to the results of the Tian equation and the self-function regression method for a chemical reaction was used to obtain the power–time curve equation, the growth rate constant, the generation time, the time constant of the calorimeter and the pre-exponential factor in this paper. The power–time curve equation consists of two parts. The amplitude is related not only to the bacterial growth rate constant and the culture time, but also to the time constant of the calorimeter. The scope of application of the power–time curve equation obtained in this paper is wider than that in the literature [2, 8].

References

1 P. Monk and I. Wadsö, Acta Chem. Scand., 22 (1968) 1842.
2 Z. Honglin, L. Yongjun and S. Haitao, Thermochim. Acta, 216 (1993) 19.
3 Z. Honglin, N. Zhaodong, S. Haitao, L. Yongjun, X. Iijun, S. Qingzhu and S. Xiufang, Thermochim. Acta, 223 (1993) 23.
4 L. Yi, W. Cunxin, X. Changli, Q. Songsheng and H. Zhongyu, Acta Physico-Chim. Sinica, 12 (1996) 659.
5 E. Calvet and H. Prat, Recent Progress in Microcalorimetry, Pergamon Press, London 1963.
6 Z. Xiancheng, M. Xiangguang, Z. Yuanqin, C. Minzhu, Q. Ziming and H. Qishan, Chem. J. Chinese Universities, 18 (1997) 581.
7 L. Jinsong, Ph.D. Thesis, Sichuan University, Chengdu, 1994.
8 X. Changli, T. Houkuan, S. Zhaohua and Q. Songsheng, Thermochim. Acta, 123 (1988) 33.
9 T. Houkuan, X. Changli, S. Zhaohua, Q. Songsheng, L. Yaoting and L. Haishui, Acta Physico-Chem. Sinica, 3 (1987) 113.
10 I. Wadsö, Thermochim. Acta, 294 (1997) 1.