Journal of Thermal Analysis and Calorimetry, Vol. 63 (2001) 423–431

THERMOKINETIC RESEARCH METHOD FOR BACTERIAL GROWTH IN CONDUCTION CALORIMETER

Z.-D. Nan[1], *Y. Xiang*[1], *S.-Q. Cheng*[1], *X.-C. Zeng*[1*] *and H.-L. Zhang*[2]

[1]Department of Chemistry, Sichuan University, Chengdu 610064, P. R. China
[2]Department of Chemistry, Qufu Normal University, Shandong 273165, P. R. China

(Received April 20, 2000; in revised form August 2, 2000)

Abstract

This paper presents the basic equations of thermokinetics and the thermoanalytical curve equation for bacterial growth in conduction calorimeter on the basis of the basic theory of thermokinetics. The bacterial growths in the log phase for *Vibro metschnikovii* and *Bacillus subtilis* at different temperatures were calorimetrically investigated. The rate constant of bacterial growth, the cooling constant of the thermokinetic system, the generation time and the pre-exponential factor at different temperature were obtained, which allowed to evaluate the activation energy of bacterial growth (E_a). According to the transition-state theory of chemical kinetics, the activation enthalpy ($\Delta S^{\neq}$), the activation Gibbs free energy ($\Delta G^{\neq}$) and equilibrium constant ($K^{\neq}$) of the activated state at different temperatures were also obtained. The above results showed that the research method suggested in this paper was reasonable.

Keywords: bacteria, microcalorimeter, thermoanalytical curve equation, thermokinetic properties, thermokinetics

Introduction

Microcalorimetry has been widely used to study the metabolic processes of living cells [1–6], whose basic principles are dependent on the theoretical model of the calorimeter [7–8]. For a conduction calorimeter, according to Calvet and Prat [9], the thermal power of bacterial growth and the amplitude of the calorimetric signal satisfy the Tian's equation, that is to say, the amplitudes of the calorimetric signal at a given time are expected to be proportional to the thermal power generated in bacterial growth [1–4]. In the literature [5], the Tian's equation was used to study the biological oxygen demand of aqueous by means of the calorimeter. In this paper, the basic equations of thermokinetics for bacterial growth in conduction calorimeter and the thermoanalytical curve equation have been derived according to the basic theory of

* Author to whom all correspondence should be addressed.

1418–2874/2001/ $ 5.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

thermokinetics. Using this thermoanalytical curve equation, we studied two kinds of bacteria, *Vibrio metchnikovii* (abbr. *V. Metchnikovii*) and *Bacilius subtilis* (abbr. *B. subtilis*), growing at 289.15, 292.15, 295.15, 298.15, 301.15, 304.15, 307.15 and 310.15 K. The growth rate constant, the cooling constant of the thermokinetic system, the generation time and the pre-exponential factor of bacterial growth in the log phase were calculated. According to the Arrhenius equation and the transition-state theory of chemical kinetics, the activation energy and all activation thermodynamic parameters were calculated.

Fundamental theory

The basic equation of bacterial growth

According to the basic theory of thermokinetics, when bacteria grow in conduction microcalorimeter, the thermal power W can be calculated using the Tian's equation [10]

$$W=K\Delta+\frac{\Lambda \mathrm{d}\Delta}{\mathrm{d}t} \tag{1}$$

where Δ is the amplitude of the calorimetric signal at time t; K and Λ are the heat constant and the heat capacity constant of a thermokinetic system, respectively.

Integrating Eq. (1) with respect to time t, we obtain

$$Q=Ka+\Lambda\Delta \tag{2}$$

$$Q_{\infty}=KA \tag{3}$$

where Q and Q_{∞} are the heat effects of bacterial growth up to t and total heat effect, respectively; a and A are the area up to t and total area under the thermoanalytical curve, respectively.

Let

$$k=\frac{K}{\Lambda} \tag{4}$$

where k is the cooling constant of a thermokinetic system.

Inserting Eq. (4) into Eq. (1), we have

$$\left(\frac{k}{K}\right)W=k\Delta+\frac{\mathrm{d}\Delta}{\mathrm{d}t} \tag{5}$$

When bacteria grow in conduction microcalorimeter, enthalpy is a function with respect to temperature T, pressure P and the bacterial number N. Therefore, we have

$$\mathrm{d}H=\left(\frac{\partial H}{\partial T}\right)_{P,N}\mathrm{d}T+\left(\frac{\partial H}{\partial P}\right)_{T,N}\mathrm{d}P+\left(\frac{\partial H}{\partial N}\right)_{T,P}\mathrm{d}N \tag{6}$$

It can be proved that [10]

$$W=-\left(\frac{\partial H}{\partial N}\right)_{T,P}\frac{dN}{dt} \tag{7}$$

$$Q=-\left(\frac{\partial H}{\partial N}\right)_{T,P}(N-N_0) \tag{8}$$

$$Q_\infty=-\left(\frac{\partial H}{\partial N}\right)_{T,P}(N_\infty-N_0) \tag{9}$$

where N_0 and N_∞ are bacterial numbers at the initial time and at the final time of bacterial growth , respectively.

From Eqs (7), (8) and (9), we have

$$\frac{Q}{Q_\infty}=\frac{N-N_0}{N_\infty-N_0} \tag{10}$$

$$\frac{W}{Q_\infty}=\frac{\frac{dN}{dt}}{N_\infty-N_0} \tag{11}$$

According to Eqs (1), (2), (3), (10) and (11), we have

$$\frac{Ka+\Lambda\Delta}{KA}=\frac{N-N_0}{N_\infty-N_0} \tag{12}$$

$$\frac{\frac{K\Delta+\Lambda d\Delta}{dt}}{KA}=\frac{\frac{dN}{dt}}{N_\infty-N_0} \tag{13}$$

From [10], we have

$$\Delta=K^{-1}e^{-kt}\int_0^t We^{kt}d(kt) \tag{14}$$

From Eqs (13) and (14), we obtain

$$\Delta=A(N_\infty-N_0)^{-1}e^{-kt}\int_0^t\left(\frac{dN}{dt}\right)e^{kt}d(kt) \tag{15}$$

Equations (12), (13) and (15) are the basic equations of thermokinetics for bacterial growth, and Eq. (15) is the thermoanalytical curve equation of bacterial growth in conduction calorimeter. There are many kinds of relationship of bacterial growth about dN/dt with respect to time t. Thus, the thermoanalytical curve equation can be obtained by inserting the relationship of bacterial growth about dN/dt with respect to time t into Eq. (15).

Thermoanalytical curve equation of bacterial growth in the log phase

Bacterial number and culture time are in accordance with exponential law in the log phase [1, 4]

$$N=N_0 e^{k_1 t} \tag{16}$$

$$\frac{dN}{dt}=N_0 k_1 e^{k_1 t} \tag{17}$$

where k_1 is bacterial growth rate constant. Inserting Eq. (17) into Eq. (15), we obtain

$$\Delta=A'(e^{k_1 t}-e^{-kt}) \tag{18}$$

$$A'=\frac{N_0 A}{N_\infty - N_0}\frac{kk_1}{k+k_1} \tag{19}$$

Equation (18) is a thermoanalytical curve equation of bacterial growth in the log phase. It is a perfect non-linear equation. A' is called pre-exponential factor, the dimension of which is the same as that of amplitude of the calorimetric signal.

Self-function recursion equation

For a perfect non-linear equation [11]:

$$f(t)=A+Be^{-k_1 t}+Ce^{-k_2 t} \tag{20}$$

The following recursion equation can be derived:

$$\frac{f_i}{f_{i+1}}=b_1+\frac{b_2 f_{i+2}}{f_{i+1}} \tag{21}$$

where $f_i=A+Be^{-k_1 t_i}+Ce^{-k_2 t_i}$, $f_{i+1}=A+Be^{-k_1 (t_i+\Delta t)}+Ce^{-k_2 (t_i+\Delta t)}$, $f_{i+2}=A+Be^{-k_1 (t_i+2\Delta t)}+Ce^{-k_2 (t_i+2\Delta t)}$, b_1 and b_2 are all constants.

The following recursion equation can be derived for Eq. (18)

$$\frac{\Delta_i}{\Delta_{i+1}}=\frac{b_1+b_2\Delta_{i+2}}{\Delta_{i+1}} \tag{22}$$

$$b_1=e^{-k_1\Delta t}+e^{k\Delta t} \tag{23}$$

$$b_2=-e^{-k_1\Delta t}\times e^{k\Delta t} \tag{24}$$

where Δ_i, Δ_{i+1}, Δ_{i+2} are three amplitudes of the same thermoanalytical curve at fixed time intervals, $\Delta t=t_{i+2}-t_{i+1}=t_{i+1}-t_i$. From some sets of data, Δ_i, Δ_{i+1}, Δ_{i+2} (i=1, 2, 3…), b_1 and b_2 can be calculated by using linear regression analysis for Eq. (22). From Eqs (23) and (24), k_1 and k can be written as

$$k_1=\frac{-1}{\Delta t}\ln\left[\frac{b_1-\sqrt{b_1^2+4b_2}}{2}\right]$$
$$k=\frac{1}{\Delta t}\ln\left[\frac{b_1+\sqrt{b_1^2+4b_2}}{2}\right] \quad (25)$$

Generation time and pre-exponential factor

Generation time G is calculated by [1]

$$G=\frac{\ln 2}{k_1} \quad (26)$$

The pre-exponential factor A' can be obtained by using Eq. (18), N_∞ can be calculated by using Eq. (19).

Thermodynamic activation parameters

Activation energy can be calculated with Arrhenius equation

$$\ln k_1=\frac{-E_a}{RT}+B \quad (27)$$

where k_1 is the bacterial growth rate constant at temperature T, E_a is activation energy, B is constant, R is gas constant. $\ln k_1$ and $1/T$ have a linear relation. E_a can be obtained by using linear regression analysis.

The biochemistry involved bacterial growth is massively complicated. In order to apply the transition-state theory of chemical kinetics, it was supposed that bacterial growth was divided into three steps [12]:

$$X+S \underset{k_b}{\overset{k_f}{\rightleftharpoons}} XS \xrightarrow{k_1} 2X+P \quad (28)$$

where X is bacterium, S is substrate, XS is bacterium-substrate complex, P is the product. According to the transition-state theory of chemical kinetics [13],

$$k_1=\left(\frac{k'T}{h}\right)\exp\left(\frac{\Delta S^{\neq}}{R}\right)\exp\left(-\frac{\Delta H^{\neq}}{RT}\right) \quad (29)$$

$$\Delta H^{\neq}=E_a-RT \quad (30)$$

where k' is Boltzman constant, h is Planck constant; $\Delta S^{\neq}$ is activation entropy of bacterial growth, $\Delta H^{\neq}$ is the activation enthalpy.

Activation Gibbs free energy $\Delta G^{\neq}$ and equilibrium constant $K^{\neq}$ of the activated state can be calculated with Eqs (31) and (32), respectively.

$$\Delta G^{\neq}=\Delta H^{\neq}-T\Delta S^{\neq} \quad (31)$$

$$K^{\neq} = \exp\left(-\frac{\Delta G^{\neq}}{RT}\right) \tag{32}$$

Materials and experimental method

Bacteria and medium

The bacteria employed were *V. metchnikovii* and *B. subtilis*.

A soluble medium (pH=7.2–7.4) was used, which contained NaCl (1 g), pepton (2 g) and beef extract (1 g) per 200 mL water. The soluble medium was sterilized in high pressure steam at 120°C for 30 min.

Instrument

A type of heat-flow microcalorimeter, the 2277 thermal acitivity monitor manufactured by ThermoMetric AB, Sweden, was used in this experiment. The experiment was performed in a stopped-flow manner.

Experimental method

In the calorimetric experiment, the flow vessel was completely cleaned and sterilized firstly. The procedure was: sterilized distilled water. 0.1 mol L^{-1} HCl, 75% alcohol solution and sterilized distilled water was pumped by a LKB-2132 microperpex peristaltic pump respectively through the vessel, each for 30 min, at a flow rate of 30 mL h^{-1}.

Once the system was cleaned and sterilized, and the baseline had been stabilized, the bacterial sample, initially containing $1.0 \cdot 10^7$ bacteria mL^{-1}, was pumped into the flow vessel system and a thermoanalytical curve of bacterial growth was recorded.

Results and discussion

The experimental results show highly reproducible under the same conditions. According to Eq. (22), the linear equations of the two kinds of bacteria at 304.12 K have been obtained as follows:

For *V. metchnikovii*
$$\frac{\Delta_i}{\Delta_{i+1}} = 1.95 - 0.927\frac{\Delta_{i+2}}{\Delta_{i+1}} \tag{33}$$

For *B. subtilis*
$$\frac{\Delta_i}{\Delta_{i+1}} = 2.12 - 1.030\frac{\Delta_{i+2}}{\Delta_{i+1}} \tag{34}$$

From Eqs (25), (26), (18) and (19), bacterial growth rate constants k_1, the cooling constants of the thermokinetic systems k, the generation time G, the pre-exponential factors A' and N_∞ can be calculated, which are listed in Table 1.

Table 1 The experimental, calculating and fitting results of bacterial growth at 304.15 K

	V. metchnikovii					*B. subtilis*				
Δ_t/μW	10.8	15.0	19.8	25.8	33.2	6.5	9.5	13.2	18.0	24.2
Δ_{i+1}/μW	15.0	19.8	25.8	33.2	42.0	9.5	13.2	18.0	24.2	32.8
Δ_{i+2}/μW	19.8	25.8	33.2	42.0	52.0	13.2	18.0	24.2	32.8	42.8
Δt/min	10					10				
r	–0.964					–0.988				
$k_1 \cdot 10^2$/min^{-1}	2.07					2.82				
$k \cdot 10^2$/min^{-1}	1.21					3.12				
G/min	33.5					24.6				
A^*/μW	0.715					0.330				
A/μW min	9270					2005				
N_0/cells mL^{-1}	$1.0 \cdot 10^7$					$1.0 \cdot 10^7$				
N_∞/cells mL^{-1}	$100 \cdot 10^7$					$91.0 \cdot 10^7$				

*: average value

From these data, the thermoanalytical curve equations of bacterial growth in the log phase at 304.15 K have been obtained as follows:

For *V. metchnikovii* $\Delta=0.715(e^{0.0207t}-e^{-0.121t})$ (35)

For *B. subtilis* $\Delta=0.330(e^{0.0282t}-e^{-0.312t})$ (36)

The thermoanalytical curve equations of bacterial growth in the log phase at different temperatures have been obtained with the same method. They are listed in Table 2.

Table 2 The thermoanalytical curve equations of bacterial growth at different temperatures

T/K	*V. metchnikovii*	*B. subtilis*
289.15	$\Delta=0.603(e^{0.00201t}-e^{-0..00142t})$	$\Delta=0.489(e^{0.01121t}-e^{-0..00996t})$
292.15	$\Delta=0.412(e^{0.00352t}-e^{-0..00208t})$	$\Delta=0.745(e^{0.0136t}-e^{-0..0124t})$
295.15	$\Delta=0.678(e^{0.00522t}-e^{-0..00346t})$	$\Delta=0.542(e^{0.0192t}-e^{-0..0165t})$
298.15	$\Delta=0.487(e^{0.00932t}-e^{-0..00602t})$	$\Delta=0.721(e^{0.0218t}-e^{-0..0208t})$
301.15	$\Delta=0.864(e^{0.0190t}-e^{-0..0102t})$	$\Delta=0.605(e^{0.0248t}-e^{-0..0265t})$
304.15	$\Delta=0.715(e^{0.0207t}-e^{-0..0121t})$	$\Delta=0.330(e^{0.0282t}-e^{-0..0312t})$
307.15	$\Delta=0.508(e^{0.0328t}-e^{-0..0932t})$	$\Delta=1.85(e^{0.0321t}-e^{-0..0720t})$
310.15	$\Delta=1.25(e^{0.0468t}-e^{-0..0124t})$	$\Delta=1.34(e^{0.0365t}-e^{-0..0985t})$

According to Eqs (27), (29), (30), (31) and (32), bacterial growth rate constants, activation energies, activation enthalpies, activation entropies, activation Gibbs free energies and equilibrium constants of the activated state have been calculated and listed in Table 3.

Table 3 Growth rate constants, activation energies, activation enthalpies, activation entropies, activation Gibbs free energies and equilibrium constants of activated state of bacterial growth*

Bacteria	T/K	k_1/min^{-1}	E_a/	$\Delta H^{\neq}$	$\Delta S^{\neq}$	$\Delta G^{\neq}$	$K^{\neq}\cdot 10^{17}$
			kJ mol^{-1}			kJ mol^{-1} K^{-1}	kJ mol^{-1}
V. metchnikovii	289.15	0.00201	113	111	53.6	95.5	0.559
	292.15	0.00352		111	54.2	95.2	0.951
	295.15	0.00522		111	53.5	95.2	1.42
	298.15	0.00932		111	54.5	94.8	2.46
	301.15	0.0190		110	53.3	93.9	5.16
	304.15	0.0207		110	50.3	94.7	5.44
	307.15	0.0328		110	50.5	94.5	8.48
	310.15	0.0468		110	49.9	94.5	12.1
B. subtilis	289.15	0.0112	41.1	38.7	–182	91.3	3.21
	292.15	0.0136		38.7	–182	91.9	3.70
	295.15	0.0192		38.6	–181	92.0	5.22
	298.15	0.0218		38.6	–181	92.6	5.97
	301.15	0.0248		38.6	–182	93.4	6.30
	304.15	0.0282		38.6	–182	94.0	7.18
	307.15	0.0321		38.5	–182	94.4	8.82
	310.15	0.0365		38.5	–183	95.3	8.90

*: for *V. metchnikovii* $\ln k_1 = 40.8 - 1.36\cdot 10^4/T$ $r = -0.993$

B. subtilis $\ln k_1 = 12.7 - 4.94\cdot 10^3/T$ $r = -0.986$

From the thermoanalytical curve equations of bacterial growth in the log phase, it can be seen that the amplitudes consist of two parts. Only in case $d\Delta/dt=0$, is W proportional to Δ. In the literature, it is general for authors to use W proportional to Δ, even when $d\Delta/dt$ is significantly different from zero [14]. If it satisfies $e^{k_1 t} >> e^{-kt}$, the equation of the thermoanalytical curve for bacterial growth in the log phase can be expressed as the equation presented in literature [1, 4]. Thus, the scope of application of the thermoanalytical curve equation obtained in this paper is wider than that in literature [1, 4]. In literature [5], the Tian equation was used, the cooling constants of the thermokinetic system were obtained by using electric calibration, while the cooling constant of the thermokinetic system can be obtained directly through the thermoanalytical curve in this paper.

From Table 3, we can see that the activation parameters of these two kinds of bacteria are obviously different. For *V. metchnikovii*, its growth activation energy is 113 kJ mol^{-1}, so its growth is a slow process. The effect of temperature on its growth rate constant is significant, k_1 increases about twenty times, but temperature hardly affects its activation enthalpy, activation entropy and activation Gibbs free energy. The equilibrium constant of the activated state increases about twenty times in the ex-

perimental temperature range. In contrast, the effect of temperature on *B. subtilis* growth is not obvious, k_1 only increases about three times in the experimental temperature range. The activation entropies of the activated state forming intermediate at different temperatures are negative. The decrease of entropy means that the formation of bacterium-substrate complex makes the decrease of the chaotic degree of system. The probability of forming bacterium-substrate complex relatively decreases, thus decreasing formation rate of the product. $K^{\neq}$ only increases about three times in the experimental temperature range. The values of the activation Gibbs free energy of these two kinds of bacteria are all between 92–96 kJ mol^{-1}, indicating that the values of the activation Gibbs free energy are approximately equal in the same medium. The activation Gibbs free energy is positive, which shows that bacterial growth needs to get energy in order to form bacterium-substrate complex. The orders of the magnitude of the equilibrium constants for the activated state of these two kinds of bacteria are about 10^{-17}, which indicates that the formation of activated bacterium-substrate complex is not easy.

References

1 Chang-Li Xie, Hou-Kuan Tang, Zhao-Hua and Song-Sheng Qu, Thermochim. Acta, 123 (1988) 33.
2 Hong-Lin Zhang, Hai-Tao Sun, Yong-Jun Liu, Qing-Zhu Shan and Xiu-Feng Sun, Thermochim. Acta, 223 (1993) 29.
3 P. Monk and I. Wadsö, Acta Chem. Scandinavic, 22 (1968) 1842.
4 L. Nunez Regueira, I. Gomez Orellane, N. Barros Pena, R. Chamy and J. M. Lema, Thermochim. Acta, 172 (1990) 163.
5 P. Weppen, J. Ebens, B. G. Muller and D. Schuller, Thermochim. Acta, 193 (1991) 135.
6 P. Backman, Thermochim. Acta, 172 (1990) 123.
7 E. H. Battley, Thermochim. Acta, 309 (1998) 17.
8 L. Marisonk, J. S. Liu, S. Ampuero, U. Von Stockar and B. Schenker, Thermochim. Acta, 309 (1998) 157.
9 E. Calvet and H. Prat, Recent Progress in Microcalorimetry, Pergamon Press, Oxfod 1963.
10 Yu Deng, Chem. J. Chinese Univ., 6 (1985) 621.
11 Xian-Cheng Zeng, Xiang-Guang Meng, Yuan-Qin Zhang and Min-Zu Chen, Chem. J. Chinese Univ., 18 (1997) 581.
12 Yong-Jun Liu, Yang-Jun Ding, Zhao-Dong Nan, Hai-Tao Sun and Hong-Lin Zhang, J. Thermal Anal., 50 (1997) 897.
13 W. J. Moore, Physical Chemistry, Longman Group Limited, London, 5th ed., 1972, p. 385.
14 I. Wadsö, Thermochim. Acta, 294 (1997) 1.